

Développement de dispositifs microfluidiques avec détecteurs électrochimiques intégrés utilisant la technologie d'impression 3D

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THÈSE DE DOCTORAT DE L'UNIVERSITÉ PSL

Préparée à l'École Nationale Supérieure de Chemie de Paris Dans le cadre d'une cotutelle avec Université de Campinas

Développement de dispositifs microfluidiques avec détecteurs électrochimiques intégrés utilisant la technologie d'impression 3D

Development of microfluidic devices with integrated electrochemical detectors using 3D printing technology

Soutenue par

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Reading Assistance

This doctoral manuscript describes the development project of microfluidic devices with integrated electrodes using 3D printing technology. A complete summary of the thesis is written in French and Portuguese situated in the first part of the manuscript followed by a chapter "state of art", written in English. The followed chapters are written in English as well. They consist of articles produced during this thesis, which are fully reproduced and supplemented with additional data:

- Chapter I State of the art
- Chapter II The 3D-printed microchip capillary electrophoresis containing spiral electrodes for capacitively coupled contactless conductivity detection
- Chapter III Stereolithography based 3D-printed microfluidic device with integrated electrochemical detection
- Future Perspectives and Conclusions

In addition, each chapter contains its own bibliographic section for a better understanding.

RESUMÉ DE LA THESE

1. Introduction

La miniaturisation des analyses chimiques et biomédicales est apparue ces dernières années dans le but de surpasser les performances des dispositifs d'analyse conventionnels, car elle concerne de très petits volumes d'échantillons et de réactifs, un faible coût de fabrication et la réduction de la taille physique des systèmes pour créer des dispositifs portables qui pourraient être utilisés dans n'importe quelle partie du monde sans expertise technique ou conditions expérimentales spécifiques. L'intérêt de cette miniaturisation est aussi d'intégrer les différentes étapes analytiques dans un seul dispositif [1], par exemple l'intégration d'une étape de séparation en amont avec une méthode de détection sensible qui conduit à un processus d'analyse total combinant des mesures sélectives et sensibles.

Les détections électrochimique et conductométrique sont adaptées au format des micropuces. En ce qui concerne le processus de fabrication, les microélectrodes peuvent être fabriquées directement dans le micro-dispositif en utilisant les mêmes procédures que celles utilisées pour construire les micropuces. Le couplage d'un dispositif microfluidique électrochimique (EMD) avec une étape de séparation électrophorétique favorise la création de systèmes de separation électrocinétique en micropuce (MCE, microchip electrophoresis). D'excellents travaux de recherche utilisant des matériaux émergents pour la fabrication d'électrodes ont été rapportés par plusieurs groupes [2-4], et l'utilisation d'électrodes intégrées dans les systèmes MCE suscite un intérêt croissant. La MCE représente la prochaine génération de dispositifs miniaturisés pour l'analyse de matrices d'échantillons complexes avec une instrumentation simple, puisque l'utilisation de vannes ou de pompes n'est pas nécessaire pour le contrôle électrocinétique des fluides ; elle est facilement miniaturisée, nécessite de faibles tensions et permet des temps d'analyse courts [5]. Cependant, la détection électrochimique est peu développée en CME. Tous les auteurs mentionnés précédemment ont dû faire face à des problèmes qui compliquent le développement en système microfluidique du couplage de la séparation électrocinétique avec une détection électrochimique, car des interférences entre les deux champs électriques peuvent se produire.

Le principal défi consiste donc à isoler le système de détection de la haute tension de séparation afin de préserver à la fois le rapport signal/bruit et l'intégrité du potentiostat. L'alignement et le positionnement des électrodes peuvent aussi impacter la reproductibilité de l'analyse. Le matériau des électrodes doit être compatible avec le substrat du dispositif afin d'éviter les fuites et de promouvoir un microdispositif expérimental robuste. En outre, le processus de fabrication classique intégrant des canaux microfluidiques dans ces nécessite des installations en salle blanche. Dans ce contexte, nous avons décidé d'utiliser la technologie de fabrication additive (AM, additive manufacturing), également connue sous le nom d'impression tridimensionnelle (3D), comme approche pour surmonter tous les problèmes de fabrication mentionnés précédemment. La figure 1.1 présente une illustration d'un dispositif générique contenant un canal électrophorétique de séparation et une detection électrochimique.



Figure 1.1. Dispositif MCE classique avec électrodes intégrées pour effectuer la détection électrochimique

La technologie d'impression 3D est un moyen polyvalent pour assembler des pièces complexes en étapes simples et automatisées. Cette technique permet de définir les microcanaux et les microstructures avec des avantages tels que la haute résolution, la variété des matériaux et la personnalisation dans le développement des dispositifs par rapport aux dispositifs 2D [6]. Même si la plupart des dispositifs fluidiques imprimés en 3D et leurs caractéristiques se situaient jusqu'à présent à l'échelle millifluidique, certains dispositifs véritablement microfluidiques (MD) ont été présentés [7]. Aujourd'hui, la stéréolithographie (SLA) et le traitement numérique de la lumière (DLP) constituent les approches les plus prometteuses pour la création de structures microfluidiques en raison de leur haute résolution. Ces technologies de polymérisation font appel à une résine photosensible durcie par une source lumineuse pour produire des couches solides et éventuellement des pièces trouées [8]. Alors que ces résines commerciales ne sont pas nécessairement formulées pour répondre aux exigences de la microfluidique, plusieurs groupes ont fait état d'imprimantes et de matériaux personnalisés qui ont été utilisés avec succès pour intégrer des éléments microfluidiques de taille inférieure à 50 μ m [9,10].

L'utilisation de l'AM pour l'assemblage de systèmes MD peut offrir plusieurs possibilités pour simplifier le processus de fabrication. Dans ce contexte, les lecteurs trouveront dans ce manuscrit des protocoles et des stratégies développés pour répondre aux exigences de la fabrication de MD en utilisant la technologie d'impression 3D. En même temps, des MD ont été conçus avec des électrodes insérées dans des canaux pour surmonter la nécessité d'alignement de ces électrodes pour la détection par EC. Le manuscript est structuré de la façon suivante :

- un microchip MCE-C4D a été développé, basé sur la technologie DLP-SLA utilisant une résine personnalisée et une imprimante 3D adéquate pour assembler les microcanaux (Fig. 1.2.a). Le système proposé contient des canaux en spirale remplis de gallium fondu pour servir d'électrodes. Le dispositif a été caractérisé en utilisant des cations inorganiques et employé pour déterminer le nitrate dans des échantillons biologiques.

- un microchip EMD a été développé par impression 3D par SLA à l'aide d'une résine commerciale et d'une imprimante 3D utilisant une approche d'impression 3D à canaux ouverts pour assembler les microcanaux (Fig. 1.2.b). Les microcanaux ont été scellés par un ruban de silicone. Le système proposé contient un canal d'électrode en forme de triangle avec une surface rectangulaire remplie d'une résine conductrice personnalisée pour servir d'électrode de travail (WE). Par la suite, une technique de séparation électrocinétique a été intégrée à la plateforme pour réaliser un dispositif MCE-EC imprimé en 3D (Fig. 1.2.c). La faisabilité de l'électrode intégrée dans les systèmes EMD et MCE-EC a été étudiée en utilisant le ferrocèneméthanol (FcMeOH) et l'hexaammineruthénium (III) (Ru(NH3)63+) comme preuve de concept.

- Le dispositif MCE-EC a été fabriqué à l'aide d'une résine commerciale et d'une imprimante 3D basée sur la technologie DLP sans le processus de scellement. Les paramètres d'impression ont été optimisés pour fabriquer des canaux microfluidiques étroits. D'autres travaux seront effectués en utilisant ce nouveau dispositif (Fig. 1.2.d).



Figure 1.2. Dispositifs imprimés en 3D conçus et fabriqués dans le cadre de ce projet. (a) MCE-C4D imprimé en 3D par DLP/SLA ; (b) EMD imprimé en 3D par SLA ; (c) MCE-EC imprimé en 3D par SLA ; (d) MCE-EC imprimé en 3D par DLP.

Les méthodologies proposées se veulent des alternatives simples et peu coûteuses aux méthodes actuelles de microfabrication, telles que la lithographie douce. L'impression 3D est destinée à accroître la facilité de production, ce qui permettra aux chercheurs de prototyper rapidement de nouveaux DM avec des électrodes intégrées sans avoir besoin de salles blanches ou d'équipements coûteux.

2. Le dispositif MCE imprimé en 3D contenant des electrode en spirale pour la detection par C⁴D

1. Conception et fabrication du microsystème

Nous avons conçu le microdispositif pour les impressions 3D en utilisant OpenSCAD (openscad.org), un logiciel gratuit de conception assistée par ordinateur (figure 2.1.a). La disposition standard du MCE à canaux croisés, avec des canaux de 45 µm de large et 50 µm de haut, a été adaptée à partir d'un modèle publié précédemment [10]. Nous avons ajouté à cette conception deux canaux indépendants en spirale pour le C4D, qui ont été enroulés autour du canal de séparation au point de détection (Figure 2.1.b).



Figure 2.1. Conception OpenSCAD du dispositif d'électrodes spirales C4D. (a) Vue du dessus et (b) vue orthogonale du dispositif avec les réservoirs d'électrophorèse représentés en violet, les canaux en vert, et les électrodes C4D en spirale en turquoise.

Les dispositifs ont été fabriqués à l'aide d'une imprimante 3D personnalisée décrite précédemment [11]. En bref, cette imprimante 3D à stéréolithographie DLP utilise une lumière de 385 nm pour polymériser séquentiellement des couches de résine à motifs de 10-µm d'épaisseur. La résine était composée de 97 % de poly(éthylène glycol) diacrylate (PEGDA, MW 250), de 2 % d'absorbeur d'UV 2-nitrophényl phényl sulfide (NPS) et de 1 % de photoinitiateur Irgacure 819. Une fois le dispositif polymérisé, les canaux ont été lavés de l'excès de résine sous pression négative, puis rincés à l'alcool isopropylique. Le dispositif microfluidique a ensuite été exposé à traitement UV pendant 20 minutes pour polymériser tout excès de résine (Fig. 2.2.a). Après la préparation des micropuces, le gallium a été fondu par chauffage à environ 35 °C et injecté dans les canaux en spirale en remplissant une extrémité du canal à l'aide d'une seringue en plastique pour former des électrodes métalliques autour du canal de séparation (Fig. 2.2.b). Des fils de cuivre ont été utilisés pour le contact électrique avec les électrodes de gallium et le circuit de détection (Fig. 2.2.c). Enfin, le MCE-C4D est prêt pour les applications (Fig. 2.2.d).



Figure 2.2. Schéma d'assemblage d'un dispositif électrophorétique entièrement microfluidique. (a) Dispositif exposé à un traitement UV. (b) Gallium injecté dans les canaux en spirale en remplissant une extrémité du canal. (c) Fils de cuivre servant de contact électrique avec les électrodes de Gallium et le circuit de détection. (d) Dispositif prêt pour les applications.

Le dispositif en T simple avait des dimensions de 12 mm de largeur et 23 mm de longueur, avec un canal d'injection de 5,3 mm de longueur et un canal de séparation de 13 mm de longueur, avec une distance de séparation effective de 9,5 mm (Fig. 2.3.a). En ce qui concerne les deux canaux spiralés indépendants pour le C4D (Fig. 2.3.b), le rayon de chaque canal spiralé était de 100 μ m, avec une amplitude de spirale de 300 μ m et un pas de 300 μ m, et produisant une longueur totale du fil de gallium de 11 mm après remplissage (Fig. 2.3. c).



Figure 2.3 (a) Dispositif imprimé montrant les connexions avec les électrodes en spirale et la taille de la micropuce. (b) Vue CAD de la micropuce. (B1) Détail du canal d'écoulement en serpentin utilisé pour injecter le gallium. (B2) Réservoirs d'entrée et de sortie connectés au canal en serpentin pour l'injection de métal. (B3) Réservoir de déchets tampon. (B4) Réservoir d'échantillon. (B5) Réservoirs tampons. (c) Canaux d'électrode en serpentin 3D entourant le canal de séparation dans un dispositif microfluidique fabriqué par DLP (A1) sans gallium fondu et (A2) rempli de gallium fondu pour former des électrodes en spirale pour la C⁴D.

2. Resultats et discussion

La réalisation de dispositions complexes, dans ce cas de canaux en spirale autour d'un canal de séparation central, serait très compliquée en utilisant le prototypage conventionnel de l'AM, comme la lithographie et la lithographie douce en raison de sa caractéristique soustractive. En outre, la plupart des techniques d'AM n'ont pas la résolution nécessaire pour produire un véritable MD. Ainsi, SLA et DLP sont les approches les plus prometteuses à utiliser dans ce travail pour créer des structures microfluidiques complexes en raison de leur haute résolution. Tout d'abord, nous avons essayé de reproduire une conception tubulaire classique d'électrodes pour effectuer une détection C⁴D. Cependant, pour cette conception d'électrodes, il était laborieux de remplir les canaux de liquides de manière homogène en raison de l'air piégé. Dans ce contexte, nous avons créé une conception en spirale, dont les performances peuvent être comparables à celles d'une électrode tubulaire puisqu'elle entoure également le canal. De plus, les canaux de l'électrode peuvent être facilement remplis de métaux liquides sans compromettre la connexion de l'électrode ou même son intégration.

1. Caractérisation et optimisation de l'electrode

Dans les détecteurs C⁴D, la tension d'excitation et la fréquence de fonctionnement sont des paramètres qui affectent directement la réponse analytique. Ainsi, ces deux paramètres ont été optimisés avant de maximiser la sensibilité du détecteur. Le signal C⁴D a été étudié en utilisant des valeurs de fréquence allant de 84 à 628 kHz en maintenant les tensions d'excitation constantes à des valeurs de 3,0, 5,0 et 7,0 Vp (voir Fig. 5) tandis que le canal de séparation était rempli d'un tampon MES/histidine à 5 mmol L⁻¹.



Figure 2.4. Réponse en fréquence pour le dispositif C4D-MCE à différentes amplitudes du signal d'excitation. Le canal de séparation a été rempli d'une solution tampon contenant 5 mmol L-1 d'acide 2-(N-morpholino)éthanesulfonique et d'histidine.

D'après nos précédents travaux [12] sur le développement du C⁴D, la meilleure fréquence de fonctionnement dépend principalement des paramètres géométriques de la cellule de détection, bien que la conductivité du BGE exerce également une certaine influence. De plus, la mesure de la tension de sortie de la ligne de base est en corrélation directe avec la surface du pic, ce qui constitue une méthode plus pratique pour optimiser la fréquence [12]. Comme on peut le voir sur la figure 2.4, en augmentant la fréquence de fonctionnement, la tension de sortie augmente jusqu'à une valeur maximale et à partir de ce point, les tensions de sortie du canal de paroi domine et bloque la plupart du signal d'excitation, tandis qu'à haute fréquence, les capacités parasites (y compris la capacité d'entrée de l'amplificateur de transimpédance) offrent des chemins alternatifs pour le signal. Habituellement, la fréquence est fixée à ce maximum pour obtenir la meilleure réponse. De plus, ce maximum est déplacé vers des fréquences plus basses en augmentant l'amplitude du signal. Les valeurs maximales

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mesurées à 3,0, 5,0 et 7,0 Vp ont été obtenues pour le MCE-C⁴D à 293, 238 et 207 kHz, respectivement. En résumé, pour une amplitude et une composition BGE données, la fréquence est balayée et fixée au maximum pour les expériences ultérieures.

Pour étudier les performances analytiques du MCE-C⁴D ainsi que son utilisation pour le suivi des espèces cationiques et anioniques dans les échantillons biologiques, la fréquence de fonctionnement et les valeurs de la tension d'excitation ont ensuite été fixées à 250 kHz et 3,5 Vp, respectivement. Nous avons ensuite cherché à évaluer les valeurs de fréquence de fonctionnement et de tension d'excitation pour déterminer les meilleures conditions de séparation pour K⁺, Na⁺ et Li⁺ concernant la concentration du tampon et la tension appliquée. Un mélange standard de ces cations inorganiques a été choisi comme système analytique modèle car ce sont des ions couramment utilisés pour fournir des informations sur le comportement du système C⁴D, en raison de la simplicité du système [13,14]. En ce qui concerne les micropuces basées sur la DLP, les meilleurs résultats ont été obtenus en utilisant un tampon composé de 20 mmol L⁻¹ MES/histidine (pH = 6,0) à 25 °C. La tension de séparation de 800 V, la tension d'injection de 500 V et le temps d'injection de 3 s ont été sélectionnés pour la séparation de K⁺, Na⁺ et Li⁺ avec une efficacité et une résolution de pic adéquates (Fig. 2.5).



Figure 2.5. Electrophérogrammes de solutions standards contenant les ions K⁺, Na⁺, et Li⁺ (25-200 μ mol L⁻¹). Les courbes d'étalonnage respectives sont également représentées. Injection en mode "gated" en appliquant +500 V et +800 V aux réservoirs d'échantillon et de tampon.

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De plus, la linéarité de la méthode a été évaluée en utilisant des solutions standards de K^+ , Na⁺ et Li⁺ (25-200 µmol L⁻¹). Une linéarité adéquate (r > 0.996) a été obtenue pour tous les tracés d'étalonnage (Fig. 2), ce qui montre une réponse appropriée du détecteur à la concentration des analytes. Il s'agit d'une meilleure performance par rapport à d'autres détecteurs fabriqués par des métaux liquides et précédemment rapportés dans la littérature. Par exemple, Thredgold et al. [15], ont rapporté une électrode de gallium avec une conception en plan pour le C⁴D. Cette électrode a fourni un coefficient de corrélation linéaire de 0,9729, 0,9794 et 0,9982 pour K⁺, Na⁺ et Li⁺, respectivement. Même si les électrodes étaient fabriquées dans le même matériau, la géométrie en spirale de l'électrode présentée ici offrait une meilleure réponse linéaire que la conception de l'électrode dans le plan.

2. Choix du milieu pour la quantification de nitrate dans la salive

La détermination du nitrate a été réalisée dans les conditions précédemment rapportées [16]. Pour optimiser le détecteur, une solution tampon composée d'acide lactique et d'histidine a été préparée à différentes proportions afin d'inspecter sa qualité de détection. La concentration d'acide lactique était fixe (30 mmol L⁻¹) et la concentration en histidine variait dans une gamme de 10 à 20 mmol L⁻¹ (avec des incréments de 5 mmol L⁻¹). Bien que toutes les proportions aient montré avec succès un signal pour le nitrate, la meilleure performance en considérant le temps d'analyse, le rapport signal/bruit, la force ionique/la capacité tampon et la détectabilité a été obtenue en utilisant le tampon composé de 30 mmol L⁻¹ d'acide lactique et de 15 mmol L⁻¹ d'histidine (pH 3.8) à 25 °C. Les solutions standard et d'échantillon ont été introduites à l'intérieur des microcanaux par un protocole d'''injection flottante''. La tension de séparation de -700 V, la tension d'injection de -500 V et le temps d'injection de 3 s ont été sélectionnés pour la détermination du nitrate dans la salive.

3. Performance analytique et application

Des études ont été menées afin d'examiner systématiquement les performances analytiques du MCE-C⁴D pour la détermination du nitrate dans les échantillons biologiques. Un problème courant concernant l'utilisation des puces d'électrophorèse est l'injection électrocinétique, qui peut compromettre la répétabilité de l'injection de l'échantillon. Nous avons donc injecté une solution standard de nitrate pour atteindre une concentration de 100 μ mol L⁻¹ dans l'échantillon de salive, puis nous avons effectué dix injections successives pour étudier la répétabilité de l'injection (Fig. 2.6).

L'écart-type relatif intrajournalier (RSD) de la surface du pic et du temps de migration était de 8,6 et 5,0 %, respectivement. Sur la base des résultats statistiques, on peut déduire que cette méthode MCE-C⁴D offre une répétabilité convenable pour dix injections successives. Un résumé des caractéristiques analytiques de cette méthode impliquant la MCE-C⁴D est présenté dans le tableau 1.



Figure 2.6. Electrophérogramme obtenu pour dix injections consécutives d'une solution d'échantillon de salive contenant 100 µmol L-1 de nitrate. BGE : 30/15 mmol L-1 d'acide lactique/His (pH 3,8). Injection de l'échantillon flottant à -500 V pendant 3 s. La tension de séparation était de -700 V. Détecteur C4D fonctionnant à 250 kHz et 3,5 Vp.

Parameter	Values
Migration time (s) ^a	17.8 ± 0.9
Peak Area ^b	0.040 ± 0.003
LOD (μ mol L ⁻¹)	2.0
LOQ (µmol L ⁻¹)	6.0
Linear range (μ mol L ⁻¹)	6-200
Analytical frequency (h ⁻¹)	105

Table 1 Performance analytique pour la détermination du nitrate en utilisant laméthodologie MCE-C4D.

^a (n=3); ^b average \pm standard deviation (n=10)

La méthode montre une performance analytique appropriée pour la détermination du nitrate dans la salive. Un bon coefficient de corrélation (r = 0,994), des paramètres d'étalonnage et une large gamme linéaire (Tableau 1) ont été obtenus dans une étude de linéarité utilisant des courbes d'étalonnage externes (Fig. 2.7.a), permettant ainsi au dispositif d'effectuer une analyse quantitative. Nous avons également caractérisé la limite de détection (LOD) et la limite de quantification (LOQ) pour le nitrate en utilisant une approche empirique, qui impliquait des mesures successives de solutions standard plus diluées jusqu'à ce qu'un rapport signal/bruit de 3:1 soit atteint.

La LOD est adaptée à l'analyte cible dans la salive puisque les niveaux trouvés dans la salive de personnes saines et malades (par exemple, les patients atteints de maladies parodontales) peuvent présenter des niveaux de nitrate entre 800 et 1500 µmol L⁻¹ [17]. De plus, notre LOD est inférieure ou comparable aux valeurs rapportées dans les protocoles existants utilisant MCE-C⁴D [16] et CE-UV [18].

Par la suite, la performance de la micropuce et de la méthode optimisée a été évaluée par la quantification du composé cible dans un échantillon biologique. Nous avons ajouté une solution standard de nitrate pour atteindre une concentration de 20 μ mol L⁻¹ dans l'échantillon de salive. De plus, nous avons effectué un test de spécificité en comparant les électrophérogrammes des échantillons avec et sans l'analyte (Fig. 2.7.b). Ces données démontrent que notre méthode a la capacité de distinguer les composés couramment présents dans la salive de l'analyte cible.



Figure 2.7. (a) Courbe d'étalonnage obtenue à partir de l'introduction de solutions standards contenant du nitrate (6 - 200 μ mol L⁻¹) en utilisant le μ CE-C4D. BGE : 30 mM acide lactique/15 mM His (pH = 3.8). (b) Electrophérogrammes obtenus pour l'introduction d'un échantillon contenant 20 μ mol L⁻¹ de nitrate standard (–) et échantillon sans nitrate (–). Injection de l'échantillon en mode " flottant" à -500 V pendant 3 s. La tension de séparation était de -700 V. Détecteur C⁴D fonctionnant à 250 kHz et 3,5 Vpp.

Sample	Analyte	Expected (µmol L ⁻¹)	<i>MC</i> E-C ⁴ D (μmol L ⁻¹)	Relative Difference
Saliva	NO ₃ -	20	21 ± 2	-5%

Table 2 Determination du nitrate dans la salive par MCE- C⁴D.

Le résultat obtenu par notre procédure est similaire au résultat attendu (Tableau 2), avec une différence relative de 5%. Par separation électrophorétique rapide et detection électrochimique simple, notre dispositif microfluidique permet d'obtenir des informations sur les niveaux de nitrate dans une matrice biologique.`

3. Conclusion

Dans ce travail, nous avons développé des microsystèmes par impression 3D basée sur la DLP de MD avec la fabrication de canaux de 40 µm pour l'analyse MCE des espèces azotées réactives dans les échantillons biologiques. Pour la première fois, nous avons présenté un dispositif microfluidique imprimé en 3D basé sur la DLP avec détection C⁴D appliqué au dépistage du nitrate dans les fluides biologiques avec une fréquence d'analyse élevée adaptée au dépistage de routine, et une méthode qui ne nécessite pas de préparation laborieuse des échantillons. Nous montrons l'utilisation de l'impression 3D DLP pour fabriquer des DM destinés à l'analyse d'un biomarqueur médicalement pertinent. Ces MD ont le potentiel pour être utilisés comme un outil de diagnostic largement disponible pour le nitrate.

3. Dispositif microfluidique imprimé en 3D par stereolithographie avec detection électrochimique intégrée

1. Fabrication et conception de l'EMD

Une imprimante 3D commerciale Formlabs 2 SLA 3D (Formlabs, Somerville, USA) a été utilisée tout au long de ce travail. Afin d'étudier la résolution de l'imprimante 3D, un dispositif microfluidique avec sept canaux droits de largeurs variables a été imprimé avec des dimensions de 200 μ m (profondeur) et de 50 à 300 μ m (largeur). Les dispositifs ont été imprimés dans huit positions différentes avec des angles d'inclinaison de 15, 60 et 85° sur les axes Y et X, 45° sur les deux axes XY et perpendiculairement à l'axe Z.

Les MD pour l'impression 3D ont été conçus à l'aide du logiciel de conception Fusion 360 (Autodesk, San Rafael, USA). Le dispositif pour la caractérisation et les analyses consiste en un canal unique de 20 mm de long, $95 \pm 6 \mu m$ de large et $180 \pm 11 \mu m$ de haut et un canal d'électrode en forme de triangle avec une surface rectangulaire de $172 \pm 12 \mu m$ de large et 180 $\pm 11 \mu m$ de haut touchant le canal de séparation (Fig. 3.1). Le dispositif de canal à face ouverte a été imprimé en utilisant les paramètres optimisés.



Fig. 3.1. (a) Esquisse du dispositif conçu à l'aide du logiciel de conception Fusion 360. (b) Encart montrant la connexion entre le canal de l'électrode et le canal de séparation (largeur de la dimension de l'interface) et la distance entre le réservoir de sortie et le canal de l'électrode.

2. Fabrication et conception du MCE-EC

Nous avons fabriqué un dispositif MCE en utilisant les conditions d'impression optimisées précédemment utilisées pour assembler l'EMD. Nous avons obtenu des canaux de 100 μ m de largeur en imprimant une largeur de 150 μ m. De plus, nous avons conservé la conception de l'électrode et le matériau conducteur fabriqué en laboratoire contenant un ratio de 9/8 de carbone et de résine, respectivement. L'EMD et le MCE présentaient plusieurs différences au niveau de la conception. Par exemple, l'EMD avait un seul canal de séparation avec une électrode de travail placée à l'intérieur du microcanal à 1,5 mm du réservoir de sortie, tandis que le dispositif MCE avait une disposition standard de canaux croisés avec une électrode de travail placée à l'intérieur du microcanal à 100 μ m du réservoir de sortie (Fig. 3.2).



Figure 3.2 (a) Esquisse de micropuce électrophorétique conçue à l'aide du logiciel de conception Fusion 360. (b) Composants du dispositif microfluidique conçu: réservoir d'échantillon (S), réservoir de déchets d'échantillon (SW), réservoir tampon (B) et réservoir de déchets tampon (BW).

Le dispositif final pour la séparation et la détection électrophorétique consiste en un simple dispositif en T avec un canal d'injection de 16,5 mm de longueur et un canal de séparation de 38 mm de longueur, 100 μ m de largeur et 300 μ m de hauteur, avec une distance de séparation effective de 29,5 mm (Figure 2.2). Le canal d'électrode est en forme de triangle avec une surface rectangulaire dont les dimensions sont de 547 μ m de largeur et 300 μ m de hauteur touchant le canal de séparation.

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3. Préparation de l'électrode

Une nouvelle conception d'électrode de travail a été créée, présentant un canal triangulaire pour la détection électrochimique qui présente un point d'ouverture (l'angle du triangle) avec sur le canal de séparation. Une nouvelle composition d'électrode a été étudiée. Un mélange de poudre de graphite et d'une résine commerciale Clear (C/résine) a été préparé sous forme de pâte conductrice dans plusieurs rapports massiques (4/8 ; 5/8 ; 6/8 ; 7/8 ; 8/8 ; 9/8), respectivement. La pâte a été fabriquée dans un flacon en plastique en mélangeant correctement les deux composants à la main avec une spatule jusqu'à intégration complète de la poudre de graphite dans une pâte homogène.

4. Intégration des électrodes dans l' EMD

Le canal de l'électrode (Fig. 3.3. a) a été rempli avec la pâte de C/résine à l'aide d'une seringue (Fig. 3.3. b), puis exposé à un rayonnement UV (à 365 nm) pendant 4 h à une dose de 50 mJ \times cm-2 (UV Biolink BLX, Vilber Lourmat, France) jusqu'à réticulation complète de la pâte (Fig. 3.3.c). Ensuite, l'excès de pâte a été soigneusement éliminé en polissant la surface avec du papier de verre humide (3M, USA) (Fig. 3.3. d). Ensuite, le dispositif a été scellé à l'aide d'un ruban de silicone (ThermalSeal RTS TM, Sigma Aldrich, Darmstadt, Allemagne) (Fig. 3.3. e). Pour éliminer les bulles et assurer l'horizontalité du dispositif, une légère pression a été appliquée sur le ruban de silicone pour une bonne adhésion. Enfin, l'EMD est prêt pour les applications (Fig. 3.3.f).



Figure 3.3 Schéma d'assemblage d'un dispositif microfluidique entièrement électrochimique : (a) dispositif imprimé en 3D avec des canaux ouverts ; (b) le canal de l'électrode est rempli de pâte de résine C/ ; (c) le dispositif imprimé en 3D contenant la pâte de résine C/ est exposé aux rayons UV ; (d) l'excès de pâte est éliminé par du papier de verre humide ; (e) l'EMD est scellé avec du ruban de silicone ; (f) l'EMD est prêt pour les applications

5. Intégration des électrodes dans le dispositif MCE

Tout d'abord, le dispositif imprimé en 3D a été scellé avec un ruban de silicone (ThermalSeal RTS TM), puis le canal de l'électrode a été rempli avec la pâte conductrice optimisée à l'aide d'une seringue. Ensuite, le dispositif complet a été exposé à un rayonnement UV à 365 nm pendant 4 heures à une dose de 50 mJ \times cm⁻² (UV Biolink BLX, Vilber Lourmat, France) jusqu'à ce que la réticulation de la pâte conductrice soit complète (Fig. 3.4).



Figure 3.4. Schéma d'assemblage d'un dispositif électrophorétique entièrement microfluidique.

6. Resultats et discussion

1. Optimisation de l'impression

La résine commerciale Formlabs Clear Resin (V4) (Formlabs, Somerville, EUA) a été utilisée pour imprimer les dispositifs et produire les électrodes. Les imprimantes 3D SLA ont une résolution limitée par la taille du spot laser de 140 μ m (résolution xy) et une couche de 25 μ m d'épaisseur (résolution z), ce qui a été le facteur limitant pour obtenir de petits canaux. Pour définir la taille minimale réalisable des canaux, un système microfluidique comportant sept canaux droits de différentes largeurs a été imprimé (Fig. 3.5.a). Chaque canal a été conçu pour avoir une profondeur de 200 μ m et des largeurs de 300 à 50 μ m. Les dispositifs ont été imprimés dans huit positions différentes (Fig. 3.5.b), avec des angles d'inclinaison de 15, 60 et 85° dans les axes Y et X chacun, 45° dans les deux axes XY et perpendiculaire (XY = 0°) (Fig. 3.5.c).



Fig. 3.5. Microdispositif (a) conception avec sept canaux droits de largeurs variables (300 ; 200 ; 150 ; 125 ; 100 ; 75 ; 50 μm) ; (b) après l'étape d'impression avec supports. (c) disposés en plusieurs positions dans la plateforme de construction pour l'impression.

Après avoir imprimé les canaux, nous avons mesuré la largeur des canaux par microscopie optique. Les dispositifs imprimés avec un angle d'inclinaison de 85°, 60°, 15° dans l'axe X ont obtenu des résultats appropriés. Le dispositif imprimé avec un angle de 85° dans l'axe X a obtenu des canaux ouverts bien définis dans les largeurs conçues entre 300-150 µm. Cependant, les dimensions imprimées étaient significativement différentes des dimensions conçues. Tous les canaux ont une largeur considérablement plus faible que la largeur conçue, diminuant jusqu'à une largeur spécifiée de 50 µm où aucun canal n'est visible. Les divergences entre les dimensions concues et réelles des canaux sont dues à la taille du spot laser de 140 µm et étaient cohérentes avec d'autres canaux microfluidiques imprimés en 3D [19]. En effet, l'effet du spot laser peut être minimisé en augmentant l'angle d'inclinaison. Le dispositif imprimé avec un angle d'inclinaison de 45° avait un impact du spot laser à la fois sur la profondeur et la largeur du canal. Ainsi, les plus petits canaux étaient bouchés. Enfin, les dispositifs orientés dans l'imprimante avec un angle de 85° , 60° , 15° dans l'axe Y et perpendiculaire (XY = 0°) n'ont imprimé aucun canal, en raison de l'impact élevé de la taille du spot laser sur la largeur du canal. Notamment, nous avons imprimé le même dispositif avec des canaux entièrement fermés avec les mêmes dimensions et angles que ceux de la figure 3.5, tous les canaux étaient bouchés par la résine durcie et même le plus grand canal (300 × 200 µm) ne pouvait pas contenir de solution. Nous avons donc utilisé le dispositif avec une orientation d'impression de 85° d'angle d'inclinaison sur l'axe X. Les plus petits canaux obtenus étaient de $95 \pm 6 \mu m$ de largeur et 180 $\pm 11 \mu m$ de hauteur (n = 10) avec un système conçu de 150 μm de largeur et 200 μm de hauteur.

2. Conception des électrodes

La conception des électrodes à microbande a été développée pour éviter toute fuite lors de l'étanchéité de l'ensemble du dispositif. Pour cela, le matériau de l'électrode doit éviter de se répandre dans le canal de séparation en accédant à la partie interface. Par conséquent, plusieurs géométries d'électrodes et les distances entre l'électrode et le canal de séparation ont été évaluées pour obtenir une configuration appropriée. Des dispositifs avec des canaux d'électrode présentant deux géométries (cercle et triangle) et différents espaces (configurations 1 à 6 correspondant à des espaces de 60, 40, 20, 10, 0 et -10 µm, respectivement) entre eux et le canal de séparation ont été conçus puis imprimés (Fig. 3.6).



Figure 3.6. Dispositif microfluidique à canaux ouverts contenant plusieurs électrodes avec deux géométries (cercle et triangle) dans différents espaces de 1 à 6, (60, 40, 20, 10, 0 et -10 µm, respectivement).

La connexion entre le canal d'électrode et le canal de séparation (largeur de l'interface, correspondant à l'électrode accessible dans le canal de séparation) a été mesurée (n = 10) par microscopie optique, puis tracée en fonction des écarts, pour les deux géométries (Fig. 3.7). Les canaux en cercle et en triangle ont obtenu un comportement linéaire. La géométrie triangulaire a produit des largeurs d'électrodes inférieures à 400 μ m, ce qui convient à la fabrication d'électrodes à microbande. La géométrie en cercle a produit des électrodes plus larges (de 400 μ m à 1280 μ m). En évaluant la pente de la courbe, les écarts des géométries circulaires (m = -12,5) affectent énormément la largeur des électrodes par rapport aux géométries triangulaires (m = -3,23), créant des interfaces plus grandes dans les géométries circulaires.



Figure 3.7 Largeur de l'interface de l'électrode mesurée en fonction de la distance théorique entre le canal de l'électrode et le canal de séparation (écart).

Pour évaluer la géométrie de l'électrode (circulaire et triangulaire), les canaux de l'électrode ont été scellés et remplis à l'aide d'une seringue avec une solution aqueuse à 10% (p/v%) d'éosine pour suivre par imagerie de fluorescence le profil des liquides lorsqu'ils sont insérés dans le canal de l'électrode. On peut observer sur la Fig. 3.8 que les électrodes à géométrie circulaire favorisent une fuite de l'éosine dans le microcanal, car la dimension de l'interface est plus grande pour la configuration circulaire que pour la configuration triangulaire. D'autre part, les électrodes à géométrie triangulaire ont permis de maintenir le liquide dans les canaux des électrodes lorsque le liquide a été ajouté à des conditions de basse pression, malgré la connexion avec le canal de séparation. Par conséquent, la C/résine peut être maintenue efficacement dans le canal triangulaire de détection (configurations 1 à 3), ce qui a favorisé des largeurs plus petites en raison des espaces plus élevés entre les canaux. L'électrode avec la géométrie de triangle 2 a démontré une meilleure connexion avec le canal de séparation en termes de largeur d'interface ($172 \pm 12 \mu m$), plus grande que pour la configuration 1 ($123 \pm 10 \mu m$), et fournissant toujours les propriétés de microélectrode.



Figure 3.8. Images de microscopie à fluorescence des canaux d'électrodes (géométries triangle et cercle) remplis d'un colorant fluorescent.

3. Optimisation du matériau des microelectrodes

Le nouveau matériau proposé pour la préparation des microélectrodes est composé de deux éléments. Le premier est la poudre de graphite dont la taille des particules est inférieure à 15 µm. L'ajout de ce contenu dans un matériau isolant permet la formation de réseaux de percolation électrique à l'intérieur du matériau, ce qui conduit à une augmentation des propriétés électrochimiques du matériau de plusieurs ordres de grandeur. Le second est la résine transparente Formlabs, le même matériau que celui utilisé pour la fabrication des dispositifs, assurant la compatibilité de l'électrode avec le substrat du dispositif. Nous avons réalisé des expériences préliminaires avec plusieurs mélanges de poudre de graphite et de résine à différents rapports pondéraux afin d'optimiser le matériau en termes de gain de conductivité, de fabrication la plus simple et de stabilité mécanique. Nous avons évalué des mélanges C/résine avec des ratios masiques de 4/8, 5/8, 6/8, 7/8, 8/8 et 9/8, respectivement. Les rapports inférieurs ne conviennent pas car les particules de graphite sont trop dispersées, ce qui compromet le réseau conducteur entre les particules. Nous avons obtenu une pâte visqueuse avec des mélanges de 4/8, 5/8, 6/8 et 7/8, facile à ajouter dans des tubes capillaires en verre (expériences de voltampérométrie cyclique (CV)) et à incorporer dans le canal de l'électrode avant le durcissement. En ce qui concerne les rapports supérieurs à 7/8, la pâte visqueuse se présente plus dense mais avec une viscosité suffisante pour être intégrée dans le canal de l'électrode. La solidification de l'électrode est assurée par un processus de durcissement utilisant une lumière UV. Le processus de durcissement de la résine nécessite généralement environ 1 h à une dose

de 50 mJ × cm⁻² pour une réticulation complète. L'ajout de poudre de graphite dans la résine affecte la cinétique de la réticulation de la résine, en particulier lorsque le rapport C/résine est élevé (7/8, 8/8 et 9/8). Ainsi, le processus de polymérisation du nouveau matériau a été optimisé pour 4 heures à une dose de 50 mJ× cm⁻² afin de garantir la solidification complète du matériau.

La conductivité électrique des matériaux de l'électrode a été évaluée en utilisant la méthode de résistance de la sonde à 2 points (Tableau 3).

w/w ratio	Resistivity	Conductivity
	Ω.cm	S cm ⁻¹
4/8	65.79	1.52×10^{-2}
5/8	11.92	8.39 × 10 ⁻²
6/8	3.88	2.58×10^{-1}
7/8	2.06	4.84×10^{-1}
8/8	1.20	8.31 × 10 ⁻¹
9/8	1.17	8.57 ×10 ⁻¹

Table 3: Résistivité et conductivité du matériau d'électrode en function du ration massique (n = 3).

Les rapports C/résine de 4/8 et 5/8 ont été écartés car les particules de graphite se sont avérées trop dispersées, compromettant l'intégrité du réseau conducteur. Pour les autres rapports, la conductivité a été mesurée dans la gamme de 2,6 à $8,6 \times 10^{-1}$ S cm⁻¹. En ce qui concerne les matériaux polymères conducteurs de la littérature, deux matériaux à base de nanoparticules d'argent ont montré une meilleure conductivité que le matériau C/résine 9/8 [20,21]. Cela peut être attribué au fait que l'encre d'argent est intrinsèquement plus conductrice que le carbone. D'autre part, l'argent a une plage de potentiel supérieure particulièrement limitée dans laquelle il peut être utilisé avant d'être oxydé. Le carbone est un matériau moins coûteux et présente des avantages importants dans le comportement des électrodes, par exemple une réduction de l'encrassement et une grande plage de travail pour la détection par rapport aux électrodes métalliques.

Il est généralement connu que la diminution de la teneur en isolant dans le mélange de pâte de carbone peut entraîner une augmentation souhaitable de la conductance du matériau, cependant ce processus peut être saturé dans un rapport de concentration déterminé. Ce phénomène peut être observé dans la courbe exponentielle montrant la dépendance de la conductivité avec le rapport C/résine (Fig. 3.9). Lorsque les particules électriquement conductrices sont en contact multiple permanent maximal, la conductivité totale du matériau C/résine est ainsi atteinte. Dans cette situation, la résine ne fait que remplir l'espace libre entre les particules de graphite et n'a pratiquement aucun effet sur la conductivité globale, saturant le processus [22].



Figure 3.9. Dépendance de la conductivité en fonction de la composition C/résine pour plusieurs ratios de graphite et de résine, respectivement.

Ensuite, nous avons évalué l'influence de la composition de chaque composition d'électrode sur le comportement électrochimique par CV (Fig. 3.10) en utilisant le FcMeOH comme sonde. Nous avons réalisé cette étude à l'aide d'une électrode en forme de disque utilisant un tube capillaire en verre comme corps de l'électrode, d'un diamètre de 2 mm. Nous avons utilisé un système à 3 électrodes dans une cellule électrochimique classique (Fig. 3.10.a). Il est remarquable que pour une faible teneur en graphite dans le composite (6/8 ; 7/8), on observe un courant anodique en forme de vague lié à l'oxydation de Fe(+II) en Fe(+III). La surface peut former un réseau de parties conductrices micrométriques et pourrait donc être assimilée à un réseau de microélectrodes séparées avec une diffusion radiale et sans recouvrement de la couche de diffusion. Au fur et à mesure que le rapport C/résine augmente (8/8 ; 9/8), la forme des voltammogrammes cycliques passe progressivement d'un signal en forme de pic (Fig. 3.10.b). Concernant le rapport C/résine de 9/8,

le voltammogramme affiche un pic anodique pour le FcMeOH avec une intensité de pic la plus élevée de $5,36 \pm 0,06 \,\mu$ A. Le ΔE (supérieur à 57 mV) de la sonde doit être attribué à la cinétique de transfert lent de la charge caractérisée par une séparation pic à pic de $228 \pm 5,77$ mV, ce qui est en accord avec les rapports précédents pour le graphite [23]. En outre, le ΔE augmente en fonction de l'augmentation de la vitesse de balayage, une telle déviation élevée suggère le processus de transfert d'électrons quasi-réversible à des vitesses de balayage plus élevées [24].



Figure 3.10 (a) Appareil à 3 électrodes utilisé pour réaliser la caractérisation électrochimique de la pâte conductrice fabriquée en laboratoire ; WE = rapport C/résine (6/8 - 9/8), circulaire (diamètre 2 mm), RE = fil Ag-AgCl, CE = fil de Pt. (b) Voltampérométrie cyclique sur des électrodes C/résine de 1 mM FcMeOH. Electrolyte : 10 mmol L⁻¹ PBS. Vitesse de balayage 25mV s⁻¹. (c) Vitesse de balayage de 1 mM FcMeOH de 10 mV s⁻¹ à 360 mV s⁻¹.

En utilisant le matériau d'électrode avec un rapport C/résine de 9/8, les valeurs de courant de crête en fonction de la racine carrée du taux de balayage (\sqrt{v}) ont été tracées pour estimer la surface de l'électrode (en utilisant l'équation de Randles-Sevcik) (Fig. 3.10. c). Les valeurs obtenues correspondent à 90-97% de la surface géométrique théorique d'un disque de 2 mm de diamètre. La C/résine 9/8 a donc été choisie pour les expériences ultérieures en raison de ses propriétés conductrices et mécaniques acceptables pour la préparation des électrodes intégrées.

4. Couplage de la détection électrochimique dans le dispositif microfluidique 1. Integration de la pate conductrice dans le dispositif microfluidique

Nous avons intégré l'électrode dans le dispositif en remplissant le canal de l'électrode avec la pâte conductrice fabriquée en laboratoire avec la composition optimisée. Le dispositif complet a été exposé dans la chambre UV pour le processus de post-polymérisation du dispositif imprimé et pour la réticulation de la pâte d'électrode pendant 4 heures à une dose de 50 mJ × cm⁻². Il est remarquable que le processus de post-polymérisation soit une étape nécessaire pour finaliser la fabrication du dispositif, nous avons donc profité de ce processus pour obtenir les électrodes intégrées dans la même étape, minimisant ainsi le nombre de processus pour obtenir un dispositif complet. De plus, la connexion des électrodes à l'encre d'argent ou au ruban conducteur n'est pas nécessaire, puisque les fils peuvent être connectés directement dans la pâte conductrice à l'intérieur du canal ouvert de l'électrode, et qu'ils sont fixés pendant le processus de post-polymérisation. Ensuite, nous avons poli le dispositif afin d'éliminer l'excès de composite, et de lisser la surface du dispositif pour le processus de scellement à l'aide d'un ruban de silicone (ThermalSeal RTS TM). Enfin, nous avons réalisé une électrode à microbande intégrée à la paroi latérale pour fonctionner comme une electrode de travail en mode de détection dans le canal (Fig. 3.11).



Figure 3.11. Schéma de l'EMD proposé avec un encart montrant l'électrode latérale à microbande intégrée.

2. Caractérisation électrochimique

La caractérisation électrochimique de l'électrode à microbande a été réalisée en utilisant le FcMeOH comme sonde redox. Un montage à 2 électrodes, une pseudo-électrode de référence et une contre-électrode en court-circuit (C/résine contre argent/chlorure d'argent), a été utilisé pour réaliser toutes les expériences suivantes. L'électrode de travail est positionnée à l'intérieur du microcanal tandis que la pseudo-électrode de référence est positionnée dans le réservoir de sortie, qui est placé à 1,5 mm du canal de l'électrode. Tout d'abord, nous avons rempli le canal avec la solution de la sonde redox pour exécuter les expériences CV. Entre chaque acquisition, le canal a été lavé avec de l'eau et du tampon avant de renouveler la solution pour certifier la répétabilité des mesures, accomplissant un écart type relatif de \pm 2% dans les valeurs de courant de pointe (n = 3) (Fig. 3.12).



Figure 3.12. (a) Electrode C/résine intégrée au triangle dans le dispositif scellé avec du ruban de silicone. (b) Voltampérométrie linéaire de 1 mmol L⁻¹ FcMeOH sur cette électrode intégrée dans un dispositif imprimé en 3D (n = 3). Électrolyte : PBS 0,01 mol L⁻¹ (pH = 6,7). WE : rapport C/résine 9/8, RE/CE = fil Ag-AgCl. Vitesse de balayage 25 mV s⁻¹

L'étude de voltamétrie cyclique du FcMeOH a montré des caractéristiques avec un potentiel d'oxydation de demi-vague de 260 ± 16 mV et vague de $77,02 \pm 0,76$ nA (Fig. 3.13.a). Nous avons tracé les valeurs de courant en fonction de la racine carrée de la vitesse de balayage (\sqrt{v}) (Fig. 3.13.b) et nous avons utilisé la valeur de la pente pour calculer la surface dynamique $(0,027 \text{ mm}^2)$ de la plateforme de détection par l'équation de Randles-Sevcik. La densité de courant expérimentale est plus prononcée sur le format microélectrode que sur le format millimétrique, passant de 1,74 μ A × mm² à 2,84 μ A × mm² pour le FcMeOH avec un taux de balayage à 50 mV s⁻¹, renforçant les motivations pour la miniaturisation des électrodes.



Figure 3.13. Voltampérométrie cyclique sur des électrodes C/résine de (-) 1 mmol L⁻¹ de FcMeOH dans du PBS 10 mmol L⁻¹ et (-) une solution de meme PBS. WE = rapport C/résine 9/8, RE/CE = fil Ag-AgCl. Vitesse de balayage 50 mV s⁻¹. (b) Courant anodique en fonction de la racine carrée des tracés de vitesse de balayage de l'électrode intégrée C/résine (9/8).

Après avoir mis en place un protocole simple pour la création d'EMD à l'aide d'une imprimante 3D basée sur SLA, nous avons fait la démonstration de la détection électrochimique en employant la configuration à 2 électrodes pour étudier la linéarité de réponse du matériau transducteur par voltammétrie à onde carrée (SWV). La figure 3.14 a montré plusieurs pics de réponse anodique contre différentes concentrations de FcMeOH, allant de 15 μ m à 1000 μ m obtenus sur l'électrode intégrée. Des pics symétriques, nets et bien définis autour de 0,15 V ont été observés. L'électrode de détection a une réponse linéaire lorsque nous avons augmenté la concentration de la sonde, la courbe d'étalonnage a accompli un coefficient de régression de 0,995. Les valeurs LOD et LOQ étaient de 4,5 μ M et 15 μ M, respectivement.



Figure 3.14. (a) SWV à la microélectrode C/résine (9/8) intégrée dans un dispositif imprimé en 3D dans du PBS 10 mM contenant 1 mM de FcMeOH (de 15 à 1000 μ M) dans des conditions statiques. (b) Tracé d'étalonnage correspondant du courant en SWV en fonction des concentrations de la sonde. Paramètres : $\Delta E = 10 \text{ mV}$; f = 30 Hz ; Estep : 6 mV.

3. Rénovation de l'électrode intégrée

L'un des objectifs de ce travail était de parvenir à un dispositif réutilisable, de cette façon nous avons développé des stratégies pour renouveler la surface de l'électrode après plusieurs expériences exhaustives. Comme les électrodes sont placées à l'intérieur du dispositif avec un scellement irréversible, il n'est pas possible de rénover la surface par un polissage classique. De plus, les électrodes de micropuces ne peuvent pas être nettoyées en utilisant les méthodes agressives communément appliquées aux électrodes de disques car plusieurs procédures de nettoyage chimique, par exemple l'utilisation d'une solution de piranha, d'acide nitrique chaud et de fortes concentrations d'acide sulfurique ne sont pas compatibles avec le matériau du substrat des micropuces [25]. Pour surmonter ce problème, nous avons développé une stratégie pour rénover la zone de l'électrode, à l'intérieur du canal, en introduisant de l'acide sulfurique (0,1 M) dans le canal de séparation et en réalisant 100 voltammogrammes cycliques à 1 V s⁻¹, 50 à 0,5 V s⁻¹, 25 à 0,25 V s⁻¹ et 10 à 0,1 V s⁻¹. L'électrode a été réutilisée jusqu'à 30 fois jusqu'à ce que son courant commence à diminuer par passivation (Fig. 3.15). Une meilleure

réversibilité (séparation pic à pic plus faible) et une augmentation de l'intensité des pics voltammétriques pour le couple redox FcMeOH ont été observées, confirmant la propreté de la surface de l'électrode.



Figure 3.15. Comportement en voltamétrie cyclique du FcMeOH 1 mM dans du PBS au niveau de la microélectrode en C/résine (9/8) intégrée dans un dispositif imprimé en 3D (a) après réutilisation de l'électrode pendant 30 fois (n =3) et (b) après traitement électrochimique du WE par une solution d'acide sulfurique (0,1 M) (n =3), voir le texte pour plus de détails. Vitesse de balayage : 25 mV s^{-1} .

4. Application de l'EMD imprimé en 3D

Afin de prouver l'utilité d'un EMD aussi simple et bon marché créé à l'aide d'une imprimante 3D SLA, la réponse électrochimique d'une sonde, injectée de manière continue et hydrodynamique dans le canal de séparation, a été mesurée sur le canal de détection intégré fabriqué en laboratoire et optimisé précédemment. Pour cela, une solution de $Ru(NH_3)_6^{3+}$ (5 mM) a été injectée en continu à un débit allant de 0 (aucun débit) et 55 µL min⁻¹ et analysée par voltampérométrie cyclique (Fig. 3.16). Ces valeurs de débit ont été choisies pour imiter l'écoulement électro-osmotique à l'intérieur du microcanal, qui est favorisé par les techniques de séparation à commande électrique.



Figure 3.16. (a) Influence du débit sur le voltamograme cyclique (forward scan) pour une electrode de travail de C/résine 9/8 dans une solution de $Ru(NH_3)_6^{3+}$ 5 mM dans du PBS. Débit allant de 5 à 55 µL/min. Vitesse de balayage 50 mV/s. (b) Dépendance du courant sur le débit à l'intérieur du microcanal.

Comme le montre la Fig. 3.16, l'augmentation du flux hydrodynamique augmente le courant de réduction du $Ru(NH_3)_6^{3+}$ Ceci est en effet dû au rétrécissement de la couche de diffusion, qui augmente le gradient de concentration, et à la convection de la solution assurée par le flux continu, renouvelant la solution et donc l'analyte. Les conditions d'écoulement hydrodynamique à l'intérieur du canal offrent des avantages par rapport au mode statique, tels que l'évolution du courant et une amélioration d'environ 4 fois des valeurs LOD et LOQ

5. Couplage du détecteur ampérométrique avec la separation électrophorétique dans le microdispositif

Nous avons créé un dispositif d'électrophorèse sur micropuce avec un canal de détection rempli de la pâte conductrice optimisée fabriquée en laboratoire et scellé avec un ruban de silicone (ThermalSeal RTSTM) (Fig. 3.17).


Figure 3.17. Vues inférieure et supérieure du dispositif proposé.

1. Caractérisation du flux électroosmotique

D'autres caractérisations de la surface du matériau imprimé en 3D ont été développées afin d'évaluer le flux électro-osmotique généré à l'intérieur de ces microcanaux imprimés en 3D du système MCE. Les surfaces ont été caractérisées par angle de contact (WCA) et par spectroscopie infrarouge à transformée de Fourier (FTIR) avec module d'échantillonnage à réflexion totale atténuée (ATR). Les mesures d'angle de contact ont permis de caractériser les propriétés hydrophiles du substrat du dispositif (Fig. 3.18).



Figure 3.18. Mesures de l'angle de contact de la surface du dispositif propose

Pour le matériau imprimé en 3D, la valeur mesurée de l'angle de contact est égale à $78,7\pm0,3^{\circ}$ (n = 3), cette valeur est 33% inférieure à celle du PDMS (WCA = 117°) [26]. Ces résultats indiquent que le matériau imprimé en 3D a un comportement plus hydrophile que le PDMS, un matériau couramment utilisé pour fabriquer des dispositifs MCE. Ce détail est potentiellement intéressant pour la fabrication de micropuces puisqu'il indique une meilleure mouillabilité du dispositif imprimé en 3D lors de l'utilisation de solutions aqueuses, permettant d'envisager des flux électro-osmotiques élevés.

La figure 3.19 présente le spectre FTIR-ATR de la résine non polymérisée (avant impression) et de la résine polymérisée (matériau imprimé en 3D). Les deux spectres, celui de la résine durcie et celui de la résine non durcie, présentent des bandes d'absorption similaires situées à 2952 et 2868 cm⁻¹, qui sont attribuées aux modes de vibration d'étirement du carbone et de l'hydrogène des groupes -CH₂ et -CH₃ du squelette du polymère. La bande située autour de 1450 cm⁻¹ est fournie par le mode d'oscillation des groupes -CH₃. La bande située à 1704 cm⁻¹ est attribuée au groupe carbonyle, et la bande située à 3373 cm⁻¹ est attribuée au groupe hydroxyle (mode d'étirement). Comme on peut le noter, le spectre présente une absorption de déformation axiale O-H intense avec une large bande entre 3300 - 2500 cm⁻¹, ce phénomène s'explique par la présence de dimères d'acide carboxylique dans la résine durcie et non durcie. La présence de groupes carboxyliques en surface est favourable à la generation de flux électro-osmotiques élevés à l'intérieur du canal. La bande située à 1639 cm⁻¹ liée au groupe C=C (mode d'étirement) n'est présente que dans la résine non polymérisée, ceci était attendu puisque la réaction de polymérisation en chaîne a commencé à rompre les doubles liaisons C=C des monomères.



Figure 3.19. Spectre FTIR-ATR de la résine FormLabs non polymérisée (-) et polymérisée (-).

Le flux électro-osmotique d'un microcanal rectangulaire unique imprimé en 3D, d'une largeur de 100 μ m, d'une hauteur de 300 μ m et d'une longueur de 38 mm, a été déterminé par la méthode du marqueur neutre. La méthode du marqueur neutre implique la mesure du temps nécessaire à une molécule de marqueur neutre pour migrer vers le système de détection sous un champ électrique. La mobilité électro-osmotique a été déterminée en utilisant 1 mM de FcMeOH préparé dans des tampons phosphate (pH 2, 6, 12), borate (pH 10) et borate (pH 8), à 20 mM (Fig. 3.20).



Figure 3.20. Dépendance au pH du flux électro-osmotique mesuré dans des microcanaux en résine FormLabs pour une gamme de pH allant de 2 à 12, par la méthode du marqueur neutre. Marqueur neutre : Ferrocène méthanol ; BGE : 20 mM phosphate et borate.

Pour les pH 8, 10 et 12, la mobilité électro-osmotique a été évaluée entre 5,7 et 8,6 $\times 10^{-5}$ cm2 V⁻¹ s⁻¹ (n=3), mettant en évidence une charge négative sur la surface de la paroi du microcanal imprimé en 3D. Cela peut probablement être dû à la présence de groupes acide carboxyliques mis en évidence dans le spectre FTIR-ATR du dispositif imprimé en 3D.

2. Canal de séparation électrophorétique

L'effet du chauffage par effet Joule sur le dispositif imprimé en 3D a été évalué en contrôlant le courant électrique pour plusieurs valeurs du potentiel appliqué (Fig. 3.21). Nous



avons calculé la résistance électrique (1,14 M Ω cm⁻¹) en utilisant l'inverse de la pente de la courbe courant versus tension dans la région linéaire (de 0 à 210 V cm⁻¹).

Figure 3.21. Courbe ohmique (courant en fonction du champ électrique) pour le dispositif microfluidique imprimé en 3D dans une solution tampon phosphate 50 mM.

Nous avons observé un comportement linéaire jusqu'à un champ électrique de 210 V cm⁻¹, pour des valeurs de champ électrique plus élevées, l'effet de chauffage par effet Joule est plus prononcé, entraînant une déviation de la linéarité. La dissipation du chauffage par effet Joule est moins efficace en comparaison avec d'autres matériaux MCE, par exemple la silice fondue ou le verre. Cet inconvénient peut être surmonté en utilisant un tampon de séparation plus dilué (force ionique plus faible, le chauffage par effet Joule peut être minimisé mais la capacité tampon peut être compromise). Par conséquent, nous avons utilisé le potentiel de séparation de 700 V (184 V cm⁻¹), et nous avons diminué la concentration du tampon phosphate de 50 mM à 10 mM, la force ionique de 10 mM est appropriée pour atteindre un bon accord entre l'effet Joule et la capacité du tampon.

3. Caractérisation de l'électrode

La caractérisation électrochimique de l'électrode à microbande a été réalisée en utilisant $Ru(NH_3)_6^{3+}$ comme sonde redox dans des conditions statiques. Un montage à 2 électrodes, avec une pseudo-électrode de référence et contre-électrode en court-circuit (argent/chlorure d'argent), a été utilisé pour réaliser toutes les expériences suivantes. L'électrode de travail est positionnée à l'intérieur du microcanal tandis que la pseudo-électrode de référence

est positionnée dans le réservoir de sortie, qui est placé à 100 μ m de l'électrode de travail. Nous avons calculé la surface active de l'électrode en traçant les valeurs de réduction du pic de la sonde (Ru(NH₃)₆³⁺) en fonction de la racine carrée du taux de balayage (Fig. 3.22). Une bonne linéarité a été trouvée (R² = -0,998), nous avons utilisé la valeur de la pente pour calculer la surface dynamique (0,30 mm²) de la plate-forme de détection par l'équation de Randles-Sevcik



Figure 3.22. (a) CV sur des électrodes en C/résine de Ru(NH₃) $_{6}^{3+}$ 5 mM dans un tampon phosphate 10 mM à plusieurs vitesses de balayage (10-250 mV/s) et courant cathodique en fonction de (V/s)^{1/2}. (b) SWV de Ru(NH₃) $_{6}^{3+}$ (de 15 à 1000 μ M) et tracé d'étalonnage entre le courant de SWV et les valeurs des concentrations de la sonde. WE = microbande 9/8 (largeur 547 μ m), RE/CE = fil Ag-AgCl.

Les séparations électrophorétiques sont effectuées par l'application d'un champ électrique qui génère une mobilité électrophorétique pour les espèces charges et donc leur migration dans le canal de séparation. Le couplage de l'électrophorèse sur micropuce avec la détection électrochimique peut poser des problèmes en raison des interférences qui peuvent se produire entre les champs électriques générés par les deux techniques. Ce champ électrique peut avoir un fort impact sur le détecteur électrochimique placé à l'intérieur du canal de séparation. En ce qui concerne la détection ampérométrique, le courant électrique produit par le potentiel de séparation (10-30 kV) à l'intérieur du canal peut être supérieur de six ordres de grandeur ou plus aux courants électrochimiques mesurés au niveau du détecteur ampérométrique [27]. Cet effet a été étudié en effectuant une voltampérométrie cyclique Ru(NH₃)₆³⁺ dans du PBS placé dans un microcanal, avec ou sans l'application d'une tension de séparation pour l'électrophorèse et en utilisant une électrode C/résine à l'extrémité du canal.

Nous avons utilisé une configuration à 2 électrodes (pseudo-RE et CE (Ag/ AgCl) en court-circuit) placées dans le réservoir de sortie. La voltampérométrie cyclique a été réalisée sous tension de séparation à différentes valeurs à l'intérieur du canal (Fig. 3.23)



Figure 3.23. Influence de la tension de séparation sur les voltammogrammes cycliques de 5 mM $Ru(NH_3)6^{3+}$ dans la configuration à canal terminal. WE : microbande 9/8 (largeur 547 µm), RE/CE = fil Ag-AgCl. Tension de séparation : 0 à +700 V (entrée). Vitesse de balayage : 50 mV s⁻¹.

Le potentiel de réduction de la sonde est déplacé vers des valeurs plus positives lorsque la tension de séparation augmente. En l'absence de la tension de séparation, la sonde a été réduite à une valeur de potentiel de -0.37V/ Ag-AgCl ; avec la tension de séparation de 700 V (côté entrée) le potentiel de réduction se déplace à -0.2V/ Ag-AgCl. Le décalage du potentiel peut être expliqué par l'interaction entre le champ électrique pour la séparation et celui pour la détection. De plus, nous avons obtenu un signal avec des niveaux de bruit acceptables même sous des champs électriques plus élevés. Cela peut être attribué au fait que l'alignement des canaux d'extrémité explore la stratégie consistant à placer l'électrode de travail à la sortie du canal de séparation, à 5-100 μ m de l'extrémité de sortie. En procédant ainsi, l'influence du champ électrique de séparation sur la détection électrochimique est évitée. Dans ce contexte, l'installation de 2 électrodes dans une configuration de canal d'extrémité est efficace pour la détection -0,2V/ Ag-AgCl.

Le canal terminal peut souffrir d'un autre inconvénient que nous devons vérifier : puisque l'électrode de travail est placée à l'extérieur du microcanal, l'échantillon subit une dilution dans le réservoir, ce qui compromet la performance analytique [28]. Dans ce contexte, nous avons étudié la configuration du canal d'extrémité, où l'électrode de travail est placée à l'intérieur du microcanal de séparation pour éviter la dilution de l'échantillon. De plus, nous avons testé la stabilité du courant sous plusieurs champs électriques (Fig. 3.24). Pour cette configuration, nous utilisons un potentiostat sans fil pour les mesures chronoampérométriques car l'utilisation d'un potentiostat classique peut être endommagée par la fonction de la tension de séparation pendant l'application de la séparation électrophorétique sans mise à la terre. Nous avons effectué des mesures chronoampérométriques pour indiquer toute influence du champ électrique sur un montage à 2 électrodes dans un système en forme de croix dans une réduction de potentiel de -0,2V. Le montage comprenait une électrode C/résine de 547 µm de largeur placée juste avant l'extrémité du canal de séparation (environ 100 µm de distance) et une pseudo-RE/CE constituée d'un fil Ag/AgCl immergé dans le puits le plus proche.



Figure 3.24. Influence du champ électrique de séparation sur le courant chronoampérométrique du BGE 10 mM tampon phosphate (pH = 7,4) dans une configuration en canal. WE : microbande 9/8 (largeur 547 µm), RE/CE = fil Ag-AgCl. Tension de séparation : 0 à +700 V (entrée). Potentiel de détection : -0.2V.

En l'absence de tension de séparation, la valeur de courant est faible. En appliquant une tension de séparation, le courant augmente pour les valeurs négatives et le bruit augmente proportionnellement lorsque la tension de séparation augmente. Ceci peut être attribué à un effet Joule plus important sous une valeur de champ électrique plus élevée (100 V cm⁻¹). En outre, ce comportement peut être lié à l'interférence électrique dans l'électrode de travail. Le potentiel de l'électrode de travail augmente sensiblement avec l'augmentation du champ électrique, ce qui peut provoquer des effets secondaires dans l'électrode de travail, par exemple la génération de gaz dans l'électrode en raison de l'oxydation de H₂O. Malgré l'augmentation du bruit obtenue en appliquant des tensions élevées (700 V), nous pouvons toujours avoir un bon rapport signal/bruit S/N.

4. Application de la MCE-EC imprimée en 3D

Nous avons utilisé la configuration à 2 électrodes pour la séparation et la détection chronoampérométrique du $Ru(NH_3)_6^{3+}$ dans le dispositif microfluidique. Le tampon de séparation était composé d'un tampon phosphate 10 mM puisque ce milieu a été utilisé pour toutes les études concernant ce dispositif. Pour cela, nous avons utilisé un mode d'injection " flottant " (500 V pendant 5s), suivi d'une séparation électrophorétique (700 V pendant 750 s) et d'une détection chronoampérométrique à -0.2V/ Ag-AgCl. L'électrophérogramme est présenté dans la Fig. 3.25.



Figure 3.25. Electrophérogrammes de BGE 10 mM de tampon phosphate (pH = 7,4), 500 μ M Ru(NH₃) $_{6}^{3+}$ dans la configuration canal terminal. Le graphique d'étalonnage entre les surfaces des pics et les valeurs des concentrations de la sonde (100 à 700 μ M) est également représenté. WE : 9/8 microbande (547 μ m de largeur), RE/CE = fil Ag-AgCl. Potentiel d'injection : +500V (entrée échantillon), potentiel de séparation : +700 V (entrée du tampon) et potentiel de détection : -0,2V/ Ag-AgCl.

D'après la figure 3.25, nous avons obtenu un rapport signal/bruit S/N approprié malgré le bruit de la tension de séparation. Le pic de réduction fourni par l'introduction de la sonde a un temps de migration de 114 s. Le dispositif a fourni une performance analytique

appropriée pour la détermination du Ru(NH₃) $_{6}^{3+}$. Un bon coefficient de corrélation (r = -0,996), des paramètres d'étalonnage et une gamme linéaire (100 à 700 μ M) ont été obtenus en utilisant la courbe d'étalonnage externe (Figure 3.25). Par conséquent, l'électrode à base de C/résine s'est avérée appropriée pour la séparation électrophorétique couplée à la détection chronoampérométrique.

7. Conclusion

Nous avons fait la démonstration d'une imprimante 3D basée sur SLA pour fabriquer deux types de dispositifs, EMD et MCE couplés à une détection ampérométrique. Nous avons prouvé le concept de l'impression 3D basée sur SLA pour l'EMD avec une conception d'intégration d'électrode facile et une nouvelle formulation d'électrode pour la détection de la CE. Nous avons caractérisé cette électrode par électrochimie, fournissant une bonne reproductibilité et une réponse linéaire. La méthode analytique a conduit à une LOD et LOQ de 4,5 μ M et 15 μ M, Ru(NH₃)₆³ respectivement. Les dispositifs proposés sont réutilisables avec une surface d'électrode qui peut être rénovée sans avoir à ouvrir le dispositif, ce qui favorise la robustesse du système.

8. Perspectives

Dispositif de micropuce électrophorétique imprimée en 3D par DLP (Digital Light Processing) avec detection électrochimique intégrée

1. Conception et fabrication des microdispositifs

Une imprimante 3D Solus Pro avec un projecteur SprintRay a été utilisée tout au long de ce travail. Les MD pour l'impression 3D (Fig. 4.1.a) ont été conçus à l'aide du logiciel de conception Fusion 360 (Autodesk, San Rafael, USA). Le dispositif pour la caractérisation et les analyses consiste en un canal unique de 38 mm de longueur, $103 \pm 7 \mu m$ de largeur (Fig. 4.1.b) et un canal d'électrode en forme de triangle avec une surface rectangulaire de $352 \pm 6 \mu m$ de largeur et de hauteur touchant le canal de séparation (Fig. 4.1.c).



Figure 4.1 (a) Dispositif MCE complet imprimé en 3D. (b) Vue de dessus du canal fermé du dispositif. (c) Canal d'électrode en forme de triangle à paroi latérale touchant le canal de séparation.

Le dispositif à canal fermé a été imprimé en utilisant la résine Versapic 1147 Clear. Les spécifications de l'imprimante ont été optimisées : épaisseur de couche de 10 μ m, résolution de 12 μ m, temps d'exposition de 30 s pour les couches de base et de 2 s pour construire les canaux, la pièce de couverture et les réservoirs, avec un décrément de transition de 5 s.

2. Resultats et discussions

1. Omptimisation de l'impression

La résolution des imprimantes 3D DLP est limitée par plusieurs paramètres : la taille des pixels du projecteur (résolution xy), le temps d'exposition et l'épaisseur de la couche (résolution z), qui sont autant de facteurs limitant la réalisation de petits canaux. Pour définir la taille minimale réalisable des canaux, nous avons étudié plusieurs temps d'exposition et décréments de transition pour assembler les MD. Le temps d'exposition a été fixé à 30 s (couches de base) / 1s (autres) avec 3 s de décrément de transition, permettant d'obtenir des canaux de 127 \pm 12 µm (Fig. 4.2.a). Ainsi, le temps d'exposition et le décrément de transition ont été augmentés, 30 s (couches de base) / 2 s (autres) avec 5 s de décrément de transition, pour atteindre une polymérisation plus élevée conduisant à des canaux plus petits. En conséquence, des canaux de 103 \pm 7 µm ont été réalisés (Fig. 4.2.b).



Figure 4.2. Images de microscopie optique de canaux imprimés en 3D d'environ (a) 127 μ m et (b) 103 μ m de largeur.

Dans les perspectives d'avenir, des électrodes seront intégrées dans le dispositif MCE entièrement imprimé. Ensuite, le nouveau dispositif sera caractérisé et optimisé pour servir de plateforme pour le dépistage de la sérotonine et du fer dans les échantillons biologiques.

4. Conclusion générale

Les technologies d'impression 3D basées sur la photopolymérisation ont été utilisées dans le même but (microcanaux et électrodes intégrées) mais en utilisant plusieurs approches différentes selon l'imprimante 3D utilisée. Nous avons publié des articles scientifiques rapportant l'utilisation d'imprimantes 3D pour assembler plusieurs DM. Ces protocoles guideront les nouveaux utilisateurs d'imprimantes 3D pour atteindre une taille minimale de canal d'écoulement en utilisant ces imprimantes 3D spécifiquement commerciales ou pour aller plus loin dans leur amélioration à la lumière de la description du comportement des paramètres.

5. Bibliography

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RESUMO DA TESE

1. Introdução

A miniaturização em análises químicas e biomédicas tem surgido nos últimos anos com o objetivo de superar o desempenho de dispositivos analíticos convencionais, pois lidam com volumes de amostras e reagentes muito pequenos, possui baixo custo de fabricação, e a diminuição do tamanho físico dos sistemas contribui para a criação de dispositivos portáteis que poderão ser usados em qualquer parte do mundo sem conhecimento técnico ou condições experimentais específicas. A ideia de miniaturização torna-se mais atrativa após a integração de etapas analíticas em uma única plataforma, por exemplo, a integração de uma etapa de separação com um método de detecção sensível, levando a um processo de análise total com medidas seletivas e sensíveis [1].

A detecção eletroquímica (EC) e condutométrica são adequadas para o formato microchip. Considerando o processo de fabricação, os microeletrodos podem ser fabricados diretamente na plataforma usando os mesmos procedimentos que são usados para construir microchips. O acoplamento de dispositivos microfluídicos eletroquímicos (EMD) com plataformas de separação eletroforética promove a criação de sistemas de eletroforese capilar de microchip (MCE). E excelentes trabalhos de pesquisa utilizando materiais emergentes para fabricação de eletrodos têm sido relatados por vários grupos [2–4], e há um aumento de interesse no uso de eletrodos integrados em sistemas MCE. O MCE representa a próxima geração de dispositivos miniaturizados para as análises de amostras com matrizes complexas utilizando uma simples instrumentação, uma vez que o uso de válvulas ou bombas não são necessários para o controle eletrocinético dos fluidos, é facilmente miniaturizado, e promove curtos tempos de análises [5]. No entanto, a detecção EC é mal desenvolvida em MCE devido a ocorrência de algumas interferências relevantes que podem ocorrer. Todos os autores mencionados anteriormente tiveram que lidar com questões que dificultam o desenvolvimento da microfluídica, eletroforese capilar e eletroquímica de forma conjunta.

O principal desafio depende do isolamento do sistema de detecção da alta tensão de separação, a fim de preservar tanto a relação sinal-ruído quanto a integridade do potenciostato. O alinhamento e posicionamento dos eletrodos pode refletir na reprodutibilidade da análise. O material do eletrodo deve ser compatível com o substrato do dispositivo para evitar vazamentos e promover uma plataforma experimental robusta. Além disso, o processo clássico de

fabricação para a produção de dispositivos com canais microfluídicos demanda acesso à sala limpa. Nesse contexto, decidimos utilizar uma tecnologia de manufatura aditiva (AM), também conhecida como impressão em três dimensões (3D), como abordagem para superar todas essas questões de fabricação mencionadas anteriormente que possam refletir no desempenho analítico dos dispositivos obtidos. Uma ilustração de um dispositivo genérico contendo canal de separação e detecção de EC é exibida em Fig. 1.1.



Figura 1.1. Dispositivo MCE clássico com eletrodos integrados para realizar a detecção de CE.

A tecnologia de impressão 3D é uma maneira versátil de montar peças complexas em etapas simples e automatizadas. Esta técnica oferece boa precisão para definir microcanais e microestruturas com benefícios, por exemplo, alta resolução, variedade de materiais e personalização no desenvolvimento de dispositivos quando comparados aos dispositivos 2D [6]. Embora a maioria dos dispositivos fluídicos impressos em 3D tenham sido na escala milimétrica, alguns dispositivos microfluídicos (MD) foram demonstrados [7]. Atualmente, a Estereolitografia (SLA) e a Processamento de Luz Digital (DLP) são as abordagens mais promissoras para a criação rotineira de estruturas microfluídicas devido à alta resolução. Essas tecnologias de polimerização envolvem uma resina fotossensível curada por uma fonte de luz para produzir camadas sólidas e, eventualmente, orifícios e outras estruturas internas [8]. Considerando que essas resinas comerciais não são necessariamente formuladas para atender aos requisitos do campo da microfluídica, vários grupos têm relatado impressoras e materiais

personalizados para alavancar com sucesso o design microfluídico com elementos de tamanhos <50 μm [9,10].

O uso do AM para a montagem de MD pode fornecer várias possibilidades para simplificar o processo de fabricação. Nesse contexto, os leitores encontrarão neste manuscrito protocolos e estratégias desenvolvidas para atender aos requisitos de fabricação de MD utilizando tecnologia de impressão 3D. Ao mesmo tempo, estamos criando MD com canais de eletrodos para superar a necessidade de alinhamento destes eletrodos para detecção EC. O conteúdo do manuscrito consiste em:

- MCE-C⁴D impresso em 3D com base em DLP-SLA empregado uma resina e impressora 3D personalizadas e adequadas para montar microcanais (Fig. 1.2.a). O sistema proposto contém canais espiral preenchidos com gálio para atuar como eletrodos. O dispositivo foi caracterizado a partir da separação de cátion inorgânicos e empregado para determinar nitrato em amostras biológicas.
- EMD impresso em 3D com base em SLA utilizada uma resina e impressora 3D comerciais. Empregou-se uma abordagem de fabricação de canais abertos (Fig. 1.2.b). Os microcanais foram selados por uma fita de silicone. O sistema proposto contém um canal de eletrodo em forma de triângulo com uma superfície retangular preenchida por uma resina condutora customizada para atuar como eletrodo de trabalho (WE). Posteriormente, uma técnica de separação eletroforética foi integrada à plataforma criando um dispositivo MCE-EC impresso em 3D (Fig. 1.2.c). A viabilidade do eletrodo integrado nos sistemas EMD e MCE-EC tem sido investigada utilizando metanol ferroceno (FcMeOH) e hexaammineruthenium (III) ([Ru(NH₃)₆]³⁺) como prova de conceito.
- Dispositivo MCE-EC fabricado usando uma resina comercial e impressora 3D baseada em DLP sem o processo de selagem. Os parâmetros de impressão foram otimizados para fabricar canais microfluídicos. Mais trabalhos serão realizados com este novo dispositivo (Fig. 1.2.d).



Figura 1.2. Dispositivos impressos em 3D projetados e fabricados neste projeto. (a) MCE-C⁴D impresso em DLP/ SLA; b EmD impresso em 3D baseado em SLA; c McE-EC impresso em 3D com base em SLA; d McE-EC impresso em 3D com base em DLP.

As metodologias propostas estão comprometidas em ser alternativas simples e baratas aos métodos atuais de microfabricação, como a litografia macia. A impressão 3D é dedicada a aumentar a facilidade de produção, o que permitirá aos pesquisadores rapidamente prototipar um novo MD com eletrodos integrados sem a necessidade de uso de sala limpa ou equipamentos caros.

2. O dispositivo MCE impresso em 3D contendo eletrodos em espiral para detecção C⁴D

2.1 Design e Fabricação de Microchips

Projetamos MD para impressões 3D usando o OpenSCAD (openscad.org), um programa gratuito de design (Figura 2.1.a). O layout padrão canais cruzados MCE, com canais de 45 μ m de largura e 50 μ m de altura, foi adaptado de um trabalho publicado anteriormente [10]. Adicionamos a este design dois canais espirais independentes para C⁴D, que estão envoltos do canal de separação no ponto de detecção (Figura 2.1.b).



Figura 2.1. Design OpenSCAD para o dispositivo de eletrodo espiral C⁴D. (a) Visão de dispositivo ortogonal de cima para baixo e (b) com reservatórios de eletroforese mostrados em roxo, canais em verde e eletrodos C⁴D com espirais em turquesa.

Os dispositivos foram fabricados usando uma impressora 3D customizada [11]. Resumidamente, esta impressora 3D de estereolitografia DLP emprega luz de 385 nm para polimerizar sequencialmente camadas de resina padrão de 10-µm de espessura. A resina é composta de 97% de diacrilato de poli (etileno glicol) (PEGDA, MW 250), 2% de absorvedor UV 2-nitrofenil fenil sulfeto (NPS), e 1% de fotoiniciador Irgacure 819. Uma vez que o dispositivo foi polimerizado, o excesso de resina foi lavado sob pressão negativa e, em seguida, enxaguados com álcool isopropílico. O dispositivo microfluídico foi então exposto à luz UV por 20 minutos para polimerizar qualquer excesso de resina (Fig. 2.2.a). Após a preparação do microchip, o gálio foi derretido pelo aquecimento a cerca de 35 °C e injetado nos canais em espiral, preenchendo uma extremidade de canal com gálio enquanto a pressão negativa foi aplicada na extremidade do outro canal usando uma seringa plástica para formar eletrodos metálicos ao redor do canal de separação (Fig. 2.2.b). Fios de cobre foram utilizados para contato elétrico com eletrodos de gálio e circuitos de detecção (Fig. 2.2.c). Finalmente, o MCE-C⁴D está pronto para aplicações (Fig. 2.2.d).



Figura 2.2. Esquema para montar um dispositivo eletroforético microfluídico. (a) Dispositivo exposto a uma etapa de poscura UV. (b) Ga injetado nos canais em espiral preenchendo uma extremidade de canal. (c) Fios de cobre como contato elétrico com os eletrodos de Ga e os circuitos de detecção. (d) Dispositivo pronto para aplicações.

Resumidamente, o dispositivo tem dimensões de 12 mm de largura e 23 mm de comprimento, com canal de injeção de 5,3 mm de comprimento e canal de separação de 13 mm de comprimento, com uma distância de separação efetiva de 9,5 mm (Fig. 2.3.a). Em relação aos dois canais de espiral independentes para C⁴D (Fig. 2.3.b), cada raio do canal da bobina foi de 100 μ m, com uma amplitude espiral de 300 μ m, produzindo um comprimento total de 11 mm do fio de gálio após o enchimento (Fig. 2,3. c).



Figura 2.3. (a) Dispositivo impresso mostrando as conexões com os eletrodos em espiral e o tamanho do microchip. (b) Visão CAD renderizada do microchip. (B1) Detalhe do canal de fluxo serpentino usado para injetar gálio. (B2) Reservatórios de entrada e saída conectados ao canal serpentino para injeção de metal. (B3) Reservatório de resíduos de tampão. (B4) Reservatório de amostra. (B5) Reservatórios tampão. (c) Canais de eletrodos serpentinas 3D ao redor do canal de separação em um dispositivo microfluídico fabricado em DLP (A1) sem gálio derretido e (A2) com gálio derretido para formar eletrodos espiral para C⁴D.

2.2 Resultados e discussão

Alcançar layouts complexos, neste caso canais em espiral em torno de um canal de separação central, seria muito complicado usando a prototipagem convencional de MD, como litografia e litografia macia devido à sua característica subtrativa. Além disso, a maioria das técnicas AM não tem a resolução desejada necessária para produzir verdadeiramente MD. Assim, SLA e DLP são as abordagens mais promissoras a serem usadas neste trabalho para criar estruturas microfluídicas complexas devido à sua alta resolução. Primeiro, tentamos reproduzir um design tubular clássico de eletrodos para realizar a detecção C⁴D. No entanto, para este design de eletrodo, foi trabalhoso encher os canais com líquidos de forma homogênea devido ao ar preso. Nesse contexto, criamos um design espiral, para o qual o desempenho pode ser comparável a um eletrodo tubular, uma vez que circunda o canal também. Além disso, os canais de eletrodos podem ser facilmente preenchidos com metais líquidos sem comprometer a conexão do eletrodo ou mesmo sua integração.

2.2.1 Caracterização e otimização do eletrodo

Em C⁴D, a tensão de excitação e a frequência de operação são parâmetros que afetam diretamente a resposta analítica. Assim, ambos os parâmetros foram otimizados antes de maximizar a sensibilidade do detector. O sinal C⁴D foi investigado utilizando valores de frequência que variavam de 84 a 628 kHz mantendo as tensões de excitação constantes em valores de 3,0, 5,0 e 7,0 V_p (ver Fig. 2.4) enquanto o canal de separação estava preenchido com 5 mmol L⁻¹ MES/histidina, o eletrólito de fundo (BGE).



Figura 2.4. Resposta de frequência para o dispositivo C⁴D-MCE em diferentes amplitudes do sinal de excitação. O canal de separação foi preenchido com uma solução tampão contendo 5 mmol L⁻¹ de MES e histidina.

De acordo com nosso trabalho anterior [12] sobre o desenvolvimento de C⁴D, a melhor frequência de operação depende principalmente dos parâmetros geométricos da célula de detecção, embora a condutividade do BGE também exerça alguma influência. Além disso, a medição da tensão de saída da linha de base correlaciona-se diretamente com a área de pico, sendo um método mais conveniente para otimização de frequência [12]. Como pode ver em Fig. 2.4, aumentando a frequência de operação, a tensão de saída aumenta até o valor máximo e a partir deste ponto as tensões de saída diminuem. Isso se deve às capacitâncias da célula de detecção: em baixas frequências, a capacitância do canal de parede domina e bloqueia a maior parte do sinal de excitação, enquanto em altas frequências outras capacitâncias (incluindo a

capacitância de entrada do amplificador de transimpedância) oferecem caminhos alternativos para o sinal. Normalmente, a frequência é definida neste máximo para a melhor resposta. Além disso, esse máximo é deslocado para frequências mais baixas aumentando a amplitude do sinal. Os valores máximos medidos em 3,0, 5,0 e 7,0 V_p foram obtidos para o MCE-C⁴D foram de 293, 238 e 207 kHz, respectivamente. Em resumo, para uma determinada amplitude e composição BGE, a frequência é escaneada e definida no máximo para outros experimentos.

Para estudar o desempenho analítico do MCE-C⁴D, bem como seu uso para o monitoramento de espécies catiônicas e aniônicas em amostras biológicas, os valores de frequência operacional e tensão de excitação foram então fixados em 250 kHz e 3,5 Vp, respectivamente. Em seguida, procuramos avaliar os valores de frequência operacional e tensão de excitação para a determinação das melhores condições de separação para K⁺, Na⁺ e Li⁺ em relação à concentração do tampão e à tensão aplicada. Uma mistura padrão desses cátions inorgânicos foi selecionada como um sistema de analito modelo, uma vez que são comumente usados íons para fornecer informações sobreo comportamento do sistema C⁴D, devido à falta de complexidade do sistema [13,14]. Em relação aos microchips baseados em DLP, os melhores resultados foram obtidos utilizando-se BGE composto de 20 mmol L⁻¹ MES/histidina (pH = 6,0) a 25 °C. A tensão de separação de 800 V, a tensão de injeção de 500 V e o tempo de injeção de 3 s foram selecionados para a separação de K⁺, Na⁺ e Li⁺ com eficiência adequada e resolução de pico (Fig. 2. 5).



Figura 2.5. Eletroferogramas fornecidos pela introdução de soluções padrão contendo os íons K⁺, Na⁺e Li⁺ (25-200 μ mol L⁻¹). Também são mostradas respectivas curvas de calibração. Injeção de modo "gated injection" aplicando +500 V e + 800 V em reservatórios de amostra e tampão.

Além disso, a linearidade do método foi avaliada utilizando soluções padrão de K⁺, Na⁺ e Li⁺ (25-200 μ mol L⁻¹). A linearidade adequada (r > 0,996) foi alcançada para todas as curvas de calibração (Figura 2.5), o que mostra uma resposta adequada do detector à concentração dos analitos. Quando comparado com outros detectores fabricados com metais líquidos e previamente relatados na literatura, o sistema proposto apresentou um melhor desempenho. Por exemplo, Thredgold et al. [15], relataram um eletrodo planar de gálio para C⁴D. Este eletrodo forneceu um coeficiente de correlação linear de 0,9729, 0,9794 e 0,9982 para K⁺, Na⁺ e Li⁺, respectivamente. Embora os eletrodos fossem feitos do mesmo material, a geometria em espiral do eletrodo demonstrada oferece uma resposta linear melhor do que o design do eletrodo no plano.

2.2.2 Otimização do método para determinação de nitrato na saliva

Posteriormente, a determinação do nitrato foi alcançada em condições previamente relatadas [16]. Na otimização do detector, uma solução tampão composta de ácido láctico e histidina foi preparada em diferentes proporções para inspecionar sua qualidade de detecção. A concentração de ácido láctico foi fixada (30 mmol L^{-1}) e a concentração de histidina variou em uma faixa de 10 a 20 mmol L^{-1} (com incrementos de 5 mmol L^{-1}). Apesar de todas as proporções mostrarem com sucesso um sinal de nitrato, o melhor desempenho considerando o tempo de análise, relação sinal-ruído, força iônica/capacidade tamponante e detectabilidade foi alcançado utilizando o BGE 30 mmol L^{-1} de ácido láctico/15 mmol L^{-1} histidina (pH 3.8) a 25 °C. Soluções padrão e amostra foram introduzidas dentro de microcanais através de um protocolo de injeção "floating injection". A tensão de separação de -700 V, a tensão de injeção -500 V e o tempo de injeção 3 s foram selecionados para determinação de nitrato na saliva.

2.2.3 Desempenho analítico e aplicação

Foram realizados estudos para investigar sistematicamente o desempenho analítico do MCE-C⁴D para a determinação do nitrato em amostras biológicas. Uma questão comum sobre o uso de chips de eletroforese é a injeção eletrocinética, que pode comprometer a repetibilidade da injeção amostral. Por isso, adicionamos um volume da solução padrão de nitrato para alcançar uma concentração de 100 µmol^{L-1} na amostra de saliva e, em seguida, executamos dez injeções sucessivas para investigar a repetibilidade da injeção (Figura 2.6).

O desvio padrão relativo intradia (RSD) da área de pico e o tempo de migração foram de 8,6 e 5,0%, respectivamente. Com base nos resultados estatísticos, pode-se inferir que este método MCE-C⁴D fornece repetibilidade adequada para dez injeções sucessivas. Um resumo



das características analíticas desta metodologia envolvendo o MCE-C⁴D é mostrado na Tabela 1.

Figura 2.6. Eletroferograma obtido através de dez injeções consecutivas de uma solução amostra de saliva contendo 100 µmol L⁻¹ de nitrato. BGE: 30/15 mmol L⁻¹ ácido láctico/His (pH 3.8). Injeção de amostra a -500 V para 3 s. A tensão de separação foi de -700 V. C⁴D operando a 250 kHz e 3,5 Vp.

Parâmetro	Valores	
Tempo de migração (s) ^a	$17,8 \pm 0,9$	
Área do pico ^b	$0,040 \pm 0,003$	
LOD (µmol L ⁻¹)	2,0	
LOQ (µmol L ⁻¹)	6,0	
Faixa linear (µmol L ⁻¹)	6-200	
Frequência analítica (h ⁻¹)	105	

Tabela 4 Desempenho analítico para a determinação do nitrato utilizando a metodologia MCE-C⁴D.

^a (n=3); ^b média ± desvio padrão (n=10)

O método mostra desempenho analítico adequado para determinação de nitrato na saliva. Um bom coeficiente de correlação (r = 0,994), parâmetros de calibração e ampla faixa linear (Tabela 1) foram obtidos em um estudo de linearidade utilizando curvas externas de calibração (Fig. 2.7.a), permitindo assim que o dispositivo realize análise quantitativa. Também caracterizamos o limite de detecção (LOD) e o limite de quantificação (LOQ) para nitrato utilizando uma abordagem empírica, que envolvia medidas sucessivas de soluções padrão mais diluídas até que uma relação sinal-ruído de 3:1 fosse atingida.

O LOD é adequado para o analito alvo na saliva, uma vez que os níveis encontrados na saliva de saúde e doença são maiores que o LOD (por exemplo, pacientes com doença periodontal podem apresentar níveis de nitrato entre 800 e 1500 μ mol L⁻¹) [17]. Além disso, nosso LOD é menor ou comparável aos valores relatados nos protocolos existentes usando MCE-C⁴D [16] e CE-UV [18].

Posteriormente, o desempenho do microchip e do método otimizado foi avaliado pela quantificação do composto alvo na amostra biológica. Nós adicionamos uma solução padrão de nitrato para alcançar uma concentração de 20 µmol L⁻¹ na amostra de saliva. Além disso, realizamos um teste de especificidade comparando eletroferogramas de amostras com e sem o analito (Fig. 2.7.b). Esses dados demonstram que nosso método tem a capacidade de discriminar compostos comumente presentes na saliva do analito alvo.



Figura 2.7. (a) Curva de calibração obtida a partir da introdução de soluções padrão contendo nitrato $(6 - 200 \ \mu mol \ L^{-1})$ utilizando MCE-C⁴D. BGE: 30 mM ácido láctico/15 mM Histidina (pH = 3,8). (b) Eletroferogramas obtidos para a introdução de uma amostra contendo 20 $\mu mol \ L^{-1}$ de nitrato padrão (---) e amostra sem nitrato (---). Injeção de amostra a -500 V para 3 s. Tensão de separação foi -700 V. C⁴D operando a 250 kHz e 3,5 V_{pp}.

Amostra	Analito	Esperado (µmol ^{L-1})	<i>MC</i> E-C ⁴ D (μmol ^{L-1})	Diferença Relativa
Saliva	NO ₃ -	20	21 ± 2	-5%

Tabela 5. Determinação de nitrato na saliva por MCE-C⁴D.

O resultado obtido a partir do nosso procedimento é semelhante ao resultado esperado (Tabela 2), com uma diferença relativa de 5%. Em uma corrida eletroforética rápida, nosso dispositivo microfluídico permite obter informações sobre os níveis de nitrato em uma matriz biológica.

2.3 Conclusão

Neste trabalho, demonstramos a impressão 3D baseada em DLP de MD com canais de 40 µm para análise de MCE de espécies reativas de nitrogênio em amostras biológicas. Pela primeira vez, apresentamos um dispositivo microfluídico impresso em 3D baseado em DLP com detecção C ⁴D aplicada à triagem de nitrato em fluidos biológicos com alta frequência de análise, adequada para triagem de rotina, e um método que não requer preparação de amostra laboriosa. Mostramos o uso da impressão DLP 3D para fazer MD para análise de um biomarcador medicamente relevante. Estes MD têm potencial para uso como uma ferramenta de diagnóstico amplamente disponível para nitrato.

3. Dispositivo microfluídico impresso em 3D baseado em estereolitografia com detecção eletroquímica integrada

3.1 Fabricação e design do EMD

Uma impressora 3D comercial Formlabs 2 SLA 3D (Formlabs, Somerville, EUA) foi empregada durante todo este trabalho. Para estudar a resolução da impressora 3D, um dispositivo microfluídico com sete canais de larguras variadas foi impresso com dimensões de 200 μ m (profundidade) e de 50 a 300 μ m (largura). Os dispositivos foram impressos em oito posições diferentes com inclinação angular de 15, 60 e 85° nos eixos Y e X cada, 45° tanto nos eixos XY quanto perpendicular ao eixo Z.

MD para impressão 3D foram desenhados usando o software de design Fusion 360 (Autodesk, San Rafael, USA). O dispositivo para caracterização e análise consiste em um único canal de 20 mm de comprimento, $95 \pm 6 \mu m$ de largura e $180 \pm 11 \mu m$ de altura e um canal de

eletrodo em forma de triângulo com uma superfície retangular com dimensões de $172 \pm 12 \mu m$ de largura e $180 \pm 11 \mu m$ de altura tocando o canal de separação (Fig. 3.1). O dispositivo de canal aberto foi impresso usando os parâmetros otimizados.



Fig. 3.1. (a) Esboço do dispositivo projetado usando software de design Fusion 360. (b) Zoom mostrando a conexão entre o canal de eletrodo e o canal de separação (largura da dimensão da interface) e a distância do reservatório de saída e do canal de eletrodo.

3.2 Fabricação e design do MCE-EC

Fabricamos um dispositivo MCE pelas condições de impressão otimizadas anteriormente usadas para montar o EMD. Alcançamos canais de 100 µm de largura, imprimindo 150 µm de largura projetada. Além disso, mantivemos o projeto do eletrodo e o material condutor feito em laboratório contendo proporção de carbono e resina de 9/8, respectivamente. O EMD e o MCE possuem diversas diferenças considerando o design, tais como: O EMD tem um único canal de separação com um eletrodo WE posicionado dentro do microcanal 1,5 mm de distância do reservatório de saída, enquanto o dispositivo MCE possui um layout transversal padrão com um eletrodo WE colocado dentro do microcanal com 100 µm de distância do reservatório de saída, Fig. 3.2).



Figura 3.2 (a) Esboço da eletroforese capilar do microchip projetada usando software de design Fusion 360. (b) Componentes do dispositivo microfluídico projetado: reservatório de amostra (S), reservatório de resíduos amostrais (SW), reservatório do tampão (B) e reservatório de resíduos do tampão (BW).

O dispositivo final para separação e detecção eletroforética consiste em um simples dispositivo T com canal de injeção de 16,5 mm de comprimento e um canal de separação de 38 mm de comprimento, 100 µm de largura e 300 µm de altura, com uma distância de separação efetiva de 29,5 mm (Figura 3.2). O eletrodo em formato de triângulo possui superfície retangular com dimensões de 547 µm de largura e 300 µm de altura tocando o canal de separação.

3.3 Preparação dos eletrodos

Foi criado um design de eletrodo de trabalho, um canal triangular para detecção eletroquímica com configuração "in-channel". Uma nova composição de eletrodo foi investigada. Uma mistura de pó de grafite e resina "Clear FormLabs" (C/ resina) foi preparada como pasta condutora em várias razões de w/w (4/8; 5/8; 6/8; 7/8; 8/8; 9/8), respectivamente. A pasta foi feita em um frasco de plástico, misturando corretamente ambos os componentes à mão com uma espátula até a integração completa do pó de grafite em uma pasta homogênea.

3.4 Integração de eletrodos em EMD

O canal do eletrodo (Figura 3.3. a) foi preenchido com a pasta C/resina utilizando uma seringa (Fig. 3.3. b) e posteriormente exposto à radiação UV (365 nm) por 4 h a uma dose de 50 mJ \times cm⁻² (UV Biolink BLX, Vilber Lourmat, França) até a reticulação completa da pasta (Figura 3.3.c). Em seguida, o excesso de pasta foi removido cuidadosamente polindo a superfície com uma lixa molhada (3M, EUA) (Figura 3.3. d). Posteriormente, o dispositivo foi selado usando uma fita de silicone (ThermalSeal RTS[®], Sigma Aldrich, Darmstadt, Alemanha) (Figura 3.3. e). Para remover bolhas e garantir a horizontalidade da configuração, foi aplicada uma pressão suave na fita de silicone para uma adesão adequada. Finalmente, o EMD está pronto para aplicações (Figura 3.3.f).



Figura 3.3 Esquema para montar um dispositivo microfluídico totalmente eletroquímico: (a) dispositivo impresso em 3D com canais abertos; (b) O canal de eletrodo é preenchido com pasta C/ resina; (c) Dispositivo impresso em 3D contendo pasta C/ resina é exposto em radiação UV; (d) O excesso da pasta é removido por uma lixa molhada; (e) O EMD é selado com fita de silicone; (f) O EMD está pronto para aplicações.

3.5 Integração de eletrodos no dispositivo MCE

Primeiro, o dispositivo impresso em 3D foi selado com uma fita de silicone (ThermalSeal RTS[®]), em seguida, o canal de eletrodo foi preenchido com a pasta condutora otimizada usando uma seringa. Posteriormente, o dispositivo completo foi exposto em radiação

UV a 365 nm por 4 h em dose de 50 mJ \times cm⁻² (UV Biolink BLX, Vilber Lourmat, França) até completar a reticulação da pasta condutora (Figura 3.4).



Figura 3.4. Esquema para montar um dispositivo eletroforético totalmente microfluídico.

3.6 Resultados e Discussão

3.6.1 Otimização de impressão

A resina comercial Formlabs Clear Resin (V4) (Formlabs, Somerville, EUA) foi utilizada para imprimir os dispositivos e produção dos eletrodos. A impressora 3D baseada em SLA têm a resolução limitada pelo tamanho do ponto laser de 140 μ m (resolução XY) e 25 μ m de espessura (resolução Z), que foi o fator limitante para alcançar canais de menor dimensão. Para definir o tamanho mínimo do canal viável, foi impresso um MD com sete canais retos de larguras variadas (Figura 3.5. a). Cada canal foi projetado para ter uma profundidade de 200 μ m e larguras de 300 a 50 μ m. Os dispositivos foram impressos em oito posições diferentes (Figura 3.5. b), com inclinação angular de 15, 60 e 85° no eixo Y e X cada um, 45° tanto eixo XY quanto perpendicular (XY = 0°) (Figura 3.5.c).



Figura 3.5. Design MD (a) com sete canais retos de larguras variadas (300; 200; 150; 125; 100; 75; 50 μm); (b) Após a etapa de impressão com suportes. (c) Disposto em várias posições na plataforma de construção para impressão.

Após a impressão dos canais, medimos os canais de largura por microscopia óptica. Os dispositivos impressos com ângulo de inclinação de 85°, 60°, 15° no eixo X alcançaram resultados adequados, ou seja, alcançamos canais nessas condições. O dispositivo impresso com ângulo de 85° no eixo X alcançou canais abertos bem definidos nas larguras projetadas entre 300-150 µm. No entanto, as dimensões impressas eram significativamente diferentes das dimensões projetadas. Todos os canais possuem uma largura consideravelmente mais rasa do que a projetada, diminuindo até uma largura especificada de 50 µm onde nenhum canal é visível. As discrepâncias entre as dimensões projetadas e reais nos canais devem-se ao tamanho do ponto do laser de 140 µm e são consistentes com outros canais microfluídicos impressos em 3D [19]. De fato, o efeito do ponto laser pode ser minimizado aumentando o ângulo de inclinação. O dispositivo impresso com ângulo de inclinação de 45° teve um impacto do ponto laser tanto na profundidade quanto na largura do canal. Assim, os canais menores estavam entupidos. Por fim, a orientação dos dispositivos na impressora com ângulo de 85°, 60°, 15° no eixo Y e perpendicular (XY = 0°) não imprimiu nenhum canal, devido ao alto impacto do tamanho do ponto do laser na largura do canal. Notavelmente, imprimimos o mesmo dispositivo com canais totalmente fechados com as mesmas dimensões e ângulos que os da Figura 3.5, todos os canais estavam entupidos com resina curada e nem mesmo o maior canal (300 × 200 µm) poderia passar alguma solução. Assim, utilizamos o dispositivo com orientação de impressão de ângulo de inclinação de 85° no eixo X. Os menores canais alcançados foram 95

 \pm 6 µm de largura e 180 \pm 11 µm de altura (n = 10) com um sistema projetado de 150 µm de largura e 200 µm de altura.

3.6.2 Design de eletrodos

O design do eletrodo foi desenvolvido para evitar qualquer vazamento sobre a vedação de todo o dispositivo. Para isso, o material do eletrodo deve evitar a propagação através do canal de separação acessando a partir da interface. Assim, foram avaliadas diversas geometrias de eletrodos e as distâncias entre o eletrodo e o canal de separação para fornecer uma configuração adequada. Dispositivos com canais de eletrodos que guardam duas geometrias (círculo e triângulo) e diferentes "gaps" (configurações 1 a 6 correspondentes a "gaps" de 60, 40, 20, 10, 0 e -10 µm, respectivamente) entre eles e o canal de separação foram projetados e depois impressos (Fig. 3,6).



Figura 3.6. Dispositivo microfluídico de canais abertos contendo eletrodos com duas geometrias (círculo e triângulo) em diferentes "gaps" de 1 a 6, (60, 40, 20, 10, 0 e -10 µm, respectivamente).

A conexão entre o canal do eletrodo e o canal de separação (largura da interface, correspondente a parte acessível do eletrodo com o canal de separação) foi medida (n = 10) por microscopia óptica e, em seguida, plotada versus as "gaps", para ambas as geometrias (Fig. 3.7). Canais de círculo e triângulo alcançaram um comportamento linear. A geometria do triângulo produziu larguras de eletrodos menores que 400 μ m, adequadas para fabricar microeletrodos. A geometria do círculo produziu eletrodos maiores (de 400 μ m a 1280 μ m). Avaliando a inclinação da curva, os "gaps" das geometrias circulares (m = -12,5) afetaram enormemente a largura do eletrodo quando comparadas com as geometrias do triângulo (m = -3,23), criando interfaces maiores em geometrias circulares.



Figura 3.7 Curvas correspondentes às dimensões de interface do eletrodo (largura) em função da distância teórica entre o canal do eletrodo e o canal de separação (gap).

Para avaliar a geometria do eletrodo (circular e triângulo), o canal de cada eletrodo foi lacrado e preenchido utilizando uma seringa com uma solução aquosa 10% (w/v%) de Eosin para monitorar por imagens fluorescentes o perfil dos líquidos quando inseridos no canal do eletrodo. Pode ser observado em Fig. 3.8 que os eletrodos com geometria de círculo promovem um vazamento da eosina para o microcanal de separação, pois a dimensão da interface é maior para círculo do que para configuração triangular. Por outro lado, os eletrodos com geometria triângulo permitiram manter o líquido nos canais de eletrodos quando o líquido foi adicionado em condições de baixa pressão, apesar da conexão com o canal de separação. Portanto, a C/resina pode ser eficientemente segurada no canal de formato triangular (configurações 1 a 3), o qual promoveu larguras menores devido a maiores "gaps" entre os canais. O eletrodo com geometria triângulo 2 demonstrou uma melhor conexão com o canal de separação 1 (123 \pm 10 μ m), e ainda fornecendo as propriedades de microeletrodos.



Figura 3.8. Imagens de microscopia por fluorescência dos canais dos eletrodos (geometrias de triângulo e círculo) preenchidas com um corante fluorescente.

3.6.3 Otimização do material do microeletrodo

O novo material proposto para a preparação do microeletrodo é composto por dois compostos. O primeiro é o pó de grafite com tamanho de partículas inferior a 15 µm. A adição de conteúdo em um material isolador permite a formação de redes de percolação elétrica dentro do material, levando ao aumento das propriedades eletroquímicas do material em várias ordens de magnitude. A segunda é a resina Clear Formlabs, o mesmo material usado para fabricar os dispositivos, garantindo a compatibilidade do eletrodo com o substrato do dispositivo. Realizamos experimentos preliminares com várias misturas de pó de grafite e resina em diferentes proporções de massa para otimizar o material em termos de ganho em condutividade, fabricação mais simples e estabilidade mecânica. Foram avaliadas misturas de C/resina com relações de 4/8, 5/8, 6/8, 7/8, 8/8 e 9/8, respectivamente. Proporções mais baixas não são adequadas, uma vez que as partículas de grafite estão muito dispersas comprometendo a rede condutora entre as partículas. Conseguimos uma pasta viscosa com misturas de 4/8, 5/8, 6/8 e 7/8, fácil de adicionar em tubos capilares de vidro (experimentos de voltametria cíclica (CV) e incorporar no canal do eletrodo antes de curar. Em relação a proporções maiores que 7/8, a pasta viscosa apresentou-se mais densa, mas com a viscosidade suficiente para ser integrada ao canal do eletrodo. A solidificação do eletrodo é garantida pelo processo de cura usando luz UV. O processo de cura da resina "Clear" geralmente precisa de cerca de 1 h na dose de 50 mJ \times cm⁻² para reticulação completa. A adição de grafite em pó afeta a cinética de reticulação da resina, especialmente quando a razão C/resina é alta (7/8, 8/8 e 9/8), desta forma o processo de cura do novo material foi otimizado para 4h na dose de 50 mJ× cm⁻² para garantir a solidificação completa do material.

A condutividade elétrica dos materiais dos eletrodos foi avaliada utilizando-se o método de resistência da sonda de 2 pontos (Tabela 3).

Razão m/m (Carbono/ resina)	Resistividade (Ω.cm)	Condutividade (S cm ⁻¹)
4/8	65,79	$(1,52\pm0,02)\times10^{-2}$
5/8	11,92	$(8,39\pm0,03) imes 10^{-2}$
6/8	3,88	$(2,58\pm0,02)\times10^{-1}$
7/8	2,06	$(4,84 \pm 0,01) \times 10^{-1}$
8/8	1,20	$(8,31 \pm 0,01) \times 10^{-1}$
9/8	1,17	$(8,57\pm0,01) imes10^{-1}$

Tabela 6: Resistividade e condutividade da resina personalizada (n = 3).

As relações de C/resina de 4/8 e 5/8 foram descartadas, uma vez que as partículas de grafite foram comprovadamente dispersas comprometendo a integridade da rede condutora. Para as demais proporções, a condutividade foi medida na faixa 2,6 a $8,6 \times 10^{-1}$ S cm⁻¹ . Em relação aos materiais poliméricos condutores na literatura, dois materiais que possuem em sua composição nanopartículas de prata demonstraram uma condutividade melhor do que a C/resina 9/8 [20,21]. Isso pode ser atribuído ao fato de que a tinta prateada é intrinsecamente mais condutora do que o carbono. Por outro lado, a prata tem uma faixa de potencial notavelmente limitada a qual pode ser utilizada antes de ser oxidada. O carbono é um material menos caro e combina importantes benefícios no comportamento dos eletrodos, por exemplo, a redução do processo de passivação e a grande faixa de trabalho para detecção quando comparado aos eletrodos metálicos.

É geralmente conhecido que a diminuição do conteúdo do isolador na mistura de pasta de carbono pode causar um aumento desejável na condutância do material, porém esse processo pode ser saturado em determinada concentração. Esse fenômeno pode ser observado na curva exponencial mostrando a dependência da condutividade com a razão C/resina (Fig. 3.9). Quando as partículas eletricamente condutoras estão em contato múltiplo permanente máximo, a condutividade total do material de C/resina é alcançada. Nesta situação, a resina denota apenas

um preenchimento de espaço livre entre as partículas de grafite e não tem basicamente nenhum efeito sobre a condutividade geral, saturando o processo [22].



Figura 3.9. Dependência da condutividade na composição de C/resina para várias razões de grafite e resina, respectivamente.

Posteriormente, avaliamos a influência de cada composição de razão no comportamento eletroquímico por CV (Fig. 3.10) utilizando o FcMeOH como sonda. Realizamos este estudo usando eletrodo em forma de disco a partir do uso de um tubo capilar de vidro como corpo do eletrodo, com um diâmetro de 2 mm. Usamos um sistema de 3 eletrodos em células eletroquímicas clássicas (Fig. 3.10.a). É notável que para menor teor de grafite no composto (6/8; 7/8), observa-se uma corrente anódica em forma de onda relacionada à oxidação de Fe (II) para Fe (III). A superfície pode formar uma rede de partes condutoras micrométricas e, assim, pode ser assimilada como uma rede de microeletrodos separados com uma difusão radial e sem sobreposição da camada de difusão. À medida que a relação C/resina aumenta (8/8; 9/8), a forma dos voltamogramas gradualmente muda de um sinal em formato onda para um formato pico (Fig. 3.10. b). Quanto à relação C/resina de 9/8, o voltamograma apresenta pico anódico para FcMeOH com intensidade máxima de 5,36 \pm 0,06 μ A. O Δ E (acima de 57 mV) da probe deve ser atribuído à cinética lenta de transferência de carga caracterizada pela separação de pico ao pico de 228±6 mV, o que está de acordo com trabalhos anteriores que abordam eletrodos de grafite [23]. Além disso, o AE aumenta de acordo com o aumento da taxa de varredura, um desvio tão alto sugere o processo de transferência de elétrons quase reversível e taxas de varredura mais altas [24].


Figura 3.10 (a) 3 eletrodos utilizados para realizar a caracterização eletroquímica da pasta condutora feita em laboratório; WE = razão C/resina (6/8 - 9/8), circular (diâmetro 2 mm), RE = Ag-AgCl, CE = Pt. (b) Voltametria cíclica em eletrodos C/resina de 1 mmol L⁻¹ FcMeOH. Eletrólito: 10 mmol L⁻¹ fosfato. Taxa de varredura 25mV s⁻¹. (c) Taxa de varredura de 1 mM FcMeOH de 10 mV s⁻¹ a 360 mV s⁻¹.

Utilizando o material eletrodo com razão C/resina de 9/8, os valores de corrente de pico versus raiz quadrada da taxa de varredura (\sqrt{v}) foram plotados para estimar a área de eletrodo (usando a equação de Randles-Sevcik) (Fig. 3.10. c). Os valores obtidos são de 90-97% da área geométrica teórica de um disco de 2 mm de diâmetro. C/resina 9/8 foi, portanto, escolhida para outros experimentos devido às suas aceitáveis propriedades condutivas e mecânicas adequadas para preparar os eletrodos integrados.

3.6.4 Acoplamento da detecção eletroquímica em dispositivo microfluídico

3.6.4.1 Integrando a pasta condutora no dispositivo microfluídico

Integramos o eletrodo no dispositivo preenchendo o canal eletrodo com a pasta condutora feita em laboratório com a composição otimizada. O dispositivo foi exposto na câmara UV para o processo de pós-cura do dispositivo impresso e para reticulação da pasta de eletrodo por 4h na dose de 50 mJ \times cm⁻². É notável que o processo pós-cura é uma etapa necessária para finalizar a fabricação do dispositivo, por isso aproveitamos esse processo para obter os eletrodos integrados na mesma etapa, minimizando o número de processos para alcançar um dispositivo completo. Além disso, a conexão eletrodo com tinta prateada ou fita condutiva não é necessária, uma vez que os fios podem ser conectados diretamente na pasta condutora dentro do canal de eletrodo aberto, e é fixado durante o processo de pós-cura. Depois disso, polimos o dispositivo com o objetivo de remover o excesso de composto, e suavizar a superfície do dispositivo para o processo de vedação usando uma fita de silicone (ThermalSeal



RTS[®]). Finalmente, realizamos um eletrodo de microbanda integrado para atuar como um WE no modo de detecção no canal (Fig. 3.11).

Figura 3.11. Esquema do EMD proposto com ampliação mostrando o eletrodo de microbanda integrado lateralmente.

3.6.4.2 Caracterização eletroquímica

A caracterização eletroquímica do eletrodo da microbanda foi realizada usando FcMeOH como sonda redox. Uma configuração de 2 eletrodos, pseudo-RE e CE em curtocircuito (C/resina versus cloreto de prata/prata), tem sido usada para realizar todos os experimentos seguintes. O eletrodo WE está posicionado dentro do microcanal, enquanto o eletrodo pseudo-RE está posicionado no reservatório de saída, que é colocado 1,5 mm do canal do eletrodo. Primeiro, preenchemos o canal com a solução da sonda redox para executar os experimentos CV. Entre cada aquisição, o canal foi lavado com água e tampão antes de renovar a solução para certificar a repetibilidade das medidas, atingindo um desvio padrão relativo de $\pm 2\%$ nos valores de pico de corrente (n = 3) (Fig. 3.12).



Figura 3.12. (a) Eletrodo no formato de triângulo integrado C/resina no dispositivo selado com fita de silicone. (b) Voltametria linear de 1 mmol L⁻¹ FcMeOH neste eletrodo integrado em um dispositivo impresso em 3D (n = 3). Eletrólito: PBS 0,01 mol L⁻¹ (pH = 6,7). WE: C/resina proporção 9/8, RE/CE = Fio Ag-AgCl. Taxa de varredura 25 mV s⁻¹

O CV da FcMeOH demonstrou características com um potencial de oxidação de meia onda de 260±16 mV e onda 77,0±0,8 nA (Fig. 3.13.a). Plotamos os valores de pico de corrente versus a raiz quadrada da taxa de varredura (\sqrt{v}) (Fig. 3.13.b) e utilizamos o valor da inclinação para calcular a área dinâmica da superfície (0,027 mm²) da plataforma de sensoriamento pela equação de Randles-Sevcik. A densidade de corrente experimental é mais acentuada no formato microeletrodo do que no formato macroeletrodo, aumentando de 1,74 µA × mm² para 2,84 µA× mm² para FcMeOH com taxa de varredura de 50 mV s⁻¹, fortalecendo as motivações para a miniaturização do eletrodo.



Figura 3.13. Voltametria cíclica em eletrodos C/resina de (-) 1 mmol L⁻¹ FcMeOH em PBS 10 mmol L⁻¹ e (-) branco. WE = Proporção C/resina 9/8, RE/CE = Fio Ag-AgCl. Taxa de varredura 50 mV s⁻¹. (b) Relação da corrente de pico anódica versus a raiz quadrada de taxa de varredura de eletrodo C/ resina integrado (9/8).

Depois de configurar um protocolo simples para a criação de EMD usando impressora 3D baseada em SLA, demonstramos a detecção eletroquímica empregando a configuração de 2 eletrodos para estudar a linearidade de resposta do material transdutor por voltametria de onda quadrada (SWV). Figura 3.14 demonstrou várias respostas de pico anódico versus diferentes concentrações de FcMeOH, variando de 15 μ mol L⁻¹ a 1000 μ mol L⁻¹ obtidos com o eletrodo integrado. Foram observados picos simétricos e bem definidos em torno de 0,15 V. O eletrodo de detecção tem uma resposta linear quando aumentamos a concentração da sonda, a curva de calibração atingiu um coeficiente de regressão de 0,995. Os valores LOD e LOQ foram de 4,5 μ mol L⁻¹ e 15 μ mol L⁻¹, respectivamente.



Figura 3.14. (a) SWV em C/resina (9/8) microeletrodo integrado em um dispositivo impresso em 3D em PBS 10 mmol L⁻¹ contendo FcMeOH (de 15 a 1000 μ mol L⁻¹) em condições estáticas. (b) Gráfico de calibração correspondente à corrente de pico SWV em função das concentrações da sonda. Parâmetros: $\Delta E = 10 \text{ mV}$; f = 30 Hz; E_{step}: 6 mV.

3.6.4.3 Renovação do eletrodo integrado

Um dos objetivos deste trabalho é alcançar um dispositivo reutilizável, desta forma, desenvolvemos uma estratégia para renovar a superfície do eletrodo após vários e exaustivos experimentos. Uma vez que os eletrodos são colocados dentro do dispositivo com uma vedação

irreversível, não é possível renovar a área da superfície pelo polimento clássico. Além disso, eletrodos de microchip não podem ser limpos usando os métodos agressivos comumente aplicados aos eletrodos de disco, pois diversos procedimentos de limpeza química, por exemplo, o uso de solução de piranha, ácido nítrico quente e altas concentrações de ácido sulfúrico não são compatíveis com o material dos microchips [25]. Para superar essa questão, desenvolvemos uma estratégia para renovar a área de eletrodos, dentro do canal, introduzindo ácido sulfúrico (0,1 mol L⁻¹) dentro do canal de separação e realizando 100 voltamogramas cíclicos em 1 V s⁻¹, 50 em 0,5 V s⁻¹, 25 em 0,25 V s⁻¹ e 10 em 0,1 V s⁻¹. O eletrodo foi reutilizado aproximadamente 30 vezes até que seu sinal atual comece a diminuir por passivação (Fig. 3.15). Observou-se uma melhor reversibilidade (menor separação de pico ao pico) e uma intensidade de pico voltamétricos aprimorada para o par redox FcMeOH, confirmando a limpeza da superfície do eletrodo.



Figura 3.15. Comportamento CV de FcMeOH 1 mmol L⁻¹ em PBS em C/resina (9/8) microeletrodo integrado em um dispositivo impresso em 3D (a) após a utilização do eletrodo por 30 vezes (n =3) e (b) após tratamento eletroquímico do WE usando uma solução de ácido sulfúrico (0,1 mol L⁻¹) (n =3), consulte o texto para obter detalhes. Taxa de varredura: 25 mV s^{-1} .

3.6.4.4 Aplicação de EMD impresso em 3D

Para comprovar o uso de um EMD tão barato e simples criado com uma impressora 3D baseada em SLA, a resposta eletroquímica de uma sonda, injetada continuamente e hidrodinâmicamente no canal de separação, foi avaliada no canal de detecção integrado feito

em laboratório previamente otimizado. Para isso, uma solução $[Ru(NH_3)_6]^{3+}$ (5 mmol L⁻¹) foi continuamente injetada em uma vazão que variava de 0 (sem fluxo) a 55 µL min⁻¹ e analisada por voltametria cíclica (Fig. 3.16). Esses valores de fluxo foram escolhidos para mimetizar o fluxo eletrosmótico (EOF) dentro do microcanal, que é promovido por técnicas de separação impulsionadas eletricamente.



Figura 3.16. (a) Influência da taxa de fluxo no CV em C/resina 9/8 WE em uma solução de $[Ru(NH_3)_6]^{3+}$ 5 mmol L⁻¹ em PBS. Vazão variando de 5 a 55 µL/min. Taxa de varredura 50 mV/s. (b) Dependência da corrente na taxa de fluxo dentro do microcanal.

Como evidência na Fig. 3.16, o aumento no fluxo gerado hidrodinâmicamente aumenta a corrente de redução de $[Ru(NH_3)_6]^{3+}$. Isso se deve, de fato, ao estreitamento da camada de difusão, que aumenta o gradiente de concentração e a convecção da solução, assegurada pelo fluxo contínuo, renovando a solução e, portanto, o analito. As condições de fluxo hidrodinâmico dentro do canal oferecem benefícios quando comparadas com o modo estático, como a evolução da corrente de pico e a melhoria em torno de 4 vezes nos valores LOD e LOQ.

3.6.5 Acoplamento detector amperométrico com eletroforese em um microdispositivo

Criamos um dispositivo eletroforético de microchip com um canal de detecção preenchido com a pasta condutora otimizada em laboratório e selado com uma fita de silicone (ThermalSeal RTS[®]) (Fig. 3.17)



Figura 3.17. Vista inferior e superior do dispositivo proposto.

3.6.5.1 Caracterização do fluxo eletroosmótico (EOF)

Caracterização da superfície do material impresso em 3D foram desenvolvidas para avaliar o fluxo eletrosmótico gerado dentro do microcanal impresso em 3D do sistema MCE. As superfícies foram caracterizadas pelo ângulo de contato da água (WCA) e espectroscopia no infravermelho por transformada de Fourier (FTIR) com modo de reflexão total atenuada (ATR). As medições da WCA permitiram caracterizar as propriedades hidrofílicas do substrato do dispositivo (Fig. 3. 18).



Figura 3.18. Medidas de ângulo de contato com a água na superfície do dispositivo proposto.

Para o material impresso em 3D, o valor WCA é igual a $78,7\pm0,3^{\circ}$ (n = 3), o valor é 33% menor que o valor para PDMS (WCA = 117°) [26]. Esses resultados indicam que o material impresso em 3D tem um comportamento mais hidrofílico do que o PDMS, um material comumente empregado para fabricar dispositivos MCE. Este detalhe é potencialmente interessante para a fabricação de microchips, pois é um indicativo de melhor molhabilidade em dispositivos impressos em 3D ao usar soluções aquosas, promovendo altas taxas de EOF. Certamente, não será necessário usar um surfactante para melhorar a capacidade de molhabilidade nos experimentos subsequentes.

Figura 3.19 exibe espectro FTIR-ATR da resina não curada (antes da impressão) e resina curada (material impresso em 3D). Ambos os espectros de resina curada e não curada apresentaram bandas de absorção semelhantes localizadas em 2952 e 2868 cm⁻¹ que são atribuídas aos modos de vibração de estiramento de carbono/ hidrogênio de grupos -CH₂ e - CH₃ da espinha dorsal do polímero. A banda em torno de 1450 cm⁻¹ é fornecida pelo modo de movimentação de grupos -CH₃. A banda localizada em 1704 cm⁻¹ é atribuída ao grupo carbonila, e a banda localizada em 3373 cm⁻¹ é atribuída ao grupo hidroxila (modo de estiramento). Como pode-se notar, o espectro apresentou uma absorção de deformação axial intensa de O-H com uma grande faixa entre 3300 — 3500 cm⁻¹, este fenômeno é explicado devido à presença de dímeros de ácido carboxílico na resina curada e não curada. A potencial presença de grupos carboxílicos promove a criação de altas taxas de EOF dentro do canal. A banda localizada a 1639 cm⁻¹ relacionada ao grupo C=C (modo estiramento) é apresentada apenas na resina não curada, o que era esperado desde que a polimerização de reação em cadeia começou a quebrar as ligações duplas C=C dos monômeros.



Figura 3.19. Espectro FTIR-ATR de resina clara (—) e curada (—) FormLabs.

O fluxo eletroosmótico de um microcanal retangular impresso em 3D, largura de 100 μ m, 300 μ m de altura e 38 mm de comprimento, foi determinado com método do marcador neutro. O método de marcador neutro envolve a medição do tempo necessário para que uma molécula de marcador neutro migrem para o sistema de detecção sob um campo elétrico. A mobilidade eletroosmótica foi determinada utilizando-se 1 mmol L⁻¹ de FcMeOH preparado em tampões fosfato (pH 2, 6, 12), carbonato (pH 10) e borato (pH 8), na concentração de 20 mmol L⁻¹ (Fig. 3. 20).



Figura 3.20. Dependência da mobilidade eletroforética (μ_{eo}) pelo pH em microcanais fabricados a partir do uso da resina "Clear" FormLabs, faixa de pH utilizada de 2 a 12. μ_{eo} monitorado com o método marcador neutro. Marcador neutro: FcMeOH; BGE: fosfato, borato e carbonato (20 mmol L⁻¹).

Para pH 8, 10 e 12 a mobilidade eletroosmótica foi calculada entre 5,7 e $8,6 \times 10^{-5}$ cm² V⁻¹ s⁻¹ (n=3), evidenciando uma carga negativa na superfície da parede microcanal impressa em 3D. Esse fenômeno endossa a presença de grupos de ácido carboxílico evidenciados no espectro FTIR-ATR do dispositivo impresso em 3D.

3.6.5.2 Canal de separação eletroforética

O efeito de aquecimento Joule no dispositivo impresso em 3D foi avaliado pelo monitoramento da corrente elétrica em vários valores do potencial aplicado (Fig. 3.21).



Calculamos a resistência elétrica (1,14 M Ω cm⁻¹) utilizando a 1/inclinação da corrente da curva versus tensão na região linear (de 0 a 210 V cm⁻¹).

Figura 3.21. Curva ôhmica (campo elétrico vs corrente) fornecida pelo dispositivo microfluídico impresso em 3D com uma solução tampão fosfato de 50 mmol L⁻¹.

Observamos um comportamento linear até o campo elétrico de valor 210 V cm⁻¹, em valores maiores de campo elétrico, o efeito de aquecimento joule é mais pronunciado, causando um desvio da linearidade. A dissipação do aquecimento Joule é menos eficiente quando comparada com o material MCE de outra natureza, por exemplo, sílica fundida ou vidro. Essa desvantagem pode ser superada usando BGE mais diluído (menor resistência iônica, o aquecimento joule pode ser minimizado, mas a capacidade do tampão pode ser comprometida. Por isso, utilizamos o potencial de separação de 700 V (184 V cm⁻¹), e diminuímos a concentração tampão de 50 mmol L⁻¹ para 10 mmol L⁻¹ de tampão fosfato, a força iônica de 10 mmol L⁻¹ é adequada para alcançar uma boa relação entre o efeito Joule e a capacidade tamponante.

3.6.5.3 Caracterização do eletrodo

A caracterização eletroquímica do eletrodo da microbanda foi realizada utilizando [Ru(NH₃)₆]³⁺como sonda redox em condições estáticas. Uma configuração de 2 eletrodos, pseudo-RE e CE em curto-circuito (cloreto de prata/prata), tem sido usada para realizar todos os experimentos seguintes. O eletrodo WE está posicionado dentro do microcanal, enquanto o eletrodo pseudo-RE está posicionado no reservatório de saída, que é posicionado a 100 μm do WE. Calculamos a área de superfície ativa do eletrodo, plotando os valores de redução de pico da sonda $[Ru(NH_3)_6]^{3+}$ versus a raiz quadrada da taxa de varredura (Fig. 3.22). Foi encontrada uma clara linearidade ($R^2 = -0,998$), utilizamos o valor da inclinação para calcular a área dinâmica da superfície ($0,30 \text{ mm}^2$) da plataforma de sensoriamento pela equação de Randles-Sevcik.



Figura 3.22. (a) CV em eletrodos C/resina de 5 mmol L⁻¹ [Ru(NH₃)₆]³⁺em 10 mmol L⁻¹ de tampão fosfato em várias taxas de varredura (10-250 mV/s) e correntes de pico catódico vs (V/s)^{1/2}. (b) SWV de [Ru(NH₃)₆]³⁺ (de 15 a 1000 µmol L⁻¹) e curva de calibração entre a SWV corrente de pico e os valores das concentrações da sonda. WE = 9/8 microbanda (547 µm de largura), RE/CE = Fio Ag-AgCl.

Em separações eletroforéticas, a aplicação de uma diferença de potencial é necessária para a migração dos íons sob um campo elétrico dentro do microcanal. O acoplamento da eletroforese de microchip com detecção eletroquímica pode causar desafios devido a interferências que podem ocorrer entre os campos elétricos gerados por ambas as técnicas. Este campo elétrico pode ter um forte impacto no detector eletroquímico colocado dentro do canal de separação. Quanto à detecção amperométrica, a corrente elétrica produzida pelo potencial de separação (10-30 kV) dentro do canal pode ser seis ou mais ordens de magnitude maior do que as correntes eletroquímicas medidas no detector amperométrico [27]. Este efeito foi estudado através da execução da voltametria cíclica de $[Ru(NH_3)_6]^{3+}$ em PBS dentro do microcanal, com ou sem a aplicação de uma tensão de separação para eletroforese e utilizando um eletrodo C/resina na configuração "end-channel".

Utilizamos uma configuração de 2 eletrodos com pseudo-RE e CE (Ag/AgCl) em curtocircuito posicionado no reservatório de saída. A voltametria cíclica foi realizada concomitante à aplicação da tensão de separação em diferentes valores dentro do canal (Fig. 3.23)



Figura 3.23. Influência da tensão de separação nos voltamogramas cíclicos de 5 mmol L⁻¹ [Ru(NH₃)₆]³⁺ na configuração "end-channel". WE: microbanda 9/8 (largura de 547 μ m), RE/CE = Fio Ag-AgCl. Tensão de separação: 0 a +700 V (entrada). Taxa de varredura: 50 mV s⁻¹.

O potencial de redução da sonda é deslocado para valores mais positivos quando a tensão de separação aumenta. Na ausência da tensão de separação, a sonda é reduzida no potencial de valor -0,37 V *vs.* Ag/AgCl, com a tensão de separação de 700 V (lado da entrada) o potencial de redução é alterado de -0,37 V *vs.* Ag/AgCl para -0,2 V *vs.* Ag/AgCl. A mudança no potencial pode ser explicada pela interação entre os campos elétricos do potencial de separação e o potencial de detecção. Além disso, obtivemos um sinal com níveis de ruído aceitáveis mesmo sob campos elétricos mais altos. Isso pode ser atribuído ao fato de que o alinhamento "end-channel" explora a estratégia de colocar o WE na saída do canal de separação, 5-100 µm de distância da extremidade do reservatório de saída. Ao fazê-lo, evita-se a influência do campo elétrico de separação na detecção eletroquímica. Neste contexto, a configuração de 2 eletrodos em uma configuração "end-channel" é eficiente para a detecção da sonda redox no potencial de detecção -0,2V/ Ag-AgCl.

O "end-channel" pode sofrer com outra desvantagem: uma vez que o WE pode ser posicionado fora do microcanal, a amostra pode passar por uma diluição no reservatório, o que compromete o desempenho analítico [28]. Nesse contexto, estudamos a configuração do "endchannel", onde o WE está posicionado dentro do microcanal de separação para evitar a diluição da amostra. Além disso, testamos a estabilidade da corrente sob um campo elétrico (Fig. 3.24).

utilizamos Para esta configuração, um potenciostato sem fio para medições cronoamperométricas, uma vez que o uso de um potenciostato clássico pode ser danificado pela função da tensão de separação durante a separação eletroforética sem aterramento. Desta forma, realizamos medições cronoamperométricas para monitorar qualquer influência do campo elétrico em uma configuração de 2 eletrodos com o potencial de redução fixado em -0.2 V. A configuração consistia em um eletrodo C/resina de largura de 547 µm colocado pouco antes do fim do canal de separação (cerca de 100 µm de distância) e um pseudo-RE/CE feito de um fio Ag/AgCl imerso no reservatório mais próximo.



Figura 3.24. Influência do campo elétrico de separação na corrente de detecção cronoamperométrica do tampão de fosfato de 10 mmol L⁻¹ (pH = 7,4) em uma configuração end-channel. WE: microbanda 9/8 (largura de 547 μ m), RE/CE = Fio Ag-AgCl. Tensão de separação: +300 V a +700 V (entrada). Potencial de detecção: -0.2 V.

Na ausência da tensão de separação, temos um valor de corrente próximo a zero. Aplicando uma tensão de separação, a corrente aumenta para valores negativos e o ruído aumenta proporcionalmente com a tensão de separação. Isso pode ser atribuído ao maior efeito joule sob maior valor de campo elétrico (100 V cm⁻¹). Além disso, esse comportamento pode estar relacionado à interferência elétrica no eletrodo WE. O potencial WE está aumentando visivelmente com o aumento do campo elétrico, o que pode causar efeitos indesejáveis no WE, por exemplo, geração de gases no eletrodo devido à oxidação de H₂O. Apesar do aumento do ruído alcançado pela aplicação de altas tensões (700 V), ainda podemos ter uma boa relação S/N sinal-ruído.

3.6.5.4 Aplicação de MCE-EC impresso em 3D

Utilizamos a configuração de 2 eletrodos para a separação e detecção cronoamperométrica de $[Ru(NH_3)_6]^{3+}$ no MCE. O BGE é composto por 10 mmol L⁻¹ tampão fosfato, uma vez que este meio tem sido utilizado para todos os estudos relativos a este dispositivo. Para isso, utilizamos um modo de injeção "floating" (500 V por 5s), seguido de uma separação eletroforética (700 V por 750 s) e detecção cronoamperométrica a -0,2 V *vs*. Ag-AgCl. O eletroferograma e a curva de calibração do $[Ru(NH_3)_6]^{3+}$ são apresentados na Figura 3.25.



Figura 3.25. Eletroferogramas de BGE 10 mmol L⁻¹ de fosfato (pH = 7,4), 500 μ mol L⁻¹ [Ru(NH₃)₆]³⁺ na configuração do canal final. Curva de calibração do [Ru(NH₃)₆]³⁺, concentrações da sonda (100 a 700 μ M). WE: Microband 9/8 (largura de 547 μ m), RE/CE = Fio Ag-AgCl. Potencial de injeção: +500V (entrada de amostra), potencial de separação: +700 V (entrada tampão) e potencial de detecção: -0,2V vs. Ag-AgCl.

De acordo com a Figura 3.25, alcançamos uma relação s/n de sinal-ruído adequada, apesar do ruído da tensão de separação. O pico de redução da sonda apresenta tempo de migração de 114 s. O dispositivo forneceu desempenho analítico adequado para determinação de $[Ru(NH_3)_6]^{3+}$. Foram obtidos bons coeficientes de correlação (r = -0,996), parâmetros de calibração e faixa linear (100 a 700 µmol L⁻¹) utilizando-se curva de calibração externa (Figura 3.25). Portanto, o eletrodo à base de C/resina mostrou-se adequado para separação eletroforética juntamente com a detecção cronoamperométrica.

3.7 Conclusão

Demonstramos um processo utilizando impressora 3D baseada em SLA para fabricar dois tipos de dispositivo, EMD e MCE, juntamente com a detecção amperométrica. Provamos o conceito de impressão 3D baseada em SLA para MD com design fácil de integração de eletrodos e nova formulação de eletrodos para detecção de CE. Caracterizamos este eletrodo por eletroquímica, proporcionando uma boa reprodutibilidade e resposta linear. O método analítico levou a um LOD e LOQ de 4,5 µmol L⁻¹ e 15 µmol L⁻¹ do $[Ru(NH_3)_6]^{3+}$, respectivamente. Os dispositivos propostos são reutilizáveis com uma superfície eletrodo que pode ser renovada sem a necessidade de abrir o dispositivo, promovendo robustez no sistema.

4. Perspectivas Futuras: Dispositivo de eletroforese impresso em 3D baseado em processamento de luz digital com detecção eletroquímica integrada

4.1 Design e Fabricação de Microchips

Uma impressora Solus Pro 3D com projetor SprintRay foi usada durante o trabalho. MD para impressão 3D (Fig. 4.1.a) foram projetados usando software de design Fusion 360 (Autodesk, San Rafael, USA). O dispositivo para caracterização e análises consiste em um canal de injeção de 16,5 mm de comprimento, canal de separação de 38 mm de comprimento e 103 \pm 7 µm largura de (Fig. 4.1.b) e um canal eletrodo em forma de triângulo com uma superfície retangular com dimensões de 352 \pm 6 µm de largura tocando o canal de separação (Fig. 4.1.c).



Figura 4.1 (a) Dispositivo MCE impresso em 3D. (b) Visão superior do canal próximo do dispositivo. (c) Canal de eletrodo em forma de triângulo de parede lateral tocando o canal de separação.

O dispositivo de canal próximo foi impresso usando a resina "Versapic 1147 Clear resin". As especificações da impressora foram otimizadas em espessura de camada 10 µm, resolução $12 \mu m$, tempo de exposição de 30 s para camadas de base e 2 s para construção de canais, parte de cima e reservatórios, com decremento de 5 s.

4.2 Resultados e Discussão

4.2.1 Otimização de impressão

As impressoras 3D baseadas em DLP têm a resolução limitada por vários parâmetros, tamanho do pixel do projetor (resolução XY), tempo de exposição e espessura da camada (resolução Z), que são fatores limitantes para a obtenção de microcanais. Para definir o tamanho mínimo do canal viável, estudamos vários tempos de exposição e o decremento para montar MD. O tempo de exposição foi fixado em 30 s (camadas base) / 1s (outros) com 3 s de decremento, alcançando canais de $127 \pm 12 \mu m$ (Fig. 4.2.a). Assim, aumentou-se o tempo de exposição e o decremento, para alcançar uma polimerização mais elevada levando à canais de menor largura. Como resultado, foram realizados canais com $103 \pm 7 \mu m$ (Fig. 4.2.b).



Figura 4.2. Imagens de microscopia óptica de canais impressos em 3D com cerca de (a) 127 μm e (b) 103 μm de largura.

Como perspectivas futuras, os eletrodos serão integrados ao dispositivo MCE impresso. Em seguida, o novo dispositivo será caracterizado e otimizado para atuar como uma plataforma de triagem de serotonina e ferro em amostras biológicas.

5. Conclusão geral e perspectivas futuras

As tecnologias de impressão 3D baseadas na fotopolimerização têm sido utilizadas para o mesmo propósito (microcanais e eletrodos integrados), mas usando várias abordagens diferentes dependendo da impressora 3D empregada. Publicamos artigos científicos relatando o uso de impressoras 3D para montar vários MD. Esses protocolos guiarão os novos usuários de impressora 3D a alcançar um tamanho mínimo de canal de fluxo usando essas impressoras 3D especificamente comerciais ou para ir mais longe em sua melhoria à luz da descrição do comportamento dos parâmetros.

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GENERAL INTRODUCTION

Miniaturization in chemical and biomedical analysis has emerged in recent years aiming at surpassing the performance of conventional analytical devices through the reduction of reagents, samples, waste, analysis time, production costs and contamination risks, as well as designing automated and portable devices that integrate all the classical analytical steps (sample pre-treatment, separation, detection, along with possible chemical modifications between steps) and that would be used in any part of the world without technical expertise or specific experimental conditions. The improvements of chemical etching and photolithography have greatly contributed to the emergence of this field of research. As early as 1979, Terry et al. published a microfabrication strategy using photolithographic patterning processes for the integration of a gas chromatograph and a thermal conductivity detector onto an entire silicon wafer [8]. This work went unrecognized for over a decade before a novel concept was developed by Manz et al. [2] so-called Miniaturized Total Analysis Systems (µ-TAS), which involves sampling, sample pre-treatment, separation and detection performed within a single microdevice. This miniaturized system had a tremendous impact on different research fields, for instance in analytical chemistry, as they deal with very small sample and reagents volumes, high resolution and sensitivity separations, low fabrication cost, small footprints and decrease of the physical size of the systems (portability) [9].

Regarding the separation step, integration of chromatographic and electrophoretic processes has already been performed, and particular attention has been given to electro-driven separations of target analytes because of high speed and high efficiency. Furthermore, they are more easily miniaturized compared to pressure-driven separation, as they require a single high voltage sequencer, electrodes on both ends of the separation channel, and procedural simplicity and flexibility [10]. In other words, the synergy between electrophoretic separations and the μ -TAS concept are evident. Firstly, small volumes reduce reagent and sample consumption, lowering costs. Furthermore, the use of small volumes improves heat and mass transfer, which in microchip electrophoresis (MCE) permits the employ of high voltages without Joule heating. Secondly, shorter separation channel lengths decrease analysis time, which, along with parallelization, can greatly increase throughput. Finally, application for instance planetary explorations, environmental investigation and point-of-care diagnostics can exploit the potential for portability of MCE systems. Therefore, MCE represents the next generation of miniaturized devices for the separation of complex sample matrices [5].

When it comes with the detection step, several techniques have been implemented for detection within microfluidic systems such as fluorescence (which is classically used in microsystems) [6], colorimetry [7], mass spectrometry [8], Raman [9] and infrared [10] spectroscopies, and electrochemistry [11]. Each of them has its own advantages and limitations but electrochemical (EC) detection is the most versatile and low-cost one. For example, since no optical window is necessary, one gains in flexibility by using opaque substrates. This consequently facilitates the fabrication process. Also, high selectivity can be provided by proper choice of detection potential and/or electrode material and high sensitivity, down to the nanomolar range, can be achieved in comparison to optical detection such as UV absorption [12]. In addition, the microelectrodes may be fabricated directly in the channel platform using the same fabrication techniques as to assemble to microfluidic device itself. EC detection is however poorly developed in MCE as significant electrical interferences between the electric fields generated for both separation and detection, and manufacturing challenges may occur. The classical fabrication processes to assemble microfluidic devices with integrated electrodes requires clean room facilities. The alignment and position of these electrodes can induce some issues in the analysis reproducibility. In addition, the electrode material must be compatible with the device substrate to avoid leakage and promote a robust experimental platform.

Concerning the choice of the substrate for the microfluidic devices, silicon is expensive and opaque to visible and ultraviolet light, so it cannot be used with conventional optical methods of detection. Concerning glass material, they imply no gas permeability and the classical microfabrication processes (e.g., chemical etching and photolithography) require clean room facilities [13]. Polydimethylsiloxane (PDMS) is one of the most extensively used materials in the fabrication of microfluidic devices. PDMS is a soft elastomer, optically transparent and widely used for exploratory research. Still, PDMS is a hydrophobic material and has a fast hydrophobic recovery after surface hydrophilization. These outcomes promote nonspecific adsorption of proteins as well as small hydrophobic molecules, compromising the analysis reproducibility using this substrate [14]. Therefore, new materials and new alternatives to produce microfluidic devices that reduce manufacturing steps, facilitate the integration of components, and increase the throughput are being explored.

When it comes to microfabrication, replication methods like injection molding and hot embossing can be used to structure thermoplastic materials. Main examples are poly(methylmethacrylate) (PMMA), polycarbonate (PC), substrates commonly used in the macroworld, facilitating the access to these materials. Cycloolefin polymers and copolymers (COP, COC) are emergent materials which have attracted much attention in recent years owing to their favorable optical properties, moldability and low water absorption. However, those methods are bulk-forming and subtractive processes, which have higher costs for acquisition of material and mold, and generate more waste in the manufacturing process as well [15].

Three-dimensional (3D) printing technology, or additive manufacturing, is a versatile way to assemble complex pieces in simple and automated steps. This technology enables the development of mass-produced electrochemical sensors and biosensors [16–18] of varying geometries and materials (hybrid-carbon-based [19,20] and stainless-steel-based [21]) for applications in numerous research fields, ranging from medicine [22] to environmental pollutants [23]. In addition, 3D printing techniques allow the production of microchannels and microstructures in a variety of materials with acceptable precision and high resolution [24]. Even though most 3D-printed fluidic devices and features have been to date on the sub-millimeter size scale, some truly microfluidic devices have been shown [25]. Nowadays, Stereolithography (SLA) and Digital Light Processing (DLP) are the most promising approaches for routine creation of MD due to their high resolution. These polymerization technologies involve a photosensitive resin cured by a light source to produce solid layer by layer objects [25].

In the μ -TAS context, the development of coupling between microfluidics, electrokinetic separations and EC is a field of research that still faces many challenges to make devices suitable for high throughput microfabrication and routine analytical applications. Therefore, the objective of this project is to develop devices, methodologies and technologies to overcome all these issues and to facilitate the fabrication process. In order to accomplish this goal, we selected the 3D printing technology to guide us in our purpose. The use of AM for assembling microfluidic systems can provide several possibilities to simplify the fabrication process and overcome the classical issues mentioned before. In this context, we aimed at developing strategies to attend the requirements to manufacture microfluidic devices with electrode channels to overcome the alignment necessity of electrodes for EC detection in electrochemical microfluidic devices (EMD) and MCE systems, with a design that would avoid and interference between the two electric fields required for the separation and detection steps

In the Chapter I, we address the state of the art of the Micro – Total Analysis System, electrophoretic separations, 3D printing and electrochemical detection fields. In addition, the readers will find the recent strategies of the last two decades employed to integrate electrodes of various shapes, compositions and materials in different microchip formats and materials with a critical comparison of their microfabrication process and their inherent

advantages and limitations for MCE. At the same time, this is a comprehensive chapter which will highlight the new approaches to deal with classical issues provided by MCE-EC systems, such as:

- How the compatibility of the electrode nature with the substrate of the microfluidic device will narrow the options of materials and impact the coupling of the techniques.
- How the three major electrode configurations for amperometric detection (in-, end-, and off- channel electrode alignment) will lead the fabrication process.

Finally, the main applications will also be described, and analytical features promoted by MCE-EC systems will be precisely discussed in the application subsection. The sections I.3, I.4, and 1.5 part concerning electrode integration in MCE devices and applications were published as a review article "Microchip electrophoresis and electrochemical detection: A review on a growing synergistic implementation" published in 2021 [26].

Chapter II describes a new development for efficient integration of electrodes for contactless conductivity detection in a MCE. For this purpose, a DLP based 3D-printed MCE-C⁴D was designed and fabricated using a custom resin and 3D printer adequate to assemble microchannels (Figure 1.a). The proposed system contains spiral channels filled with melted gallium to perform as electrodes. A classical tubular design of electrodes to perform C⁴D was tried, however, for this electrode design, it was laborious to fill the channels with liquids in a homogeneous way due to trapped air, thus, we created the spiral design to facilitate the integration process. The spiral electrodes were characterized using K⁺, Na⁺ and Li⁺, achieving a correlation coefficient of 0.995, 0.998, and 0.999, respectively. In addition, the device was employed to determinate nitrate in biological samples. In this study an artificial saliva sample was used. We evaluated this methodology according to FDA guidelines, and perform studies of repeatability, linearity, detection, limit of detection (LOD = 2 μ mol L⁻¹) and limit of quantification (LOQ = 6 μ mol L⁻¹). The LOD is suitable for the target analyte in saliva since the levels found in saliva of healthy (800 µmol L⁻¹) and ill: for example, patients with periodontal disease can present levels of nitrate around 1500 µmol L⁻¹[27]. To the best of our knowledge, this is the first report on C⁴D detection in a DLP-based 3D-printed microfluidic device. This chapter corresponds to a research article "3D-printed microchip electrophoresis device containing spiral electrodes for integrated capacitively coupled contactless conductivity detection" published in 2022 [28]

Chapter III describes the development of another MCE-EC device, which involved the efficient integration of electrodes for amperometric detection. A SLA based 3D-printed EMD manufactured using a commercial resin and 3D printer using an 3D-print open-channels

approach to assemble microchannel (Figure 1. b). The microchannels were sealed latter by a silicone tape. The proposed system contains triangle-shape electrode channel with a rectangular surface filled with a custom conductive resin to perform as WE. We performed an exhaustive optimization study of the printing conditions using a 3D printer produced to manufacture large pieces. Later, an electrokinetic separation was integrated into the platform accomplishing a 3Dprinted MCE-EC device (Figure 1.c). Electrochemical characterizations of C/ resin electrodes were performed using ferrocene methanol (FcMeOH), hexacyanoferrate(III)/(II) $[Fe(CN)_6]^{3-/4-}$ and hexaammineruthenium(III)/(II) $[Ru(NH_3)_6]^{3+}$ as model electroactive molecules by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and, square wave voltammetry (SWV), respectively. The feasibility of the integrated electrode in EMD and MCE-EC systems has been investigated using $Ru(NH_3)_6$ ³⁺ in phosphate buffer saline solution as proof-of-concept. The usefulness of the EMD was investigated using $[Ru(NH_3)_6]^{3+}$ solution (5 mmol L⁻¹) in a flow rate ranging from 0 (no flow) and 55 µL min⁻¹. The MCE-EC was tested with an end-channel chronoamperometric detection of $[Ru(NH_3)_6]^{3+}$, achieving LOD and LOQ of 4.5 μ mol L⁻¹ and 15 μ mol L⁻¹, respectively. This chapter corresponds to a research article "Stereolithography based 3D-printed microfluidic device with integrated electrochemical detection" published in 2022 [29].

Conclusion and future perspectives communicate a summary of all results and a critical comparation of the microchips developed including fabrication process and performance. Furthermore, early studies of a MCE-EC device fabricated by a commercial resin and DLP based 3D printer without the sealing process were presented. The printing parameters were optimized to fabricate close microfluidic channels. Further work will be done using this new device (Figure 1.d).



Figure 1. 3D-printed devices designed and fabricated into this project. (a) DLP/ SLA based 3D-printed MCE-C⁴D; (b) SLA based 3D-printed EMD; (c) SLA based 3D-printed MCE-EC; (d) DLP based 3D-printed MCE-EC.

The methodologies proposed is committed to be a simple and inexpensive alternative to current methods of microfabrication process, both the emerging process and the classics ones, such as soft lithography, to assemble EMDs, MCE-EC, MCE-C⁴D. The 3D-printed is dedicated

to increase ease of fabrication, which will allow researchers to rapidly prototype new microfluidic devices with integrated electrodes without the necessity of cleanroom facilities or expensive equipment.

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CHAPTER I: STATE OF THE ART

Part of this chapter corresponds to a research article published as a review article "Microchip electrophoresis and electrochemical detection: A review on a growing synergistic implementation" published in 2021.

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Symbols and abbreviations

3D three dimensional	LOD limit of detection
AE auxiliary electrode	LOQ limit of quantification
AM additive manufacturing	LSV linear sweep voltammetry
BDD boron doped diamond	MCE microchip electrophoresis
BGE background electrolyte	MD microdialysis
C ⁴ D Capacitively coupled	MES 2-(N-morpholino)ethanesulfonic
contactless conductivity detection	acid
CA catechol	MW multi-walled
CB-NPs carbon black nanoparticle	NPS 2-nitrophenyl phenyl sulfide
CCD contactless conductivity	ORMOCERs organically modified
detector	ceramic polymers
CE capillary electrophoresis	PC polycarbonate
CF carbon fiber	PDMS polydimethyl-siloxane
CFNE carbon fiber nanoelectrodes	PEGDA poly(ethylene glycol)
	diacrylate
CI carbon ink	PMMA polymethylmethacrylate
CNT carbon nanotube electrodes	POC point-of-care
CP carbon paste	PPF pyrolyzed photoresist film
CV cyclic voltammetry	PVAc poly(vinylacetate)
CySNO S-nitrosocysteine	RE reference electrode

CZE capillary zone electrophoresis	RMS root mean square
DA dopamine	RNS reactive nitrogen species
DLP digital light processing	ROS reactive oxygen species
DPV differential pulse	RSD relative standard deviation
voltammetry	
EC electrochemical	RSD relative standard deviation
EDS energy dispersive X-ray	RSNO S-nitrosothiols
spectra	
EOF electroosmotic flow	S/N signal-to-noise
FDA Food and Drug	SEM scanning electron microscopy
Administration	
FDM fused deposition modelling	SLA stereolithography
FE-SEM field emission scanning	SW single-walled
electron microscope	
GC glass carbon	Tg glass transition temperature
GO graphene oxide	WE working electrode
GSNO S-nitrosoglutathione	SEM scanning electron microscopy
HTGC heat-treated glassy carbon	SLA stereolithography
GSNO S-nitrosoglutathione	
HTGC heat-treated glassy carbon	
LC liquid chromatography	

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I.1. Micro – Total Analysis System

The constant monitoring of chemical changes, usually the concentration of chemical species, is of outstanding importance as for example in life sciences, chemical production, environmental analysis, and the industry. Commonly, the chemical target compound must be identified in sample matrices containing a significant number of other compounds, including many that probably act as interfering species. Strategies to specifically distinguish and quantify a specific analyte in this complex matrix have been developed over the years. Those based on the use of chemical sensors attempt to maximize the analysis selectivity but may lake specificity. On the other hand, approaches incorporating separation procedures focus on separation efficiency, to increase the specificity and minimize the selectivity requirements placed on the detection step [1].

The two methodologies presented above, chemical sensors and separation procedures, may be used as 'stand-alone systems', that is, an apparatus, standing in a lab, operated by a qualified technician. For monitoring purposes, the instrument needs to be fully automated, from sampling till data evaluation. This concept is the so called 'Total Analysis System (TAS)', which periodically converts chemical information into electronic information. Sampling, sample introduction, chromatographic and electrophoretic separations along with any necessary chemical modification before or after, and finally detection are automatically performed in a flowing stream, so as to avoid any contamination from external manipulation, and allow for repeatable 'real time' monitoring for many applications, such as rapidly changing industrial sample compositions (river water, chemical reaction mixtures) [2].

The main drawbacks of TAS, especially for liquid phase samples, include slow introduction of the sample in the analysis system, poor separation speed in the case of liquid chromatography or electrophoresis, and a considerable consumption of reagents and solvents in the case of chromatography. They can be addressed by miniaturization of the analytical equipment so as to minimize the transport distance between sampling and detection point, allowing to increase separation speed and lower bandbroadening, with smaller analytical channels inner diameter and length. The miniaturization of TAS has been named miniaturized total analysis system or μ -TAS. Some comparison with an ideal chemical sensor and with a TAS suggests that the μ -TAS is a hybrid combining the strong points of a TAS with the size of a sensor [1].

The early efforts in the field of micromachining and micro system technology for applications in chemistry have been restricted to valves, pumps, and sensors. The extraordinary

work by Steve Terry *et al.* on an integration of a gas chromatograph and a thermal conductivity detector into an entire silicon wafer by photolithographic patterning and wet etching processes did not obtain the necessary attention from scientific community at the time of its publication in 1979 [3]. However, it has encouraged renewed research in the integrated micro-analysis system field a decade later.

The μ -TAS concept, also known as "Lab-on-a-Chip" turns more popular with Manz *et al.* [4], introduced at Transducers '89. μ -TAS seeks to integrate distinct operations of chemical and biochemical analysis, such as sample introduction, reagent mixing, separation and detection on a single microdevice. This miniaturized system had a tremendous impact on different research fields, for instance in analytical chemistry, as they deal with very small sample and reagent volumes, high resolution and sensitivity of separations, low fabrication cost, small footprints and decrease of the physical size of the systems for portability purpose [5].

I.1.1. Integrating multiple analytical components into microchip electrophoresis

A wide range of technologies is available to achieve analytical microfluidic devices. Since the goal is to obtain the highest level of integration in the final device, the selection of a suitable technology for a specific application is not trivial. Occasionally, hybrid approaches need to be developed, involving multiple interconnected devices using different technologies. Figure I.1.1 displays several components that can be integrated for analytical purposes.



Figure I.1 1. Integration of analytical microfluidic systems [6].

The integration process is composed by sampling and sample pre-treatment (such as dilution, filtration, migration, pre-concentration) steps. In a second step, separation can be implemented by chromatography or electrophoresis in a microscale size. The third step includes mixing and additional processes for instance chemical reactions (such derivatization) and continuous monitoring (online system) of the products formed. Finally, the full integration assumes the incorporation of a detection system with an effective automatic data analysis and treatment system supported in suitable informatics tools, such as chemometrics and statistic methods.

Herein, we describe the main components presented in an electrophoretic separation platform: the sample pre-treatment (static methods and dynamic methods); separation method and the possibility to customize this step (surface chemistry modification, BGE choice, voltage applied) depending on the necessity; injection modes (hydrodynamic and electrokinetic modes) and advantages and disadvantages of each one; and strategies for detection within microfluidic channels, for instance optical detections and mass spectrometry (both are briefly commented on this section), and electrochemical detections (extensively commented on this section).

I.1.1.a. Sample pre-treatment using microfluidic systems

In order to increase the analyte concentration (pre-concentration), avoid contaminants and time-consuming steps, on-chip sample pre-treatment methods based on different working principles for improving sensitivity and/ or selectivity have been developed and implemented in microchips. These methods are classified into two categories: static methods that include filtration and extraction, and dynamic mechanisms that play on different analyte velocities to achieve selective enrichment by using electrophoretic methods.

Static methods usually require physical barriers to retain the analytes in a specific area, unlikely dynamic methods, which are based on the effect of electrokinetic phenomena. Conversely, dynamic methods and specifically electrophoretic-driven methods demand careful consideration of the electrophoretic behaviour of both the analyte, the sample matrix, and the background electrolyte (BGE). Among the electrophoretically driven pre-treatment methods performed in classical capillary electrophoresis, we can mention isotachophoresis (ITP), field amplified sample stacking (FASS) techniques, large volume sample stacking, sweeping, partial filling technique [7–9]. We will describe below some of the possible electrophoretic methods for sample pre-treatment within the channel.

ITP is based on the principle of moving boundary electrophoresis, thanks to a discontinuous buffer system, i.e. the introduction of the sample in-between a leading and a

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terminating electrolyte. The process is performed in the channel of the microfluidic device, in a similar way as in the classical approach. Briefly, the leading and terminating electrolytes contain ions with effective mobilities, higher and lower, respectively, than those of the analytes. Once a potential is applied, it leads to different electric fields in each region, with a high and low electric field, in the terminating and leading electrolytes, respectively. Due to this difference in electric field in the three regions, the individual analyte ions will be partitioned in the channel, after a certain time, in the order of their decreasing mobility, allowing for both compounds separation in different zones and their pre-concentration [10,11]. The ITP preconcentration stage can then be connected with a separation step in a "stand-alone ITP" configuration or combined with capillary zone electrophoresis, they can be found either in classical or microchip format [12]. Kluska et al. [13] reported an ITP method for analysis of mercury in surface waters. Mercury was determined after conversion to tetraiodomercurate by addition of KI to the sample before the analysis. Using conductivity detection, a LOD of 30 ng L⁻¹ was reached. Troška et al. [14] reported an ITP method for determination of acetylsalicylic acid, diclofenac, and ibuprofen in pharmaceutical formulation. They used a commercial poly(methyl methacrylate) (PMMA) microchip with integrated contact conductivity sensors. Due to effective sample clean-up during the ITP step, the method allows simple sample preparation and shows good linearity, accuracy, limits of detection > 750 ng mL⁻¹ and efficient separation.

FASS methods is a classical on-line pre-treatment protocol that is easily coupled to electrophoretic separation. FASS only requires the conductivity of the injection zone be lower than that of the BGE. Due to the conductivity effect, the electric field strength in the injection zone is higher than the one in the BGE zone, when a potential is applied along the channel. It then promotes a higher electrophoretic velocity of the analytes in the sample zone than in the BGE zone, leading to their stacking at the boundary between both zones, and to their preconcentration [15]. Fang *et al.* [16] demonstrated a simultaneous stacking and separation of small molecule food dyes, brilliant blue and amaranth, in a paper fluidic channel, and about 100-fold enrichment factor was obtained for each dye within 300 s. Then the authors demonstrated concentration and separation of bovine hemoglobin and cytochrome c.

Aiming the sensitivity improvement, large volume sample stacking (LVSS) can also be adopted. Briefly, large amount of sampl (dissolved in low-conductivity buffer) is loaded into capillary. In this process, the sample matrix should be removed, otherwise, the large volume of sample matrix hinders the analyte stacking, compromising the sensitivity enhancement. The excess of matrix can be removed using the polarity switching mode, generally after stacking the analytes, the polarity is inverted to push out the matrix from the capillary. Then the polarity is changed to normal and stacked analyte migrated towards detection end by the assistance of electroosmotic flow (EOF). Otsuka *et al.* [17]described the application of LVSS to increase the sensitivity (up to 1300-fold) in three chiral separation modes in CE without loss of optical resolution.

I.1.1.b. Electrophoretic separation within a microchip

Microchip electrophoresis (MCE) has been successfully designed and developed, leading to separations with high speed and high efficiency. Indeed, capillary electrophoresis is particularly well suited for miniaturization and automation due to the simplicity of its instrumental [18]. Much of the early work on μ -TAS, in fact, was focused on the development and characterization of MCE including the pioneering contributions of Manz and Harrison [19] and Jacobson and Ramsey [20]. Indeed, MCE represents the next generation of miniaturized devices, for the introduction, pre-treatment and separation of complex sample matrices. The fact is that the electrokinetic separations are easily miniaturized, require a single high voltage sequencer, provide high separation efficiency (see below) and procedural simplicity and flexibility as well as short analysis time [21].

During the electrophoretic process, a microchannel is filled with the BGE (a conductive buffer), a small sample zone is injected, and separation is then achieved by applying an electric field across the channel. Glass and silica are the widely used substrates for microchip electrophoresis-based separations. Generally, BGEs of a pH above 3 are employed in these substrates: the surface of the channel becomes deprotonated owing to the presence of ionized silanol groups, leading to a negative charge of the substrate surface. This negatively charged surface generates the classical electrical double layer: a layer of highly absorbed cations, which is called a fixed layer, is formed due to the attraction to the negatively charged surface. However, the tightly absorbed surface cations can only partially neutralize the negative charge on the channel surface. Therefore, the negatively charged surface can still attract more cations from the buffer solution, forming a diffused layer where the concentration of cations is higher than the concentration of anions. Together with the fixed layer, these two layers constitute the electrical double layer and the centre of the capillary tube is named bulk solution, where the concentration of cations and anions are about the same. When the voltage is applied across the capillary, the cations in the diffuse layer will be attracted to the negatively charged cathode, dragging the bulk solutions along with them. This phenomenon is called electroosmotic flow (EOF) (Figure I.1.2.A). The value of the EOF depends on the nature of the microsystem material, but also on the nature, the pH, and the ionic strength of the BGE.



Figure I.1 2. (a) Scheme of the double layer within a capillary tube. Although the net charge inside the channel is zero, the distribution of charge is not. The walls of the channel have an excess of negative charge, which decreases across the fixed layer and the diffuse layer, reaching a value of zero in bulk solution. (B) Flat flow profile versus parabolic profile.

One main advantage of CE is the generation of the EOF, which induces a flat flow profile, contrary to flows generated under pressure, as for chromatographic methods, which lead to a parabolic profile, therefore lowering band broadening, improving separation efficiency and resolution. (Figure I.2.B). In the last years, other materials for microchip fabrication were designed and developed (see section I.1.2). So as to generate powerful MCE, they should generate an EOF, that is why the surface nature and modification are important parameters [22]

The separation is effective and efficient if the sample is introduced in the microchannel before voltage application, with a good repeatability and with an injection volume compatible with CE separations, i.e. an injection volume equivalent to around 5% (injection plug size must be smaller than zone controlled by diffusion) of the total volume of the channel [15]. Therefore, different injection protocols were developed.

I.1.1.c. Injection modes

Similar to conventional CE injection modes, the main methods of introducing a sample into a microchannel have been described by J. M. Karlinley [23]. He grouped them into two distinct classes: injection by pressure application (or hydrodynamic injection), which can

be carried out using a syringe pump; and injection by application of voltage (or electrokinetic injection), which is conducted by applying voltages to solution reservoirs so that fluid flows towards the separation channel according to the applied electric fields.

• Hydrodynamic injection mode

Several modes of sample introduction involve a pressure difference exerted on the ends of the microchannels. It is possible to use different pressures inside the chip reservoirs to generate a flow, to create an overpressure at the entrance or a depletion at the exit to push or pull the sample into the microchannels. Generally, this mode of injection requires the use of advanced technical procedures (notably pump systems) which are not so easy to integrate in a microchip, make hydrodynamic injection less repeatable and flexible than an electrokinetic injection mode.

• Electrokinetic injection mode

The electrokinetic injection of a sample is affected by the electroosmotic mobility of the BGE and the electrophoretic mobility of the analyte, i.e. the apparent mobility of the analyte: the higher the apparent velocity, the higher the analyte quantity injected, leading to enhanced concentration. This methodology also allows in some cases to diminish the introduction of interfering species of the sample matrix The most common design used for sample introduction into the separation channel is achieved using an electrokinetically gated injection as displayed in Figure I.1.3. For sampling, the potential is applied in sample reservoir (S) with sample waste reservoir (SW) grounded and applied in buffer reservoir (B) with buffer waste reservoir (BW) grounded (Figure I.1.3.1). The electric field stopped to be applied in (B) (floating) to encourage the sample diffusion (Figure I.1.3.2). The electric field is applied again in (B) resulting a well-defined sample zone (Figure I.1.3.3). Furthermore, the volume of sample injected relies on the injection time, applied voltages, and buffer ionic strength; thus, slight changes in conditions result in differing amounts of sample introduced into the channel.



Figure I.1 3. Scheme of the "gated injection" mode in the MCE device. In (1), the electric potential is applied in sample reservoir (S) with sample waste reservoir (SW) grounded for sampling and applied in buffer reservoir (B) with buffer waste reservoir (BW) grounded. In (2), the electric potential field stopped to be applied in B to encourage the sample diffusion. In (3), the electric field is applied again in B resulting a well-defined sample zone [24].

A second injection method also widely used is the floating mode (Figure I.1.4). Briefly, the sample (S) and sample waste reservoir (SW) are positioned at the extremity of the short perpendicular channel (the injection channel), the main channel being the separation channel. First a potential is applied between S and SW so as to fill the short channel, with several volumes of the injected sample, allowing the slowest ions to be present in a representative concentration. The injected volume therefore depends directly on the geometry of the system, precisely on the dimensions of the intersection between injection channel and separation channel. During the first loading step into the injection channel, the sample can partly go through the separation channel, due to the diffusion of the analytes during the loading, which leads a dispersion of the flow profile and a loss of efficiency, compromising the injection reproducibility.

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Figure I.1 4. Scheme of the floating injection mode in the MCE device. In (1), the electric injection potential is applied in the sample reservoir (S), and the sample goes toward the sample waste reservoir (SW). In (2), the injection channel was filled with the sample. In (3), the potential applied in the injection channel is switched off and immediately switched on in the separation channel; the electric field is applied in buffer reservoir (B) resulting a sample zone well defined goes toward the detection zone.

I.1.1.d. Detection

As concerning the detection step, different techniques can be implemented. Fluorescence, which is classically provided for the microsystems, has several drawbacks, such as native fluorescence of the device material and matrix, and the general non fluorescence of the analyte, which then needs a derivatization step with a fluorophore [25,26]. Colorimetry is mainly used when a positive/negative diagnostic is required, as its sensitivity is lower. Mass spectrometry, still difficult to couple to a microfluidic system, can help for molecular identification and quantitation. Surface Enhanced Raman Spectroscopy is a highly sensitive detection mode but still has a poor reproducibility [27]. Infrared is very poorly employed for fluidic systems . Indeed, main IR processes are on solid samples. When liquid samples can by employed (with ATR-FTIR) .the absorption from the solvent can drastically saturate the IR signal [28].

Electrochemical (EC) detection has shown to be a good alternative with both conventional capillary electrophoresis (CE) and MCE, with still a challenge to overcome coming from possible interference between the two necessary electric fields for separation and detection (see below). EC detection was first implemented in classical capillary electrophoresis, the separation and detection electrodes were directly integrated into silicon and glass substrates to couple in CE capillaries and MCE platforms [29–31]. Microelectrodes can be fabricated directly in the channel platform or externally to it, and portable, isolated potentiostats are commercially available. The main electrochemical detection modes developed with CE are conductimetry and amperometry.

When it comes to use amperometry and voltammetry for selective and sensitive monitoring of redox active target analytes without any derivatization, the electric field produced inside the channel for the separation purpose can be of some orders of magnitude greater than the electrochemical one involved in the detection. Thus, there is a need to develop smart technical approaches to overcome this challenge in interfacing amperometric/voltammetric detection to CE. Walling and Ewing were the first authors to apply amperometric detection to CE [32]. They created an electrically conductive porous glass joint in the column near by the end of the capillary and the detection was accomplished at the very end of capillary, downstream the conductive joint. By this way, the constraints of interfacing high voltage electric fields are removed. In other words, conductive joint decouples the electrochemical detector from the separation voltage. The authors used a single carbon fiber (10 μ m) as working electrode, which was placed at the end of the CE capillary to detect catechol and several catecholamines, with a 5 pmol /injection as limit of detection (LOD).

Conductivity measurements allow monitoring any analyte irrespective of whether it is electroactive or not. It gains its acceptance from its universality and is particularly useful for non-electroactive species or not readily detectable by UV absorption. The main requirement is that the migrating analyte has a different conductivity from that of the carrier electrolyte. Mikkers *et al.* [33] reported the first work using the conductivity measurement as detection mode in CE. The authors separated 16 compounds, including organic and inorganic ions, using a narrow-bore tubes with 200 µm of internal diameter (i.d.).

Coupling microfluidics and electrochemical systems empowers all common advantages related to miniaturization, portability, low sample and reagent consumption and high throughput. The alliance between microfluidics and electrochemistry constitutes now an attractive approach since it offers a synergistic effect over each inherent feature enhancing their potential and offering a unique technology [34,35]. The electrochemical techniques are indeed well suited for miniaturization without losses in their analytical performances. Even more some of their analytical performances may be enhanced as the processes are confined within the microfluidic structure [36–39]. EC detection with integrated electrodes was first used in MCE systems by Mathies *et al.* [40]. The authors published a patent which includes a microfabricated electrophoretic channel with integrated amperometric detection. The device consists of a thin Pt working electrodes (10 μ m wide) placed just beyond the end of the separation channel. The channel was photolithographically defined to minimize the effect of the separation voltage on electrochemical detection. In other words, the distance between the outlet of the separation channel and the working electrode ranged from ≈ 50 to 1000 μ m. This electrode configuration decouples the detector from the separation voltage, eliminates the problem of electrolysis (gas bubbles) in the separation channel (see more details in section 3), and minimizes the background noise as well as the WE owing to separation field. It should be noted that in this alignment, target species may diffuse into the waste reservoir as they come out of the channel before being detected, which may lead to band broadening, asymmetric peak shapes, resolution loss, and reduced signal intensity because of the dilution effect. Pt wires were included in the reservoirs to provide electrical connections to the separation and injection channels in glass substrate. The authors reported then on the first study of a fully integrated separation and amperometric detection device [41] for the separation of dopamine, epinephrine, and catechol in less than 100 s and achieved LOD's of 3.7, 6.5, and 12 μ M, respectively.

The first study on conductivity detection in microfluidic devices after the introduction of the μ TAS concept was reported in 1998. Both Baldock *et al.* [42] and Fielden *et al.* [43] reported a PDMS device with integrated electrodes to perform isotachophoresis to separate three anionic dyes (amaranth, bromophenol blue and fluorescein) and transition metal ions (Mn, Co and Ni) respectively (LODs?). These microchips were sealed on top of a printed circuit board containing two parallel gold electrodes (100 μ m width) as sensing electrodes. Later on, a significant contribution to conductivity detection for CE was made by Zemann *et al.* [44] and da Silva and do Lago [45] groups in 1998 by the introduction of the capacitively coupled contactless conductivity detector (C⁴D) in CZE. In this detection mode, the electrodes are not in direct contact with the solution. In 2001, Guijt *et al.* reported the first article on contactless conductivity detector for glass microchip electrophoresis [46]. The titanium detection electrodes (30 μ m width) were covered with silicon nitrite layer to perform contactless detection. Separation of two short peptides was achieved with a 7 μ M LOD for each peptide.

EC detection is however poorly developed in MCE as significant electrical interferences may occur. All the pioneering reported studies mentioned before had to deal with problems that complicate the development of coupling between microfluidics, electrokinetic separations, and EC. Thus, this field of research still faces many challenges to make these devices suitable for high throughput microfabrication and routine analytical applications. Over the years, several strategies are employed to integrate electrodes of various shapes,

compositions and materials in different microchip formats and materials aiming platforms with a reliable analytical performance. To develop MCE-EC systems, the authors have been creating new approaches to deal with classical issues provided by MCE-EC systems, for instance: how the compatibility of the electrode nature with the substrate of the microfluidic device will narrow the options of materials and impact the coupling of the techniques; how the three major electrode configurations for amperometric detection (in-, end-, and off- channel electrode alignment) will lead the fabrication process.

I.1.2. Emergent materials used for fabricating microfluidic systems

Microfluidic devices were historically manufactured in glass [47] or silicon [48], the use of these materials steaming from their compatibility with photolithographic protocols and their similarity to fused silica. Currently, these devices are being performed in a diversity of materials and formats, including soft polymers such as polydimethylsiloxane (PDMS) [49], thermoplastic polymers [50], thermoset polymers [51], hybrid devices [52], photopolymers [53] and paper [54].

The selection of the substrate materials is evaluated through relevant physicochemical properties, for instance UV–vis absorption properties, autofluorescence levels, glass transition temperature (T_g), hydrophobicity / hydrophilicity and surface charge density allowing for electroosmotic flow (EOF). Due to the use of solvents or process or bonding the device, the substrates can undergo surface property modifications, deformation reduction, and creation of specific functional groups. Furthermore, substrate materials should be able to undergo surface modification so as to improve electrophoretic separation efficiency by reducing analyte-wall interactions and increase EOF control.

Glass substrate has therefore found widespread use in microfluidic devices owing to its surface stability, optical transparency, chemical resistance, electrical insulation, and biological compatibility. It was the first substrate to be used to carry electrophoretic separations on a chip as described by Manz *et al.* [55] and the first material for MCE platform with integrated electrodes as reported by Woolley and co-workers [41]. In this later case, Pt electrodes were photolithographically patterned onto glass in a 2-electrode configuration using end-channel alignment. However, glass microfluidic fabrication procedures are commonly complex, requiring specific clean rooms and high temperature to seal the glass device.

In order to meet the academic community demand, for inexpensive and easily microfabricated microfluidic devices, Duffy *et al.* [56] reported an easy procedure to fabricate microfluidic systems in a PDMS material. PDMS became then a widely used elastomer in early-

stage microfluidics research due to its properties, such as optical transparency, ease of microfabrication (soft-lithography), biocompatibility and possible exchange of gases with the environment. Liu *et al.* [57] were the first to design and microfabricate PDMS device with integrated electrodes. The authors illustrated a PDMS membrane that was hermetically adhered to the glass wafer containing Pt electrodes, accomplishing an integrated PDMS-glass microchip. Soft lithography is nowadays the most common manufacturing process, as it is well established in the literature [58,59]. Recently, Coltro group [60] proposed a new process for cost-effective fabrication of PDMS MCE devices using poly(vinyl acetate) (PVAc) emulsion as photoresist master. The proof of concept was demonstrated with the electrophoretic separation and C⁴D detection of inorganic cations using copper strip as sensing electrodes, with LODs ranged from 120 to 300 μ mol L⁻¹.

The use of polymeric materials shows a great potential to fabricate disposable systems in a large-scale high-quality production, since their introduction into industrial use in the 1930s. For this reason, much of the microfluidics research targeting commercial applications have dedicated their efforts to create devices using rigid polymers, as reported in the reviews by Genetick et *al.* [61] and Tsao[51]. In particular, poly(methyl methacrylate) (PMMA) is one of the most privileged materials as it is well known from the generation of micro optical devices as a low-cost material compared to silicon or glass substrate, showing a good hydrolytic stability compared to the limited stability of PDMS chips. Its main constraint is its low stability at high temperatures and high pH-values.

Hybrid microchips are achieved merging two or more materials into one device, this approach providing properties of each substrate. For example, devices were produced with PDMS and glass. PDMS is widely used for μ CE due to its low cost and ease of manufacture. However, PDMS devices have several disadvantages, including low EOF, analyte adsorption, and inconsistent analyte migration times. Glass/PDMS hybrid devices can be an effective compromise to combine the consistencies of glass with the ease of fabrication of PDMS devices. Erro and Ortiz published a work comparing the performance of a hybrid microchip (PDMS/ glass) and other all-PDMS devices. The integration of the glass material in the device allows the use of higher separation potential than in PDMS microchips, this way the use of glass improves the microchip performance. Furthermore, in contrast to PDMS devices, those made of glass exhibit a strong, reproducible EOF and, therefore, more reproducible migration times [52].

Microfluidic paper-based analytical device (µPAD) is a concept developed by Whitesides [62]. µPADs began as a simple idea with an ambitious goal: replacing substrates

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such as plastic and glass to manufacture cheap microfluidic devices. Paper brings its own inherent advantages, for instance low cost, operational simplicity, sustainable, recyclable and ease of acquisition. The development of electrochemical paper-based devices has received considerable attention since Dungchai's *et al.* publication in 2009. The authors demonstrated a paper-based device integrating screen-printed carbon based electrodes to determinate glucose, lactate and uric acid in biological samples, with LODs 0,21, 0.36 and 1.38 mmol L⁻¹, respectively [63].

Thermoplastics filaments [64] and photosensitive resin [65] have been explored by several groups as substrate for the construction of 3D microchip. Direct three-dimensional printing technology is increasingly being recognized due to its capability to fabricate prototypes in simple and automated steps offering good precision to define microchannels or microstructures with benefits such as low production cost, high printing resolution, diversity of materials such as conductive and biocompatible materials, and customization in the construction of devices in relation to 2D devices [66,67]. In addition, a major advantage of 3D-printing is that it facilitates the alignment of the integrated electrodes in microdevice systems, which is critical for the design, turning robust the manufacturing procedure of the device [68].

I.2. Additive manufacturing and 3D printing photopolymerization technologies

Industry 4.0 is a revolutionary achievement in the process of human development [69]. Among the various segments developed in this industrial revolution, we highlight additive manufacturing (AM), a technique for manufacturing objects where a material is selectively deposited in a region of space for its creation. In this way, it allows generating less waste products in the manufacturing process compared to other conventional processing techniques, such as bulk-forming or subtractive processes, which have also higher costs for acquisition of material and mold, [70]. The figure I.2.1 displays a scheme of those manufacturing processes [71].



Figure I.2. 1. Scheme diagram of the three manufacturing processes. (A) Bulk-forming process; (B) Subtractive process; (C) Additive process [71]. Reprint with permission from Springer.

Additive manufacturing (AM), also well known as solid free-form fabrication or rapid prototyping, is a transformation method of computerized models into three-dimensional objects. Since the 1980's, efforts are made to build up AM techniques. In 1983 Charles Hull coined the term stereolithography, that year he created the first-ever 3D printed part. In 1984, the term was patented for "Apparatus for production of three-dimensional objects by stereolithography" [72]. Briefly, the patent demonstrates the building process using a concentrated beam of ultraviolet light, which is focused onto the surface of a vat filled with liquid photopolymer. The light beam, moving under computer control, draws each layer of the object onto the surface of the liquid. Wherever the beam strikes the surface, the photopolymer polymerizes/crosslinks, and changes to a solid. In 1986, Hull co-founded the 3D Systems to exploit the solid imaging technology. By 1987, 3D systems produced the first 3D printer, the SLA-1. This technology has become widely used in rapid prototyping and direct fabrication, particularly after the fall of patent rights in 2014.

AM includes a range of different technologies that are used to build layer-by-layer 3D objects. In this chapter, we focus on the printing technologies developed for polymeric materials, which can be organized by the different shapes of material they are able to process (Figure I.2.2) [73]. On one hand, some manufacturing technologies employ powder materials and create a piece by depositing a binder on the layers of powder (binder jetting) or by selectively sintering or melting them (powder bed fusion). Some other technologies employ solid materials that are melted to be deposited to form each layer to create the object (material extrusion). This one is widely used due to its low cost and facility to manipulate. Finally, some technologies utilize a liquid material which is locally deposited and cured (material jetting) or

cured in a selectively way from a liquid vat (vat photopolymerization, or VP). Each technology can reach different levels of accuracy and complexity for the piece assembled. Among the other AM processes, the group of VP techniques is the most capable to reach the highest standards in terms of complexity and resolution. In other words, between all the AM available that use polymeric materials, VP printing is the most appropriate technology to fabricate microfluidic platforms. SLA and DLP are the two different approaches of VP technologies [74].



Figure I.2. 2. AM technologies for polymers, according to ASTM F2792 [73].

I.2.1. Main components and basic operation of stereolithography (SLA) and Digital lighting processing (DLP) based 3D printing

The VP technologies employ a liquid to solidify by radiation exposition, which activates the polymerization reaction. The SLA apparatus (Figure I.2.3.A) is equipped with a laser source mostly emitting radiation in the UV range that is directed by means of mirrors to a tank filled with a liquid photocurable resin. The laser beam is controlled in an XY axes (horizontal movement), and the build platform in the Z axis (vertical movement). In the printing process, the build platform is dipped into the resin vat, whose bottom surface is made of a transparent and non-adhering material (for instance Teflon). The light is projected from underneath, starting the photopolymerization of the resin between the movable platform and the bottom of the vat. Once curing the first layer, the platform moves up, and photopolymerization continues with a new layer, which is fabricated below the previous one. This bottom-up printing orientation protects the cured layers from the atmosphere, so the inhibition of the photopolymerization reaction is prevented. In addition, smaller layer techniques are achieved due to the elevator accurately, promoting higher vertical resolution and surface quality [75].



Figure I.2. 3. 3D printing SLA-based (A) and DLP-based (B) including the main components indicated.

DLP is a technique similar to SLA. The difference relies on the radiation source, i.e. DLP uses a digital light projector that simultaneous (or several flashes for large parts) flashes a single picture of every layer (Figure I.2.3.B). The picture on each layer is a digital screen, and thus a layer is made up of individual pixels within a thin layer. In contrast with SLA, DLP can accomplish faster print times, owing to a whole layer that is revealed at once instead of using a laser to trace the whole image. The image is projected over the resin with a Digital Micromirror Device (DMD) on the Light Emitting Diode (LED) screen or a UV light source (lamp). A DMD is an arrangement of small dots projected on the surface and generates de light pattern [76].

In photochemistry-based AM systems, printable materials are mostly comprised of monomers, oligomers, and photoinitiator. The VP technologies employ photopolymers, also known as UV resins as build materials, usually acrylic and epoxy resins [77]. On one hand, the acrylic resins go through photopolymerization by a radical mechanism. When exposed to UV light, the photoinitiator absorbs light and generates free radicals, which combine and react with (meth)acrylic monomers by opening carbon-carbon bond. Subsequently, reactive monomers connect to form large molecules, the polymer chain grows until two chains combine and terminate the reaction (Figure I.2.4). Most common (meth)acrylate monomer/oligomers used in 3D printing are PEGDA, UDMA, triethylene glycol dimethacrylate (TEGDMA), bisphenol A-glycidyl methacrylate (Bis-GMA), trimethylolpropane triacrylate (TTA) and bisphenol A ethoxylate diacrylate (BisEDA). On the other hand, epoxy monomers are polymerized by an ionic mechanism. Briefly, the cationic photoinitiators generate acids that react with a bond in

epoxy monomers and induce polymerization. Some commercial epoxy monomers widely used in SLA/ DLP are 3,4-epoxycyclohexylmethyl-3,4-epoxycyclohexane carboxylate (ECC), bisphenol A diglycidyl ether (DGEBA), and epoxides of aliphatic alcohols like trimethyloyl propane. Those liquid monomers/oligomers are transformed into a solid polymer within seconds or a few minutes. The polymerization is initiated by active species (e.g., free radicals or cations), formed when a photoinitiator decompose in the presence of UV (200–400 nm), visible (400–700 nm) and, not often, IR (700–1000 nm) light [78].



Figure I.2. 4. PEGDA cross-linking reaction. Radical mechanism under ultraviolet (UV) exposure and using diphenyl (2,4,6-trimethylbenzoyl)-phosphine oxide (TPO) as photoinitiator. Reprint with permission from MDPI.

I.2.1.a. The complete printing process

The AM process begins with designing a model with any professional computeraided design (CAD) software. Once a CAD model is created it should be saved in the standard triangulation language (STL) format through the CAD software. The STL file converts the surface in the CAD model to a network of triangles. The number of triangles controls the accuracy of the curved surfaces. The STL file is converted into slices and finally into G-code file. The file must be in right size, build orientation and properly placed in the build platform and then transferred to the AM machine to print layer-by-layer into 3D forms (Figure I.2.5) [79].



Figure I.2. 5. Pre-printing process using an AM machine.

At the beginning of printing the first layer, the build platform is driven down to form a gap with the bottom of the resin tank. The position of the platform must be properly placed where the first layer may be separated from the resin tank and stay on the platform firmly after being pulled off the resin tank. If the separation problem turns out, the build platform is separated from the resin tank manually and the cured material is clean up. After the problem is solved, the printing process is repeated. The first layer is monitored to guarantee that the cured layer stays on the platform after being pulled off the tank bottom. Once the first layer is successfully printed, the platform is driven down and the remaining layers are printed automatically [80]. The flow chart of the printing process is shown in Figure I.2.6.



Figure I.2. 6 The classically flow chart of the SLA/ DLP printing process [80]. Reprint with permission from Elsevier.

After the printing process is finished, the printed objects are removed from the platform and technical processing procedures are used to clean and refine the printed objects in a step named post-processing. The as-built models are rinsed in a washing solution. Firstly, we use ethanol in order to wash the superficial layer of uncured resin. Secondly, we employ isopropyl alcohol (IPA) with ultrasonic bath, to get ride of the uncured resin remained inside the channels of the printed models. Other processes can include support removal, grinding, sealing, gluing, polishing, painting, varnishing, and coating, depending on the printed object and its application. To enhance the mechanical properties, the printed objects are exposed to UV light. It is remarkable that the rising time, temperature, and duration of the curing process

have an important role in establishing the mechanical properties of the finished parts. A typical post-processing with DLP/ SLA technology is displayed in Figure I.2.7.



Figure I.2. 7. Post-printing process using VP technologies.

I.2.1.b. Main optimization parameters

During the printing process using VP technologies, several printing parameters can significantly influence the printing quality, for instance the irradiating parameters (exposure dose), operation setting (curing time of each layer, orientation, layer thickness), and the resin chemical composition [75]. In general, exposure dose (D) can be adjusted through two parameters: projector irradiance (E_e) and exposure time (Δt):

$D = E_e \times \Delta t$

Considering SLA printers, the height of the layer ranges from 12 to 150 µm. In the operation setting, the smaller the layer thickness, the higher the resolution of the printed object (z-resolution), however, smaller layer thicknesses increase the printing time. The print speed of standard SLA printers is in range of 10-20 mm/ h. The accuracy of SLA printing is also related to the diameter of the laser beam at the curing point, namely, the spot size (XY resolution). This way, the orientation of the piece into the build platform (using the supports) leads the main parameter (layer thickness or laser) that will provide a greater printing accuracy. DLP technology differs from SLA only in light source parameter. Since the DLP utilizes a digital light projector (XY plane resolution), each layer appears pixelated and the accuracy of the printed object is given by the layer thickness and the pixel matrix of the projector. In contrast to the flexibility that SLA offers, due to the free movement of the laser beam, DLP is limited to the network of pixels of the digital screen, which is illuminated only one at a time for each layer. This way, the DLP provides less accurate, similar to Minecraft-like parts in comparison with SLA. A parallel of the production accuracies is illustrated (Figure I.2.8), where a scan track in SLA is approximated with voxels (pixels) in DLP. Namely, it can be said that SLA technology can deliver higher accuracy printing curved surfaces than DLP and at a cost of production time [81].



Figure I.2. 8 Accuracy difference between SLA and DLP based on their footprint on building platform. Comparison between objects manufactured using SLA-based and DLP-based 3D printer [81,82].

For a better comprehension of SLA/ DLP resolution, it is important to know the accuracy achieved when printing features on top of the object versus printing on cantilever-like structures (for instance, closed channels). The difficulty of printing closed microfluidic channels is displayed in figure I.2.9, demonstrating the different steps for printing a cantilever structure [83].



Figure I.2. 9 Several phases of printing a cantilever structure. (A) The printing platform goes to the end of the vat, where the base of the device will be created. (B) The DLP projector exposes all the base. (C) Only the layers of channel walls are exposed by the DLP projector. (D) Once the channel is created, all the devices must be exposed to polymerize the sealing part of the microchannel. (E) The light exposed in (D) crossed through the channel and partially polymerized the channel cavity [83]. Reprint with permission from MDPI.

Printing cantilever structures become more challenging. When printing the roof of a cantilever structure, light tends to penetrate through the resin into the previous layers, affecting the Z-resolution. To prevent this kind of behaviour, the strategy is to complement the resins composition with photo absorbers in order to control the penetration depth of the incident light. Inert dyes (e.g., 2-nitrophenyl phenyl sulfide) can provide specific colours, and inhibitors aid to prevent the premature polymerization of the acrylate-based resins [84]

To improve the printability, other manipulations can be made in the composition of the resin. Photopolymers used without fillers (pigments, ceramic powders, or fibres) may produce minimum cure depths between 25 and 100 μ m. Components may be incorporate in low concentrations to the photocurable formulation to improve the properties of the resin and/or parameters for printability. The addition of fillers changes the curing behaviour in a complex way, varying on the size, optical property, filler fraction, and shape. Diluents (e.g., dipropylene glycol diacrylate) are employed to reduce the resin viscosity, leading to easy cleaning of the uncured resin inside the complex structures of printed object [84]. Furthermore, the addition of dispersion agents (e.g., trimethylolpropane triacrylate, dipropylene glycol diacrylate) improves the tinctorial strength, decreases the resin viscosity and surface tension and prevent sedimentation of particles, while stabilizers enhance shelf-life stability during resin storage and maintain a stable viscosity. Finally, the addition of fillers improved the mechanical properties of the printed object. Most often, the exact composition of a commercial resin is not publicly disclosed [76].

I.2.2. 3D printed microfluidic devices by SLA/DLP

The combination of VP techniques and microfluidics has emerged as a new field with a lot of potential to impact such important areas life sciences and health care industry. However, the integration between them is still at an early stage of development [85]. In practice, most 3D printers offer a resolution of hundreds of microns which allows the construction of fluidic systems on the millimeter scale with excellent repeatability. It is worthwhile to mention that the 3D printing fabrication can be done without cleanroom facilities, which allied to the low cost of the materials makes 3D printing very attractive for prototype development.

Efforts toward producing real microfluidic devices with channel features smaller than 100 μ m can be found [85–88]. For applications such as MCE, it is of utmost importance to keep the channel dimensions as small as possible, to avoid deleterious effects introduced by Joule heating [65,89]. The Nordin and Woolley groups have pushed the limits of the DLP technology to produce microfluidic devices with channels as small as 18 × 20 μ m in a custom DLP 3D printer [90–92]. They also demonstrated the high-throughput production of printed elements, such as integrated valves, in a single-step production of microdevices [93,94]. Conversely, it is still difficult to combine more than one material by using DLP technology, and more complete MCE devices could be produced by incorporating conductive materials to serve as electrodes for electric field application and for electrochemical detection. In view of the advantages presented by the introduction of conductive parts in microdevices, such as resistive heaters, electrodes for detection and electric field application, and electrical connections, it is desirable to establish protocols for the fabrication of more complex microfluidic devices.

I.3. Electrode materials in microsystems

On one hand, electrodes in micrometric format offer a significant advantage over the classical ones: the diffusion process of analytes at the electrode-liquid interface is different in millimetric and micrometric electrodes and leads to an increase in the signal-to-noise S/N ratio when the electrode size decreases. On the other hand, their integration onto plastic and glass chips can impressively enhance the automation, minimizes contamination for highthroughput screening and point of care (POC) devices and has promise for on-site investigation. However, the integration of electrodes within the microdevice is challenging from the process point of view, as their passivation and clean steps, isolation, geometry, and position within the microsystems constitute major issues.

The electrode material is an important consideration in MCE-EC. Namely the material selection will narrow the number of available substrates that can be used to manufacture the device. Also, the use of different materials can be explored to increase the selectivity and specificity of MCE, since the electrochemical response is highly dependent on the medium, the target analyte and electrode material.

In this section, readers will find electrodes classified into three main categories: bare metal-based electrodes; carbon-based electrodes and chemically modified electrodes. Respective information concerning the manufacturing, integration, characterization, and intrinsic properties of each material will be given.

I.3.1. Bare metal electrodes

The main metal electrodes usually employed in MCE – EC are Pt, Pd, Au, Cu, and Ag. Classical lithography techniques can be used to define the electrode area prior to depositing the metal onto the surface directly or into an etched channel. Their fabrication is well described in the literature. Even if these electrodes are expensive to buy or manufacture, they can lead to mass production [95–97].

A large number of metals require an adhesion metal layer (usually classically titanium, chromium, nichrome, or tantalum), which are metals with high binding strength with oxygen and therefore able to depassivate several surfaces of materials to adhere onto the underlying substrate. This requirement limits their use with all-PDMS devices, since the metals normally fracture due to substrate flexibility [98]. The metal electrodes are therefore commonly fabricated on glass substrates coated with chrome and positive photoresist. The desired electrode geometry is patterned using a transparency mask and a UV radiation. The photoresist is developed, the underlying chrome layer is etched away using chrome etchant. Using a buffered oxidant, the glass to be engraved. Finally, a thin layer of an adhesion metal is deposited in the engraved part followed by the deposition of the desired metal electrode, sputtering and/or evaporation (Figure I.3.1). The extra metal, photoresist, and chrome film are removed to achieve the patterning of metal electrode supported on glass.



Figure I.3. 1. Assembly of metal electrodes onto a glass substrate; **A**. Glass substrate covered with a chrome layer and negative photoresist; **B**. A mask with the desired features is placed over the substrate and exposed with UV light; **C**. The photoresist is developed; **D**. The underlying chrome layer is removed by chrome etchant and the glass exposed is etched to produce a trench by a buffered oxidant.; **E**. The adhesion metal and/or desired electrode metal is deposited onto the substrate; **F**. Finally, excess photoresist and chrome are removed from the substrate and the metal electrode remains

Concerning the deposition of the adhesion metal and/or desired metal electrode, the most classical technique is the use of a thin film deposition approach, which is a vacuum technology for applying coatings of pure materials to the surface of substrates. The thickness of the coatings is commonly in the range of angstroms to microns and the deposited electrodes

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can be of a single material, or multiple materials in a layered structure. Depending on the selected electrode material, sputtering or evaporation technique employed.

The sputtering method involves the introduction of a controlled inert gas, such as argon, into a vacuum chamber, and electrical energization of a cathode to establish a selfsustaining plasma. The exposed surface of the cathode contains the material that will coat the substrate. The gas atoms inside the plasma turn into positively charged ions, which are then accelerated directly into the target metal. The impact promotes the release of atoms of the target material that travel to the substrate, condense, and form a thin layer. Gunasekara et al. [99] reported the manufacture of dual Pt electrodes in PDMS/ glass MCE device. Before the sputtering process, the authors created a pattern etched on glass using a buffered oxide etchant. When dried, it was then exposed to oxygen plasma in order to promote adhesion of the etched glass surface. The glass plate was then placed for the sputtering process and the excess metal around the electrode was lifted off in an acetone bath. The lift-off process is widely used for dissolving the underlying positive resist layer, allowing for the patterning of the electrode material [100,101]. The metal deposition on substrates other than glass by sputtering techniques was also described. Bonabi et al. [102] developed metallization processes for organically modified ceramic polymers (ORMOCERs) for microfluidic electrochemical assays. The authors optimized all the parameters used for Cr and Pt deposition by sputtering, such as DC power, argon flow and time for a better adhesion of the layers. The deposited thin film was characterized by measuring its electrical conductivity. The feasibility of the developed metallization for implementation of microfluidic was validated by performing the determination of acetaminophen using a MCE-EC analytical system.

As concerning the evaporation process, the source material is evaporated under vacuum, allowing vapor particles to be deposited directly onto substrate, where vapor condense back to a solid state, forming a functional coating. In high-vacuum evaporation, electrical beam or electrical heating is used to melt, and evaporate the target materials. The vapor of the target material travels directly to the surface of the substrate, for example a glass, and gradually cools down to form a thin film layer [103]. Chen *et al.* [104] fabricated nanoband gold electrodes on SU-8 substrate *via* photolithography and metal vapor deposition. The strategy was to use a negative photoresist without baking step in order to avoid a strong bonding between SU-8 substrate and the wafer. After the metal deposition by using a thermal evaporator, the Au-coated SU-8 substrate was easily peeled from the wafer and the edge of the Au rather than the planar surface was used as a nanoband electrode. A characterization by an energy dispersive X-ray spectra (EDS) analysis using a field emission scanning electron microscope (FE-SEM)

confirmed the gold distribution on the edge of the substrate, with a 100 nm width of the gold band. Moreover, the authors used an adhesion layer composed of Cr for Au electrode. Indeed, Cr (and also Ti) is widely used as an adhesion promoter as it is a more reactive metal than Au itself, and thus it increases the mechanical resistance of the electrode [105].

Although these techniques allow obtaining high resolution (nanoband can be created) and appropriate material deposition for detection (homogeneous deposition), they suffer from an important limiting factor which is the substrate heating. Deposition by sputtering leads to two main sources of substrate heating: the heat for condensation and the kinetic energy of the incident atoms and ions. Due to the heating by energetic atoms and ions, sputtering heats the substrate more than evaporation at higher deposition rates. Therefore, this technique could not be used for substrates with low glass transition temperature, for instance polymers [106].

The conventional hot embossing is a process that can be used to pattern the electrodes and prepare the microchip polymeric material. Briefly, the process consists in placing the substrate, such as a polymer, on a lower plate and the master stamp is attached on an upper one. The polymeric substrate is heated above its Tg. Subsequently, the load is applied on the substrate through the upper plate and the plates are held for some time. Then temperature and load are reduced, the master stamp is separated from the substrate and the pattern on the master stamp is transferred to the substrate [107]. Wei et al [108] developed a thread-based MCE system with 3D detecting electrodes. The authors reported the integration of Cr/Au electrodes (300 µm width). in PMMA substrate with hot embossing and sputtering procedure. To manufacture the electrodes, prior to the hot embossing process, a piece of thin aluminium foil was placed between the master mold and the substrate as a sandwich structure. The aluminium foil was used as a demoulding layer that acted as a mask for metal sputtering (Figure I.3.2). This simple approach allows fabrication of 3D electrodes, resulting in a high contact surface area for electrochemical detection and finally enhancing the current response without compromising separation efficiency. Figure I.3.2 presents the separation of dopamine and catechol using both 2D and 3D electrode chips. The peaks obtained with 3D electrode exhibited a sharp and high current response. Furthermore, S/N ratio increased by a factor of five, and the calculated plate number was 1.5 times increased when a MCE is implemented with 3D electrodes for detection purpose.



Figure I.3. 2. Scheme summarizing the fabrication steps for the thread-based MCE. **1A**. In a PMMA substrate a concave master mold is fabricated in an Al alloy via micro-CNC machine; **1B**. Before the hot embossing process, a piece of thin aluminium foil is placed between the master mold and the PMMA substrate; **1C**. The Al foil is used as a mask for metal sputtering. The hot embossing process produced protruding EC electrodes, which are covered with the foil after the procedure. The Al foil on the third electrode (reference electrode) is then peeled off and sputtered with Cr/Pt layers; **1D**. The reference electrode is covered with an electrostatic tape, and the others were again sputtered with Cr/Au layers; **1E**. The residual Al foil was then stripped from the PMMA; **1F**. Polyester threads are assembled onto substrate to perform a channel. Electropherograms of a sample containing dopamine (DA) and catechol (CA) using a **2A**. bidimensional electrode and **2B**. three-dimensional electrode. Reprint by permission from Spring Nature.

The use of epoxy-embedded electrodes to integrate electrochemical detection was developed by Selimovic *et al.* [109]. Metal electrodes may be incorporated using this technique. Briefly, a metal gold wire (electrode) is placed into the hole of a Teflon mold in a vertical orientation (Figure I.3.3.A). Following the assembly of the mold and electrode, a thoroughly mixed combination of resin and an activator is poured into the mold (Figure I.3.3.B). When the mixture is cured, the electrode-containing epoxy substrate is removed (Figure I.3.3.C). The substrate is then polished using a wet grinder to create a useable substrate bearing on its underneath a disc shaped electrode (Figure I.3.3.D). Finally, the disc electrode surface is reversibly sealed under PDMS fluidic network (Figure I.3.3.E) allowing its alignment with the microchannel. To increase the surface area of the embedded 25 μ m diameter electrode (Figure I.3.3.F), gold electrodeposition was performed and then characterized by confocal image (Figure I.3.3.G). The surface area was determined using cyclic voltammetry (1.54×10⁻⁴ cm²), which is considerably larger than the geometrically calculated area (4.9×10⁻⁶ cm²). The same procedure can be used for polystyrene-embedded electrodes. This fabrication method allows the electrode to be polished before each use for surface cleaning, and a top layer containing the

separation channels can be aligned on the surface of the substrate. More recently, Jonson *et al.* [110] reported the same microfabrication embedded electrodes process to integrate a carbon - based working electrode and metal-based decoupler in a single step. The device was employed for amperometric detection of dopamine, epinephrine, catechol, and 3,4-dihydroxyphenylacetic acid. Whereas the electrode fabrication processes generally report the creation of band electrodes, the authors explored the production of disk electrodes.



Figure I.3. 3. Fabrication of embedded electrodes: **A.** A gold wire is inserted into the Teflon master mold; **B.** An epoxy mixture is placed into the mold and left to cure; **C.** The base of Teflon mold is removed from epoxy, with the outer mold still remaining; **D.** The electrode is polished to generate a fresh electrode surface. The device is assembled: **E.** The epoxy-embedded electrode is sealed to a PDMS containing the channel; **F.** Micrograph of a gold pillar electrode; **G.** Confocal image of the pillar electrode (height=27 μ m, width=65 μ m). Reprint by permission from John Wiley and sons.

I.3.2. Carbon-based electrodes

Carbon-based electrodes are relatively simple to manufacture and are of low cost and cheap production processes. They have a large electrochemical potential window, low background noise, low over-potential, and less fouling than their metal-based counterparts. Besides this, many biologically relevant analytes are organic compounds and, consequently, generate a better response at carbon-based electrodes [111]. Thus, this kind of electrode has been extensively applied in MCE- EC systems. Several articles present different types of carbon for their integration into microdevices, including pyrolyzed photoresist film (PPF) carbon, carbon fiber, carbon paste, carbon ink, and boron doped diamond electrodes. Whereas carbon

electrodes are a good choice for detection in microchips made of soft polymers, plastics, or hybrid chips, they have limited stability under high temperature and pressure conditions used for bonding all-glass devices.

I.3.1.a. Pyrolyzed photoresist film (PPF) carbon

PPF carbon is a material that does not present stability problems as the other types of carbon. In other words, PPF electrodes can be manufactured on glass substrates using photolithographic procedures. First, a photoresist is placed in the pattern of the desired electrode onto a substrate. Then, the substrate with photoresist is placed in a tube furnace under an inert gas atmosphere under temperatures up to 1100°C to produce conducting carbon films (Figure I.3.4).



Figure I.3. 4. Pyrolyzed photoresist film (PPF) carbon electrode assembly: **A.** Glass substrate; **B.** A negative photoresist is deposited on the electrode substrate; **C.** A mask with desired features is placed over the substrate and is exposed to UV light; **D.** The substrate is placed in photoresist developer and **E.** Only the electrode pattern (in photoresist) persists after development; **F.** Following pyrolysis in a tube furnace, a carbon electrode persists on the substrate. Connections are prepared with silver colloidal and copper wire.

Although PPF is similar in many ways to a smooth version of polished glassy carbon (GC), there are some significant differences which may have substantial electroanalytical consequences, to begin with its extraordinary smoothness, with a RMS (Root Mean Square of a surface measuring microscopic peaks and valleys roughness) of approximately 1-10 % of polished GC [112]. Then, the capacitance and background current are lower than for traditional GC. Merged with the ability to produce patterns on a scale of 10 - 20

μm, the near atomic flatness of PPF allows production of novel electrode structures, for instance very thin electrode layers and interdigitated carbon arrays. However, they do have a low oxygen/carbon ratio, leading to slow electron transfer kinetics for some analytes [112]. The physicochemical and electrochemical characterization of PPF carbon electrodes were explored by Ranganathan *et al.* [112]. The authors studied electron-transfer kinetics of various redox systems comparing PPF surfaces with heat-treated glassy carbon (HTGC) and glassy carbon (GC) electrodes. Briefly, the PPF provides well-defined, but less reversible, behavior on PPF than on HTGC or GC for all the redox systems investigated (Figure I.3.5).



Figure I.3. 5. Voltammetry of $Fe^{3+/2+}$ and dopamine on PPF and heat-treated GC (HT GC). All electrode surfaces were cleaned with activated carbon in IPA before voltammetry [112]. Reprint by permission from American Chemical Society.

Saylor *et al.* [113] published two different microchip substrates (PDMS/glass and PDMS/PDMS) employing different microband (33 µm width) electrode materials, PPF and carbon fiber electrodes, respectively. The authors monitored dopamine (L-DOPA) metabolism in a rat brain slice. The improvements achieved with the use of PPF electrode were higher sensitivity and lower LODs than with carbon fiber electrodes, owing to a decrease in background noise. Furthermore, PPF may be fabricated into possibly complex microstructures or in uncommon shapes that are not amenable to polishing. However, since polishing is unnecessary, mass production of PPF-based sensors is possible. Although PPF electrodes have several advantages for MCE-EC systems, other types of carbon electrodes are more indicated for thermoplastic and polymer substrates, for instance carbon fiber electrodes.

I.3.1.b. Carbon fiber electrodes

Carbon fiber (CF) electrodes are often integrated into MCE systems by placing the fiber into a PDMS substrate taking extreme care not to break the fiber. A channel whose width corresponds to the diameter of the fiber is typically created in a PDMS substrate supported on a glass for increased rigidity. The CF is then placed on the trench created in the bottom layer of the PDMS chip that is perpendicular to the separation channel and pushed into it, taking extreme care not to break the fiber. With the purpose to make connections to the fiber, a copper wire is epoxied onto the glass support, and the fiber and wire are connected by colloidal silver [114]. This approach for inserting the fiber into the trench may be laborious and time consuming. Thereby, alternative methods for integrating carbon fiber in microdevices were also described. Cheng et al. [115] reported the use of carbon fiber nanoelectrodes (CFNE), previously described by the group [116], as EC detector in MCE systems (Figure I.3. 6.A). To fabricate CFNE, a glass capillary was pulled on the flame to build a tip. A cleaned CF connected to a copper wire was inserted from the other end of the glass capillary into the tip with CF protruding from the tip (Figure I.3. 6.B). The carbon fiber was then flame-fuse-sealed in the tip of the glass capillary. The CFNE electrodes were characterized by SEM (Figure I.3.6.C) and by CV, LSV and DPV using ferrocyanide as electrochemical probe (Figure I.3.6.D). The results showed that the CFNEs have excellent electrochemical behaviour and high sensitivity. The tip of CFNE can be controlled within the range from 100 to 300 nm.



Figure I.3. 6. Microfluidic system with CNFE. (A) Configuration of a MCE system with integrated EC detection: (1) sample reservoir; (2) buffer reservoir; (3) sample waste reservoir; (4) separation channel; (5) detection cell; (6) working electrode; (7) reference electrode; and (8) epoxy; (B) Better view of the outlet of the separation channel coupled with CNFE. (C) SEM images of CFNE and tip of a CNFE. (D) Cyclic, linear sweep and differential pulse voltammograms of $1.0 \times mM$ potassium ferricyanide in 0.1 M phosphate buffer solution (pH 7.4) obtained with

a CFNE; tip diameter, 200 nm [115,116]. Reprint by permission from American Chemical Society and John Wiley and sons.

I.3.1.c. Carbon paste/ ink electrodes

Carbon paste (CP) electrodes have been employed for many years for EC detection since their introduction by Ralph N. Adams in the 1950s [117]. The first reported use of a CP electrochemical detector for microchip electrophoresis was described by Lunte's group in 2001 [118]. CP electrodes have the benefit to be integrated into more rigid substrates, for instance plastic or glass, while CF may only be placed in the more malleable PDMS.

CP electrodes are manufactured by building a channel in PDMS, PMMA or glass, where a mixture of a carbon-based material (graphite and carbon nanotubes) is placed with a binding agent to produce a working electrode. The binding agent commonly used for integration of CP electrodes is oil, but some studies have described the use of microchip material itself to improve mechanical ruggedness and substrate compatibility. Regel *et al.* developed integrated graphite/PMMA composite electrodes with 100 µm width into a PMMA substrate. The two parts of the chip sealed using a solvent-assisted thermal bonding method to form the final MCE-EC device [119]. Recently, Gouyon *et al.* [120] described the development of screen printed graphite/PDMS (C/PDMS) band electrodes of 30 µm width into a PDMS substrate for amperometric detection in MCE systems (Figure I.3.7.A). The PDMS was explored by the authors, as this material could be used as a sealing agent between the carbon composite electrode and a microfluidic system made of the same polymer, owing to its physicochemical properties, such as covalent bonding ability after plasma surface treatment and elasticity. Electrochemistry (Figure I.3.7.B) and SEM (Figure I.3.7.C) were used to characterize the microband carbon composite electrodes.


Figure I.3. 7. Development of a screen-printed electrode and its characterization. (A) Scheme of the fabrication of the 3-electrodes setup made of C/PDMS mix. (B) Electrochemical characterization by cyclic voltammetry on C/PDMS electrodes of (B) 5 mM Ru(NH₃)₆³⁺ and (C) 1 mM FcMeOH. Working Electrode = C/PDMS 8/8 (w/w) circular, pseudo-RE=Ag/AgCl wire, CE = Pt wire. Scan rate 25mV s⁻¹. (D) SEM images of C/PDMS electrode [120]. Reprint by permission from Elsevier.

The CP electrodes may also be manufactured by a commercial printer or a screen-printing technique. The microchip design is deposited on a screen of polyester or other fine mesh, with blank areas coated with an impermeable substance or on a substrate with pattern trench. The paste or ink is pressed into the mesh or substrate with a squeegee and onto the surface to be printed. The excess may be removed by pressing or/and removing a piece of Scotch[®] tape over the electrodes. Nowadays, this microfabrication technology is well-established for mass production of thick film electrodes. In addition, silver electrodes and carbon ink (CI) electrodes also may be produced using this technique, which is helpful to obtain pseudo-reference and counter electrodes [121].

CI electrodes are popular materials for commercial versions of MCE-EC systems. As discussed before, these electrodes may be fabricated by screen-printing on PDMS [122], glass, or epoxy [109] substrates. With the purpose to facilitate the electrical connection to CI electrodes, some groups previously integrated a metal electrode connector in the device by sputtering and evaporation. The micromolding structure made by soft lithography techniques is aligned and reversibly sealed over the metal connectors, and an ink mixture is aspirated through the channels. Mecker and Martin [123] reported this approach to fabricate a 13 µm length microband CI electrode with Pd connectors for amperometric detection in microdialysis sampling and microchip electrophoresis device. Recently, a novel method to fabricate screenprinted electrodes was reported by Petroni et al. [124]. Briefly, two pieces of adhesive tape were pasted onto an PMMA substrate, at a distance delimiting the electrode width. Once established the electrode region with the adhesive tapes, the carbon electrode was drawn onto the substrate and subsequently, a layer of carbon ink was gently spread over the delimited area. Lastly, the electrode was kept at room temperature to dry, leaving just the carbon ink over the acrylic substrate (Figure I.3.8). The authors monitored the thickness of the sample by polishing SEM images of the carbon electrode surface having various thicknesses are shown (1.15 - 4.33 μm) in Figure I.3. 8 (A-D). Indeed, since the carbon ink deposition and electrode polishing steps are manually performed, the electrode reproducibility can be a problem. Using the proposed method, the thickness layer was $3.18 \pm 0.51 \,\mu\text{m}$ with an RSD of 11.6% (n = 5). The authors characterized the electrodes by performing hydrodynamic voltammograms to set up an optimum detection potential value (Figure I.3.8.E) for nitrite and ascorbate. These novel

electrodes provided stable background current and suitable signal to noise ratio.



Figure I.3. 8. Scheme of the electrode microfabrication process: **1A** substrate cleaning; **1B** adhesive tapes to delimit the electrode area (an office paper mask was used as reference to define the electrode area); **1C** handmade deposition of home-made carbon ink over between the adhesive tape; **1D** electrode surface polishing; **1E**. revealed electrode through removal of adhesive tape; **1F** electrical contact made by a copper wire attached with epoxy resin; **1G** colloidal silver glue application to provide electrical contact between the copper wire and the electrode; **1H** alignment of PDMS device over the substrate containing the electrode. **2.** SEM images obtained from electrode surfaces with different thickness: **(A)** 1.15 μ m; **(B)** 2.21 μ m; **(C)** 3.18 μ m and **(D)** 4.33 μ m. **2E** hydrodynamic voltammograms constructed from electropherograms recorded at different detection potentials. Experimental conditions: 5 mM phosphate buffer (pH 6.85) containing 200 μ M CTAB; injection voltage: -700 V for 6 s; separation voltage: -1200 V [124]. Reprint by permission from Elsevier.

I.3.1.d. Pencil electrodes

Pencil drawing is the simplest and lowest-cost method for the rapid production of carbon-based electrodes on paper. Chagas *et al.* [125] published, for the first time, the fabrication of a pencil drawn electrode (PDE) on paper platforms to accomplish C⁴D detection in a PMMA electrophoresis chips. The electrodes bands (2×2 mm) were printed on the paper substrate to delimit the electrode area. Next, PDEs were drawn on the top face of the printed area by a pencil. The feasibility of the proposed device was shown with the separation of inorganic cations in tear samples. After that, the authors reported the use of PDEs on a paper-based MCE- C⁴D device for separation of bovine serum albumin and creatinine, main biomarkers in the detection of kidney disease. The authors successfully provided microfluidic devices of extremely low cost (less than \$0.10) and easy to manufacture, especially to integrate the sensitive electrodes [126].

I.3.1.e. Boron-doped diamond electrodes

The benefits of boron-doped diamond electrode material for MCE-EC systems are its ruggedness and reproducibility, offering less capacitive currents, a rapid electron transfer kinetics and a wide electrochemical potential window. These electrodes are achieved by depositing a thin film of boron-doped diamond (BDD) on silicon wafers by a high-pressure plasma-assisted chemical vapor deposition system with both boron and carbon sources. Subsequently, chemical etching is used to dissolve the silicon wafer substrate to produce a freestanding diamond thin film. This film can then be glued to the substrate with an adhesive tape. Quick-setting nonconductive epoxy may be used for shaping of the working electrode area [127]. Wang et al. [128] highlighted the attractive behavior and advantages of BDD electrode detector for MCE systems in 2003. The authors compared critically the performance of BDD electrode (6.0×0.3 mm) in band shape to a commonly used screen-printed electrode in the same operational conditions (Figure I.3.9). One advantage is the reduced fouling offered by the electrode, which reflects the negligible adsorption of organic compounds at the BDD surface. The BDD detector displays higher stability (Figure I.3.9.A), higher sensitivity (Figure I.3.9.B) and lower noise level (Figure I.3.9.C) for microchip measurements of TNT (2,4,6trinitrotoluene) response.



Figure I.3. 9. Comparison of the performances for BDD and screen-printed electrodes. (A) Reproducibility of the detection intensity for successive flow injection measurements of 22 μ M TNT using (a) BDD and (b) screen printed carbon electrodes. (B) Hydrodynamic voltammograms for 66 μ M TNT at (a) screen printed and (b) BDD electrodes. (C) Impact of separation voltage on the baseline noise for (a) BDD and (b) screen printed carbon electrodes [128]. Reprint by permission from American Chemical Society.

I.3.1.f. Nanostructured and/or chemically modified electrodes

Modification of electrode surface is often employed to increase the electronic transfer rate, amplify the current signals and/ or improve the selectivity of MCE-EC analysis toward specific analytes [129]. Electrochemical deposition is a technique widely used for modification

of electrodes in microdevices using cyclic voltammetry. The main advantage of this strategy is that electrodeposition may be conducted into the microchip, reducing the number of steps. The electrode is modified by immersion in a metal salt solution, which is electrochemically reduced on the electrode surface.

A gold nanoparticle-modified indium tin oxide microelectrode for amperometric detection in MCE was developed by Zhu *et al.* [130]. The authors optimized the deposition conditions in terms of deposition cycles, deposition potential range and scan rate, as well as concentration of the electrolyte to obtain high particle density s for the fabrication of a high-quality sensor.

Platinization is a popular electrode modification, consisting in an electrochemical deposition of platinum black nanoparticles on a platinum or a carbon working electrode. This type of modification improves the electron transfer kinetics for small molecules, for instance reactive and oxygen species, and increases the surface area of the electrode. Comparing with bare Pt electrodes, platinum black (Pt-black) can provide currents magnification about 3 times in case of hydrogen peroxide and 6 times for NO₂⁻. Pt-black is commonly used for studies involving hydrogen peroxide and nitric oxide analyses, also monitoring reactive species from cells in MCE –EC systems and in miniaturized sensors [131,132].

A material that has become popular for modified electrodes is reduced graphene oxide. This modification with graphene may be achieved by electrochemical reduction of graphene oxide (GO) over the electrode surface with an electrodeposition process in which the oxygenated functional groups are removed from GO sheets. Electrode modification with GO does not require any specific chemical reagent and is accomplished by the electron transfer between GO and the working electrode. This method avoids the use of harmful reducing agents such as hydrazine and prevents the formation of intermediates. We may find a few examples of the use of reduced graphene oxide for MCE-EC. Chua and Pumera[133] reported the performance of reduced graphene on glassy carbon electrodes to detect dopamine and catechol. Electrochemically reduced graphene was shown to exhibit highest peak resolution and greater separation efficiencies than thermally and chemically reduced graphene, and two-fold increment as compared to the bare glassy carbon. More recently, Lucca *et al.* [134] described, for the first time, the modification of Pt electrodes integrated into a commercial MCE system by deposition of reduced GO for the selective amperometric detection of ascorbic acid in a commercial medicine sample.

Single-walled (SW) and multi-walled (MW) carbon nanotube electrodes (CNT) have also been widely employed in EC detection because of their outstanding features such as large surface area, reduced surface fouling, and good electron transfer kinetics, which allows to enhance the analytical features of the analyses (repeatability, selectivity, sensitivity). The surface modification of carbon nanomaterial-based electrodes has been performed by casting a drop containing a carbon nanomaterial dispersion on a solid electrode surface. This method has an issue with the deposited film, which can be mechanically fragile, and the presented signal is dependent on two carbon sources, the carbon nanomaterial and the commercial transducer [135]. Some new strategies have been reported such as the use of the carbon nanomaterial as exclusive material to improve the analytical response and allow reliable studies of the pure nanomaterial. For instance, Martin and Escarpa [136] developed SW carbon nanotube presstransferred, as a unique conductive material, for EC detection in MCE. The new carbon nanomaterial is produced by filtration of SW carbon nanotubes on a Teflon filter. The filtered nanomaterial is homogeneously press-transferred on a nonconductive PMMA substrate, where SW carbon nanotube act as exclusive transducers. The authors explored the excellent properties of carbon nanomaterials for screening melatonin, tryptophan, and serotonin.

I.4. Main electrochemical detection techniques within MCE

I.4.1. Amperometric detection mode

Generally, a basic three-electrode (working, auxiliary and reference) system has been used, but it can also be implemented in a two-electrode (working and reference) system when using micrometric working electrodes. Even though amperometry is the most frequently used electrochemical method in microfluidic devices, pronounced shortcomings limit its applications. First, there is a necessity to improve the reproducibility of the surface of the working electrode (WE) and the reproducibility of its position and alignment within the separation channel. Second, the high voltage needed for the separation step induces interferences with the low detection electrical field used for amperometric detection, compromising the obtained results. Thus, different strategies to prevent or minimize the interaction of the separation electric field with the WE are required. The three major electrode configurations are in-, end-, and off-channel electrode alignment.

I.4.1.a. Off-channel detection

In this configuration, the current associated with the high separation voltage is minimized by a fracture (often called a decoupler or joint), which is made in the capillary and grounded prior to the detection cell. In other words, the WE is isolated from the separation voltage. Briefly, the decoupler is manufactured by a fracture of the separation channel nearby to its end. Then, this region is immobilized in order to increase the mechanical strength of the device. A porous membrane is positioned along the fracture, enabling the conduction of the current and finally, this device is placed in contact with the grounding separation electrode. There are several materials which can be used as membrane: Nafion[®], porous glass, graphite, cellulose acetate and other polymers. In the off-channel alignment, the WE is placed inside the channel and the separation electric field (\vec{E}) is efficiently moved to ground by a decoupler, promoting the electrical isolation of the detector from the separation voltage. A field-free region is created where analytes are pushed straight the WE by the residual hydrodynamic flow produced from the EOF generated up-stream the decoupler. As a result, this leads to low background noise and no voltage fluctuations in the WE owing to the separation voltage, which can result in low LODs. However, this configuration requires a strong EOF to "push" the analytes to the detector detector. Also the WE and the decoupler should be maintained very close to each other to avoid signal broadening and loss of resolution (Figure I.4.1.).



Figure I.4. 1. Electrode off-channel alignment used in MCE-EC with a single working electrode. The separation field (\vec{E}) is moved toward the ground by a decoupler. The analytes are carried toward the WE by hydrodynamic flow produced from the EOF.

The main challenge to integrate a decoupler into a MCE-EC system is that the decoupler and the WE are typically composed of different materials, which may require different fabrication methods. The disadvantage of this alignment is the generation of gas at the decoupler owing to the production of hydrogen gas at the cathode or oxidation of water at the anode, depending on the applied polarity, leading to the formation of bubbles inside the channel and consequently disturbing the separation. Metal band electrodes, such as Pd [137] and Pt [138], are widely employed as decoupler, as they can absorb the hydrogen gas produced at the

cathode, minimizing bubble formation. However, no study has yet reported the existence of decouplers for a reversed polarity separation, in which the electrode material would absorb oxygen gas in MCE-EC systems.

Beyond the metal-based decouplers, cellulose acetate decouplers have been described for MCE-EC. This new decoupler in CE was presented by Osbourn and Lunte [139]. This procedure consists in a fracture which is created within the microchannel, then covered by an ion-permeable membrane, for instance Nafion[®] or cellulose acetate, and positioned in the ground reservoir. Afterwards, the authors described the use of cellulose acetate decoupler, which was placed over a part of the separation channel which has been etched to provide an ion path (Figure I.4. 2.A). Using this configuration, noise values as a function of separation current for the designed decoupler were evaluated and low background noise (arising from the isolation of the detector cell from separation current) up to 60 µA was obtained (Figure I.4. 2.B). The authors reported on operational ability of the MCE device to perform analyses at extremely high fields strengths, enabling higher resolution in ultrafast separations (30 s). In this study, the application of 700 V/cm did not affect the detector (noise levels at or below 1 pA), resulting in LOD of 25 nM for dopamine with a carbon fiber electrode, which is a low LOD value concerning electrochemical detection with MCE system (Figure I.4. 2.C). The separation current is grounded through the fracture covered with this membrane. The target compounds are pushed from the decoupler (fracture) to the WE by the EOF generated in the separation part of the channel [140].



Figure I.4. 2.. (A) Microchip with laser-etched decoupler layout. (A): (1) decoupler holes, (2) detector reservoir, and (3) carbon fiber electrode. (B) Noise values as function of separation current for the laser-etched decoupler. (C) Electropherogram of the injection of (1) 82 nM dopamine and (2) 500 nM hydroquinone in the MCE-EC system [140]. Reprint by permission from American Chemical Society.

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I.4.1.b. In-channel detection

Martin and co-workers[141] reported on the in-channel detection mode. This approach allows the analytes migrating over the electrode while they are in the separation channel, minimizing analytes diffusion and resulting in high separation efficiencies, high sensitivities, and better defined peaks. However, this detection mode must have an isolation of the high separation voltage from that required for the detection at the WE, which may be achieved by using an electrically isolated potentiostat. Indeed, when the electrode is positioned within the separation field (\vec{E}) (Figure I.4. 3), there may be both potential shifts and increase of noise due to the separation voltage fluctuations. This results in increased background noise at the detector and higher LODs, even if the separation efficiency is improved.



Figure I.4. 3. Electrode in-channel alignment used in MCE-EC with a single working electrode. The separation field (\vec{E}) is moved toward the ground electrode. The WE electrode is placed between separation electrodes.

To overcome the LOD issue and improve the performance of the amperometric detection, Chen and Hahn [142] published a dual electrode/ dual in-channel microchip configuration to subtract the background signal. Both electrodes, WE and RE are positioned at the same distance from the outlets of two identical channels, one is used as a separation channel and the second is used as a "reference" channel. The symmetry of dual channel microchips allows the electrodes to experience the same electric potentials and fluctuations, and thus the interferences are cancelled out during the measurement of the current between the two electrode set-ups (Figure I.4. 4).



Figure I.4. 4. Scheme of a MCE-EC in dual electrodes in-channel configuration. **1B.** Photograph of the real microchip. Electropherograms of 5 mM dopamine: **2A.** in a single in-channel configuration; **2B** in a dual in-channel configuration; **2C.** in a single in-channel, head- to-head WE-RE configuration. Reprint by permission from American Chemical Society.

I.4.1.a End-channel detection

End-channel configuration was the first developed configuration and is the most common alignment for MCE-EC systems. This alignment explores the strategy of placing the WE at the exit of the separation channel, $5-20 \mu m$ away from the outlet end (Figure I.4. 5). By doing so, the influence of the separation electric field on the electrochemical detection is avoided. This configuration is predominantly developed now because its ease of microfabrication but also electrode accessibility and cleaning. But this alignment, in which the analytes diffuse into the waste reservoir as they exit the channel before being detected, induces a significant broadening of the signals and asymmetry of the peak shapes, which leads to loss of separation efficiency and reduction in sensitivity.



Figure I.4. 5. Electrode end-channel alignment in MCE-EC with a single working electrode. The analytes are widespread into the waste reservoir leaded by separation current. The separation field (\vec{E}) is moved toward the ground electrode.

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Ertl *et al.*[143] developed an integrated sheath-flow channel to be placed at 30° angles relative to the separation channel (Figure I.4. 6.A) to focus the fluid stream over the middle of the WE (Figure I.4. 6.B). Consequently, the sheath-flow subtracted the influence of separation voltages on end-column detection while preserving optimum performance. The novel device consists of an upper glass substrate containing the etched fluidic elements (separation, injection, and sheath-flow channels) and a lower glass with the metal electrodes integrated by sputtering. The authors used catechol for the performance evaluation of this sheath-flow EC detector, in the presence and absence of sheath-flow support. This system allowed obtaining efficient detection of the analyte (sensitivity = 0.18 nA/ μ M) and low background-noise level (LOD = 4.1 μ M) at distance of up to 250 μ m from the separation channel outlet (Figure I.4. 6.C). In addition, the analysis of catechol as model substance evidenced a linear detector response in the 10 to 500 μ M range (Figure I.4. 6.D). The absence of sheath-flow support resulted in a significantly low (~90%) and broader signal peaks.



Figure I.4. 6. The MCE-EC chip with sheath-flow channel (A) Scheme of a separate channel with integrated sheath-flow channels. (B) Electrodes at the detection region visualized under a microscope. (C) Catechol separations presented as relative (%) signal changing as a function of electrode distance from the separation channel (100, 150, 200, and 250 μ m) calculated in the absence and presence of sheath-flow support. (D) Catechol linear range (500, 250, 50, and 10 μ M) provided by 3.5- μ m-tall gold-plated WE placed 250 μ m from the separation channel exit [143]. Reprint by permission from American Chemical Society.

More recently, Guan and Henry[144] reported the implementation of a bubble cell at the detection zone to increase the exposed WE surface area to effectively dissipate the separation field. With this approach the authors improved detection sensitivity and decreased LODs compared with a system without a capillary expansion.

It should be noted that the end-column electrochemical detector can be manufactured in a wall-jet configuration; the electrode having an area of several mm² is being placed opposite to the channel. The wall-jet macroelectrode is easily exchangeable, allowing fast and convenient replacement if passivated. However, the dependence of the separation

efficiency upon the detection potential wall-jet macroelectrode was an unexplored subject for application studies. Indeed, there was no article dealing with the fundamental issue until Pumera *et al.* [145] reported on the study of the electrochemical response of a wall-jet detector for microchip electrophoresis. The authors demonstrated that MCE-EC in wall-jet operates in the coulometric mode, also they demonstrated how the detection potential strongly impacts the resolution of the signals.

I.4.2. Conductimetric detection

Microfluidic devices with electrochemical detection have been mostly based on amperometric mode due to its high sensitivity. However, this approach is limited to the detection of electroactive species. Conductimetry is a relevant complementary approach since it is a universal detection, applicable to charge species. Furthermore, conductivity detection remains a direct method for sensing small inorganic and organic ions that lack native fluorescence, strong chromophores, or functional groups for derivatization. In these detection systems, usually background electrolytes exhibit a comparatively lower specific conductance compared to those of the target compounds. Nevertheless, high concentration of background electrolytes is required to reduce electro-dispersion effect in MCE. This conductometric detection may be accomplished by either direct contact of the solution with electrodes (Figure I.4.7.A) or contactless method (Figure I.4.7.B) that requires capacitively coupled devices applying high frequencies.



Figure I.4. 7. Comparison between (A) contact and (B) contactless conductivity detection and the usual placement of the electrodes in each detection mode [146,147]. Reprint by permission from Elsevier and Royal Society of Chemistry.

I.4.2.a. Contact conductivity detection

The drawbacks of this detection mode are (i) unwanted electrochemical reactions at the electrodes and (ii) fouling of the detection electrodes (iii) formation of bubbles. In order to avoid them, the authors commonly perform separation with low electric fields. Shadpour et al. [148] employed 90 V cm⁻¹ separation field, reduced the distance between the electrodes and minimized their size in a 16-channel polycarbonate (PC) MCE device. The gold sensor array (microbands 60 µm) was lithographically patterned onto a PC cover plate before its thermal bonding on the fluidic network. Even though the contact conductivity sensor array was successfully developed with simultaneous detection of different samples, the LOD for this sensor array was poorer than previous publication using conductivity detection. In order to overcome these limitations and consequently improve compatibility between conductivity detection and microchip, without using current decouplers, a novel bubble cell detection zone (Figure I.4. 8.A) was designed by Noblitt and Henry[149] in a PDMS microchip with goldplated tungsten electrodes. The group evaluated the separation of inorganics anions with and without bubble cell. Resolution between the peaks was 1.50, 1.68, 1.25, and 2.50 without bubble cell, and 1.46, 1.70, 1.27, and 2.56 with bubble cell (Figure I.4. 8.B). These values demonstrated that the bubble cell has a minimal effect on the separation efficiency, and it decreases the separation field in the conductivity region reducing the voltage drop between the detection electrodes. So, this novelty allowed the use of high separation voltage (600 V. cm⁻¹), with reduced background noise and short analysis time (Figure I.4. 8.C).



Figure I.4. 8. Microchip with amplified detection by a bubble cell. (A) Bubble cell, 3-fold larger than the separation channel. (B) Electropherograms of 25 μ M chloride, nitrate, perchlorate, chlorate, and hexafluorophosphate (from left to right), using a device without the bubble cell (top) and with a bubble cell (3x the channel size) (bottom). (C) Separation of 50 μ M anions using a device with a bubble cell at the following separation fields and injection times (top to bottom): -125 V/cm with 2.2s, -200 V/cm with 1.7s, -325 V/cm with 1.5s, and -450 V/cm with 1.2s [149]. Reprint by permission from American Chemical Society.

I.4.2.b. Capacitively-coupled contactless conductivity detection

In 2001, Guijt *et al.* [46] reported the first article on contactless conductivity (CCD) detector for microfluidics. The glass microchip electrophoresis was formed by an etched channel and integrated four titanium electrodes covered with a dielectric layer composed by silicon carbide (SiC). All four electrodes are 106 μ m long, the two inner electrodes are 30 μ m wide, the outer electrodes 25 μ m. Unsuccessfully, SiC coating stability was limited to low separation fields (50 V cm⁻¹), leading to long analysis times and band broadening. Some improvements of the design were then accomplished by the same authors, allowing for higher separation voltages (at least 3-fold) [150]. Tůma *et al.* proposed a new design for electrodes, easier to achieve, and showed that CCD electrodes for CE do not need to be tubular: they can be fabricated in other shapes without noticeable influence on CCD performance [151]. This was an important step to move forward manufacturing planar electrodes. A microchip with integrated CCD was established by Pumera *et al.*[152] by placing two planar aluminium foil strips electrodes which were fixed onto PMMA cover plate of a microchip using a common epoxy.

Currently, CCD is well known as capacitively coupled contactless conductivity detection (C⁴D). The electrodes are placed outside or near to the channel instead of being inside, leading to efficient isolation from high electric separation fields, easy replacement of the separation channel or capillary and alignment of electrodes. Kuban and Hauser [153] reported a PMMA MCE with copper electrodes to supervise effects of the cell geometry and operating parameters on C⁴D detection within a MCE-EC system. The authors used inorganic cations to demonstrate that sensitivity and separation efficiency can be improved by applying lower frequency, high excitation voltage, short and narrow electrodes arranged in an antiparallel configuration and separated by a minimal gap in the C⁴D detection system.

More recently, novel approaches have been published describing microfluidic devices with integrated electrodes for C⁴D detection using emerging materials. Duarte *et al.* [154] reported the use of ionic KCl solutions as sensing electrodes for C⁴D detection in PDMS based MCE devices. Initially, two detection channels were engraved in parallel orientation onto a PMMA substrate and sealed with a thin adhesive membrane. The separation channels were fabricated in PDMS by soft lithography and reversibly sealed against the PMMA with a polymeric adhesive intermediary membrane (Figure I.4. 9.A). The authors achieved electrophoretic separations of cationic species with resolution while avoiding laborious schemes to integrate electrodes, such as metal deposition or encapsulated electrodes (Figure I.4.

9.B). The results achieved for the C⁴D with liquid electrodes (KCl solution 2 M) settle with the creative and simple electrical model.



Figure I.4. 9. Integrated MCE – C^4D (A) Setting-up of the device with ionic solutions as sensing electrodes. e1 and e2 consist of the excitation and receiver electrodes, respectively. (B) Electropherograms of the injection of K⁺, Na⁺, and Li⁺ (300 μ M each) in water using 2 M KCl as sensing electrodes [154]. Reprint by permission from John Wiley and sons.

I.5. Microchip electrophoresis with electrochemical detection applications

MCE-EC devices have found widespread use in biological, environmental, and food applications. In this section, some significant examples of studies that engage MCE-EC systems as fundamental tools to analyze real samples are presented (Tables I.5.1-I.5.3). Beyond that, we highlight the analytical improvements attained in each methodology.

I.5.1 Application in the analysis of biological samples

MCE-EC devices have been employed for several biological applications, such as for the quantitation of amino acids, proteins, peptides, DNA and inorganic compounds (Table I.5.1). Biological applications focusing on cell analysis were recently reviewed by Lunte's group [155]. In particular, there are several articles by Martin *et al.* [156] describing PC-12 cells analysis using MCE-EC [123,156–158]. The authors developed a PDMS microchip with integrated microelectrodes, peristaltic pumps, and injection valves. The combination of several analytical steps, such as injection, separation and detection simplify the analysis process and the monitoring of the release of neurotransmitters from a PC-12 cell layers immobilized in a

collagen-coated micropallet. Recently, the authors described 3D scaffolds of polystyrene (PS) fibers to immobilize PC-12 cell culture, with integrated automated pumps and a 4-port injection system in a PDMS/polystyrene electrophoretic microchip. The polystyrene substrate was embedded with a Pd decoupler and a working carbon fiber electrode for off-channel amperometric detection. The authors demonstrated the close-to-real-time analysis of multiple molecules released from the 3D cell culture integrated within the PDMS device[158].

Scott *et al.* [159] reported on a microdialysis sampling coupled directly on a glassbased MCE with amperometric detection. The electrodes were photolithographically patterned in an in-channel configuration, the glass was etched, and then the Pt metal was deposited by sputtering. In this work, the group developed an on-animal separation-based sensor to simultaneously monitor drug metabolism and its behavior in a freely roaming animal. The device was used to monitor the production of nitrite following introduction of nitroglycerin into the sheep subdermal tissue. The group compared the results obtained by the proposed device with off-line analysis by liquid chromatography (LC)-EC system. Using the proposed system, samples from the first probe (implanted subcutaneously into the epidermal tissue) were analyzed immediately online by MD-ME-EC, every 60 s, with instantly interpretable data, while those from the parallel probe were collected off-line every 10 min in a centrifuge tube, stored in a -80 °C freezer for later analysis by LC-EC system.

Tsai *et al.*[160] developed a PMMA/photoresist/PMMA microchip with amperometric detection for screening target biological compounds. The fabrication process does not require a replicate mold, and the photoresist is used as both side walls of the microfluidic channels and an adhesive layer for sealing with a PMMA to cover. The photoresist layer is simple and a low-cost alternative to other bonding protocols, which are more demanding, requiring much higher temperature and pressure or the employment of surface plasma treatment for adhesion of polymeric layers constituting the device. The graphite electrodes were manufactured by screen-printed techniques onto PMMA substrate in an off-channel configuration. The proposed device was employed to separate uric acid and ascorbic acid in human urine with high analytical frequency (72 injection h⁻¹). Furthermore, the novel device attained results similar to the reported ones using a PDMS/glass microchip [161] in terms of separation efficiency. The rapid identification of target compounds in urine successfully demonstrated the PMMA MCE-EC device is a suitable high-throughput screening system for clinical samples.

Meneses *et al.*[162] evaluated the performance of an in-channel amperometric detection strategy combined with a dual channel PDMS/glass MCE system for the quantitation

of organic anions. To produce the Pt electrodes, the glass substrate was patterned and developed using a photolithographic method, subsequently the metal was deposited by sputtering. The authors explored the performance of both two- and three-electrode configurations using two different reference electrodes. Moreover, they evaluated the effect of electrode placement. Baseline noise obtained in this dual-channel configuration (4 pA) was significantly lower than that observed in a single channel configuration (25 pA) integrating an in-channel amperometric detection. The dual-channel device was applied for nitrite detection in a sheep microdialysis sample, reaching nitrite concentration levels of 68 μ M in 90 min of continuous perfusion of nitroglycerin.

Yang *et al.* [108] reported a thread-based MCE system with 3D gold amperometric electrodes for the detection of urea in whole blood. The gold electrodes were integrated on PMMA substrate with hot embossing and sputtering procedure. Prior to the hot embossing process, a piece of thin aluminium foil was placed between the master mold and the substrate as a sandwich structure that acted as a mask for metal sputtering. The authors showed that 3D-concaved structured electrodes in an off-channel configuration enhanced current response and signal-to-noise ratio of the MCE-EC system of a factor nine and five, respectively, when compared to the responses of a sensing chip integrating conventional 2D sensing electrodes. Lately, Duarte *et al.* [163] reported a SU-8/Pyrex system with integrated Pt electrodes in an end-channel configuration, for the separation, decomposition (under metal catalysis) and detection of low molecular weight S-nitrosothiols (RSNO) in a single run (Figure I.5.1.1). For the first time, the S-nitrosoglutathione (GSNO) and S-nitrosocysteine (CySNO) were separated and quantified via efficient detection of successive nitrite zones produced from RSNO after their decomposition bys Hg^{2+} to nitrite for quantification (Figure I.5.1.2).



Figure I.5. 1. (1) Separation and quantitation of RSNOs in a MCE-EC: **1A** Electrophoretic migration of the RSNOs towards the separation channel. Addition of Hg^{2+} to the BW reservoir 15 s before the end of this step. **1B** Inversion of the separation potential, which leads to the migration of Hg^{2+} into the separation channel faster than RSNOs, allowing for RSNO decomposition, and migration of the nascent nitrite (in green) towards the electrodes. **(2)** Electropherograms of CySNO (1 mM), GSNO (1 mM) and the GSNO mixture (1 mM)/CySNO (1 mM) in blue, red, and black, respectively[163]. Reprint by permission from Royal Society of Chemistry.

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Table I.5. 1. Relevant applications of MCE-EC for biological applications

Application	BGE	Separation Voltage	Electrode material	Size (µm) ^c	Format	Detection	Detection potential	Detection mode	System (electrodes)	Concentration range (µM)	LOD (µM)	Ref.		
Dopamine	boric acid /SDS	280 V cm ⁻¹	Carbon ink	13	Microband	Amperometry	+0.9 V vs. Pt	Off- channel	2-	100 to 300	9	[123]		
Dopamine	boric acid	70014		10				off-		NA	9.3	[450]		
Norepinephrine	hrine /SDS	700V	Carbon ink	10	Microband	Amperometry	+0.9 V vs. Pt	channel	2-	NA	7.5	[156]		
Dopamine	TES/SDS	TES/SDS	TES/SDS	$160 \mathrm{V} \mathrm{cm}^{-1}$	Carbon	33	Microdisk	Amperometry	$\pm 0.0 \text{ V}$ vs. Dt	off-	2-	NΔ	NA	[157]
Norepinephrine			fiber	55	Whereast	7 imperometry	·0.9 v v3.1t	channel	2	1471	4	[137]		
Dopamine	TEC/CDC	330 V cm ⁻¹	Carbon	33	Microdisk	Amperometry	$\pm 0.0 \text{ V}$ vs. Pt	off-	2	NA	0.6	[158]		
Norepinephrine	125/505	550 V CIII	fiber	55	WICIOUISK	Amperometry	10.9 V VS.11	channel	2		NA	[130]		
Nitrite	PBS	-1600 V	Pt	15	Microband	Amperometry	+1.05 V vs. Pt	In-channel	2-	2.5 to 25	0.75	[159]		
Uric acid	PBS	100 V cm^{-1}	graphite	50	Microband	Amperometry	10.7 V vs.	off-	3_	10 to 1000	NA	[160]		
L-ascorbic acid			graphic	50			Ag/AgCl	channel	-נ	10 10 1000	1171	[100]		
Nitrite		oric acid/	1	15	Microband		+1.1 V vs.	In-channel		2 1 to 50	1.44			
T thirte	boric acid/		Pt			Amnerometry	Ag/AgCl ^a		3_	5.1 10 50	1.11	[162]		
Nitrite	TTAB						+1.1 V vs. Pt ^b			3.1 to 50	0.58	[]		

					CHAP	ΓER I: STATE	OF THE AR	T 158					
HaOa							+1.1 V vs.			NA	7 37		
11202							Ag/AgCl ^a			INA	1.57		
ЦО							+1.1 V vs.			NTA	0.75		
H_2O_2							Pt^b			INA	0.75		
							+1.1 V vs.	-			214	-	
lyrosine							Ag/AgCl ^a			NA	NA		
							+1.1 V vs.	-				-	
Tyrosine							Pt ^b			NA	0.14		
Urea										100 to 10000	100		
K ⁺												-	
Na ⁺		••••••	~ ()	• • • •			-0.23 V vs.	Off-				[100]	
Ca ²⁺	MES	300 V cm^{-1}	Cr/Au	300	Microband	Amperometry	Pt	channel	3-	NA	NA	[108]	
Mg^{2+}													
NH4 ⁺													
S-													
nitrosoglutathione	Arginine/	1000 V cm ⁻¹	Pt	50	Microband	Amperometry	+ 0.7 V vs.	End-	2-	130 to 675	20	[163]	
S-nitrosocysteine	acetic acid					Conductivity	Pt	channel					
							0.85 V vs.	End-					
Nitrite	boric acid/		Pd	40	Microband	Amperometry	Ag/AgCl	channel	3-		20		
Nitrate	TTAR	1.2 kV								NA	67	[164]	
Nitrite				Cu	1000	band	Conductivity					308	-
INITIA											300		

NA = not available

* tetradecyltrimethylammonium bromide (TTAB), 2-morpholinoethanesulfonic acid monohydrate (MES).

 $\ensuremath{^*\!a}$ one single WE electrode

*b two WE electrodes (dual)

*c diameter for microdisk or width for microband

I.5.2 Applications in the analysis of food samples

Table I.5.2 summarizes the most significant examples of MCE-EC devices for food analysis. In particular, MCE with conductivity detection was developed for the quantitative analysis of inorganic and organic ions in beverages, either alcoholic or non-alcoholic. Lee *et al.* [165] reported the production of semi-circular detection electrodes in a glass substrate using integration strategies such as sputtering for the deposition of metal electrodes and lift-off as a standard process. The semi-circular Au electrodes in the head to head geometry were assembled by the two plates bonding, with metal deposited on the cover plate and within the channel of the base plate. The authors compared the results achieved after the injection of inorganic cations, using semi-circular electrodes and planar electrodes, giving rise to a lower standard deviation (7.8%) for the semi-circular geometry compared to 18.3% for the planar one. The proposed device was applied for the screening of inorganic compounds in red wine, commercial sports drinks, and drinking waters.

Recently, Quero *et al.* [146] proposed a polyester thread based assembled in a 3Dprinted platform as a novel MCE device with C⁴D detection (Figure 21.1). This 3D printed platform was designed with four reservoirs and circular patterns to embrace the copper electrodes. The device was employed to screen Na⁺ and K⁺ ions in diet soft drink and regular soft drink (Figure I.5. 2), achieving remarkable LODs (0.50 and 0.69 μ M for K⁺ and Na⁺, respectively), which were similar or lower than those described for C⁴D in silica capillary and other microchip devices [166]. This improvement in the detectability is owing to the thin isolation layer (7.5 μ m) of the polymer that covered the commercial copper wire used as electrode, clearly indicating that the smaller the thickness of the isolation layer, the lower the electrical impedance of the capacitors formed between the electrodes and the channel solution.



Figure I.5. 2. Polyester thread based MCE-EC (1) The3D-printed platform device assembled in an aluminium cage. Top insert : injection intersection, down insert : C⁴D electrodes. (2) Electropherograms of (A) a standard solution of K⁺ and Na⁺ (75 μ M, each); (B) lemon and (C) cola diet soft drinks [146]. Reprint by permission from Elsevier.

Amperometric detection in MCE systems is also widely used to quantitate organic compounds in beverages and oils. Du and Fung[167] developed a PMMA-based microchip with dual opposite electrodes in an end-channel configuration. The carbon-fiber electrodes were integrated with epoxy using embedded strategy. The authors achieved analytical features, such as LODs (0.031–0.21 mg/L), repeatability (2.0–3.3% RSD, n=5), working range and recovery rate (94-108%), suitable for peak purity assessment of polyphenols in red wine samples. Petroni et al. [124] reported a hybrid microchip (PDMS/ acrylic) with integrated handmade graphite electrodes (3-electrode cell format in an end-channel configuration) using a screen-printed approach to separate target compounds in drinking water. The new methodology permits to discriminate nitrite from other anions naturally occurring in the sample (F⁻, Cl⁻, NO₃⁻ and SO₄⁻ ²), thus avoiding any interference. Furthermore, the group compared the results from the proposed method with the ones of a reference method and performed recovery studies (95.4%) in order to validate the methodology. Aghdam et al. [95] reported a hybrid microchip (SU-8/ glass) to separate and detect synthetic antioxidants in edible oils by commercial integrated Au electrodes (3-electrode cell format in an end-channel configuration). The proposed method was validated by a reference HPLC method. The authors applied the paired t-test revealing that there is no difference between obtained results with a confidence level of 95%.

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Table I.5. 2 Relevant applications of MCE-EC for food applications

Application	BGE	Separation voltage	Electrode material	Size ^a (µm)	Format	Detection	Detection potential (amperometry)	Detection alignment	System (electrodes)	Concentration range	LOD	Ref.	
K^+										$1.67 - 125^{1}$	0.51		
Na ⁺	MES/HIS	1 kV	copper	1000	band	Conductivity		C ⁴ D	Antiparallel	$2.30 - 125^{1}$	0.691	[146]	
Li ⁺										3.57-1251	1.071		
K ⁺ Mg ²⁺ Ca ²⁺ Na ⁺	MES/HIS	300 Vcm ⁻¹	Au	NA	Semi circular	Conductivity		C ⁴ D	Head-to- head	NA	NA	[165]	
Trans-Resveratrol	Dorato/									$0.28-20^2$	0.031^2		
(+)-Catechin	B CD/	12 kW	carbon-	20	Micro	Amnoromotru	1.0 V/0.8 V vs.	End-	2 alaatradaa	0.28-40	0.11	[167]	
(–)-Epicatechin	р-СD/ 12 KV MeOH	12 K V	fiber	50	disk	Amperometry	Ag/AgCl	channel	2-electrodes	0.28-40-	0.0502	[107]	
Quercetin		MeOH									0.83-402	0.162	_
Gallic acid										1.0-802	0.212		
Nitrite	Phosphate/ CTAB	Phosphate/	NA	graphite	400	Micro	Amperometry		End-	3-electrodes	0.5-5 2	8.21	[124]
Ascorbic acid			8		band			channel		20-200 ¹	12.71		
TBHQ					Miara			End		10-1001	0.81		
BHA	Borate	2 1 kV	.V Ti/ Au	50	Micro	Amperometry	+1.0 V vs. Au	Ena-	3-electrodes	10-100 ¹	1.41	[95]	
PG					Danu			channel		20-2001	4.31		

NA = not available

*2-morpholinoethanesulfonic acid monohydrate (MES), histidine (HIS), propyl gallate (PG), tert-butyl hydro- quinone (TBHQ), and butylated hydroxyanisole (BHA).

*1 µM *2 mg/L

*a Diameter for microdisk or width for microband

I.5.3 Application in the analyses of environmental samples

The applicability of MCE- C⁴D systems for screening degradation products in environmental samples is widely explored by Wang group[168]. The authors measured chemical warfare agent degradation products in natural (river) water using a PMMA based MCE-EC. The aluminium (Al) electrodes (in anti-parallel configuration) were fixed in PMMA substrate using a common epoxy glue. Later, the authors detected low-energy ionic explosives and nerve agent degradation products using the same microchip with a movable detection cell [169]. Ding *et al.* [170] have also demonstrated the separation of organophosphonate nerve agent degradation products using a PDMS device with embedded electrodes in an anti parallel configuration. Under optimal conditions, the analysis of five compounds was achieved in less than 2 min. This new methodology can be applied for on-site monitoring of degradation products of chemical warfare agents in water samples.

The feasibility of amperometric detection in environmental samples was also described by Pelle *et al.* [171] using a glass/PMMA microchip. The carbon black nanoparticle (CB-NPs) electrodes were transferred into the PMMA substrate by pressure (Figure I.5. 3.A). For the first time, the authors describe a MCE-EC workflow including an external sample treatment step for monitoring phenyl carbamate in real environmental samples (river, lake, and irrigation waters). The authors achieved analytical features, such as LODs (7–12 μ M), relative standard deviation (RSD, n =10) for the peak intensity and migration times below 3% and 8 %, respectively, and working range (25–125 μ M). These features showed the suitability of the press-transferred carbon black nanoparticles detectors for the screening of pesticides (Figure I.5. 3.B). Significant other applications are summarized in Table I.5.3.



Figure I.5. 3. (A) Scheme of the carbon black nanoparticulate (CB-NPs)-based electrode onto a PMMA device, with a zoom on the CB-NPs electroactive film. (B) Cyclic voltammograms of isoprocarb (red line), carbofuran (blue line) and carbaryl (green line) hydrolytic forms at 0.1 mM [171]. Reprint by permission from Springer Nature.

Targeted analytes	BG E	Separation voltage	Electrode material	Size ^c (µm)	Format	Detection	Detection mode	System	Concentration range	LOD	Ref.
PMPA IMPA EMPA MPA	ME S/H IS	-250 V cm ⁻¹	Al	800	band	Conductivity	C ⁴ D	antiparallel	0.3-100 ²	0.48-0.86 ²	[168]
CH ₃ NH ₃ ⁺ NH ₄ ⁺ Na ⁺	ME S/H IS	208 V cm ⁻¹	Al	800	band	Conductivity	C ⁴ D	antiparallel	NA	NA	[169]
IMPA PMPA EDPA EMPA MPA	ME S/H IS	1.2 kV	Al	15000	band	Conductivity	C ⁴ D	antiparallel	1.3-4.5 ²	4.5 ³ 2.0³ 2.0³ 2.9³ 1.3³	[170]
carbofuran isoprocarb carbaryl	Bor ate	1 kV	carbon black	0.030 ^d	nanoban d	Amperometr y +0.70 V vs Ag/AgCl	End-channel	3-electrodes	25–125 ¹	9 ¹ 7 ¹ 12 ¹	[171]

Table I.5. 3 Relevant applications of MCE-EC for environmental applications

*Isopropyl methylphosphonic acid (IMPA), pinacolyl methylphosphonic acid (PMPA), O-ethyl-N,N-dimethyl phosphoramidate (EDPA), ethyl methylphosphonic acid (EMPA), and methylphosphonic acid (MPA),

2-morpholinoethanesulfonic acid monohydrate (MES), histidine (HIS).

NA = no available

 $^{*_{1}}\,\mu M \;\;^{*_{2}}\,mg/L\;^{*_{3}}\,ppm$

*° width

*d nanoparticles size

This first chapter presented the state of the art of microfluidics, 3D printing technology and electrochemical detection. We addressed the recent advances in MCE-EC configurations, materials and fabrication process, focusing on the electrodes fabrication and electrochemical performance. In addition, we have demonstrated a several approaches to fabricate microchip electrophoresis with integrated electrodes. The alliance between microfluidics, capillary electrophoresis and electrochemistry showed as an attractive approach since they have a synergistic effect over each inherent features enhancing their potential and offering a technology covering these challenges. However, according with the literature, the fabrication of microfluidics using 3D printing is still in the early stages.

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CHAPTER II. 3D-PRINTED MCE DEVICE WITH C⁴D DETECTION

This chapter corresponds to a research article "3D-printed microchip electrophoresis device containing spiral electrodes for integrated capacitively coupled contactless conductivity detection" published in 2022 [28].

Summary

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3D three dimensional	MCE microchip capillary electrophoresis
AM additive manufacturing	MES 2-(N-morpholino)ethanesulfonic acid
BGE background electrolyte	NPS 2-nitrophenyl phenyl sulfide
C ⁴ D Capacitively coupled contactless	PEGDA poly(ethylene glycol) diacrylate
conductivity detection	
CE capillary electrophoresis	RNS reactive nitrogen species
DLP digital light processing	ROS reactive oxygen species
FDA Food and Drug Administration	RSD relative standard deviation
FDM fused deposition modelling	SLA stereolithography
LOD limit of detection	

Symbols and abbreviations

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THE 3D-PRINTED MCE DEVICE CONTAINING SPIRAL ELECTRODES FOR C⁴D DETECTION

Abstract

In this work we demonstrate for the first time the design and fabrication of microchip electrophoresis devices containing cross-shaped channels and spiral electrodes around the separation channel for microchip electrophoresis and capacitively coupled contactless conductivity detection. The whole device was prepared in a digital light processing based 3D-printer in poly(ethylene glycol) diacrylate resin. Outstanding X-Y resolution of the customized 3D printer ensured the fabrication of 40 μ m cross-section channels. The spiral channels were filled with melted gallium to form conductive electrodes around the separation channel. We optimize the device and the methodology, which is simple, is faster than other methods in the literature with 40 s total separation. The determination of nitrate was achieved with a background electrolyte composed of 30 mol L⁻¹ lactic acid and 15 mol L⁻¹ histidine (pH 3.8). The limit of detection for nitrate was found to be 2 μ mol L⁻¹. The device was employed in the analysis of nitrate in saliva samples, a significant biomarker in clinical diagnosis related to inflammatory diseases.

Keywords: Liquid metal electrodes, biological samples, nitrate, microchip electrophoresis, 3D printing.

II.1. Introduction

Although additive manufacturing (AM) has been extensively used in different fields to produce relatively large objects, the use of 3D printing in the production of microfluidic devices has gained increased attention only in the last few years [1]. In practice, most 3D printers offer resolution of hundreds of microns which allows the construction of fluidic systems on the millimeter scale with excellent repeatability. It is worthwhile to mention that layer-to-layer sealing steps typical for planar micromachining are not needed in 3D printing, and the fabrication can be done without cleanroom facilities, which allied to the low cost of the materials makes 3D printing very attractive for prototype development.

In view of the advantages presented by the introduction of conductive parts in microdevices, such as resistive heaters, electrodes for detection and electric field application, and electrical connections, it is desirable to establish protocols for the fabrication of more complex microfluidic devices. Indeed, fluidic devices containing conductive parts can be created in a single step by multimaterial 3D printing. Despite this benefit, multimaterial FDMbased 3D printers cannot currently compete with the resolution of structures produced by a custom DLP-based 3D printer. Alternatively, a multimaterial DLP-based 3D printer uses several resin baths, requiring cleaning steps at each resin exchange, and each layer requires multiple exposures in each resin, making the process complex and inefficient [2]. To overcome this drawback of fabrication, some groups found creative and simple alternatives to assemble complex fluidic devices with integrated conductive parts. In 2017, Yu et al. presented a method to incorporate metal connections in FDM-based structures, aiming at the construction of 3D circuit boards [3]. In their approach, a eutectic alloy of gallium and indium was injected in void channels created during the printing to form conductive wires. Khoshmanesh et al. published a comprehensive review on the use of liquid metal in microfluidics [4]. In the same direction, Khondoker and Sameoto reviewed the applications of gallium alloys and described different techniques for structurally depositing these conductive materials [5]. In work not involving 3D printing, Gaudry et al. [6] and Thredgold et al. [7] reported electrophoretic microchips containing in-plane electrodes of molten metal alloy and liquid gallium, respectively. Both groups demonstrated the electrode performance using a capacitively coupled contactless conductivity system to detect inorganic cations.

Capacitively coupled contactless conductivity detection (C⁴D) [8,9] is an interesting electrochemical detection mode due to its low cost, easy miniaturization, and intrinsic decoupling of the separation electric field, which makes it suitable for microchip capillary electrophoresis (MCE) applications [10,11]. C⁴D is a well-established detection technique and has been successfully applied to capillary electrophoresis and MCE, and many applications can be found [12]. Many configurations of the detection cell geometry have been proposed in MCE, which usually involve the deposition of thin metal films on the top or bottom of the separation channel, although some authors proposed the insertion of electrodes on the side walls [12]. The sensitivity of the detector and frequency response are directly related the cell geometry, and better results have been obtained in capillary electrophoresis by using tubular electrodes, whose positioning is facilitated by the cylindrical form of capillaries [8,9]. Conversely, the conventional protocols for microfabrication of MCE devices make it difficult

to construct detection cells that surround the separation channels. In this sense, 3D printing offers an effective way to construct C^4D detection cells directly integrated in the microchip in a single fabrication procedure.

Saliva constitutes an attractive source of biological sample that avoids causing discomfort to the patient during its collection [13]. In addition, many substances present in the blood are also present in saliva, so this biological matrix can be an indicator of the condition of the blood as well as the rest of the body. Many constituents used as biomarkers or indicators of biological condition can be found in saliva. For example, oxidative stress results from imbalance between oxidant and antioxidant species, with prevalence of oxidative species resulting in harmful action, for instance, reactive oxygen species (ROS) and reactive nitrogen species (RNS), in cells [14]. These reactive species are signaling molecules that are involved in the regulation of cellular function. Moreover, redox imbalance in an organism can be associated with inflammatory diseases caused by eating habits or some biological dysfunction including sepsis, asthma, tuberculosis, and periodontal disease [14]. A key strategy in reducing inflammatory disease would be to create a widely available diagnostic tool for reactive species determination in body fluids, such as nitrate, which is a nitric oxide metabolite [15]. Microfluidic devices are well suited to address this need because of their advantages, for instance, low reagent and sample consumption, small size, portability and enabling multiprocess integration. In addition, for these analyses we can apply MCE for determination because this technique is commonly used for inorganic ion analysis as an alternative to chromatography[16,17] or electrochemistry [18,19].

In this work, we demonstrate a novel approach to integrate electrodes into MCE devices for C⁴D detection. We develop a simple way to assemble a fully microfluidic device using liquid metals and DLP 3D printing technology. The suitability of the microfluidic device is demonstrated through a fast, low-cost and simple methodology to screen nitrate in saliva. We evaluate this methodology according to FDA guidelines, and perform studies of repeatability, linearity, detection, LOD and LOQ. To the best of our knowledge, this is the first report on C⁴D detection in a DLP-based 3D-printed microfluidic device.

II.2. Materials and methods

II.2.1. Reagents and materials

All reagents used were of analytical grade and were used as received. The solutions were prepared with deionized water of 18.2 M Ω ·cm. We used lactic acid (Synth, Diadema Brazil), 2-(N-morpholino)ethanesulfonic acid (MES) and histidine (Sigma-Aldrich) to prepare the background electrolyte (BGE) buffer solutions. Sodium nitrate, sodium nitrite, potassium chloride, sodium chloride and lithium chloride from Sigma-Aldrich were utilized to prepare stock standard solutions. For 3D printing, poly(ethylene glycol) diacrylate (PEGDA, MW 250) and phenylbis (2,4,6-trimethylbenzoyl) phosphine oxide (Irgacure 819) was acquired from Sigma-Aldrich and 2-nitrophenyl phenyl sulfide (NPS) was purchased from TCI Chemicals (Portland, OR). Metallic gallium was purchased from Sigma-Aldrich. Artificial human saliva was purchased from Biochemazone (Alberta, CA).

II.2.2. Microchips Design and Fabrication

We designed microfluidic devices for 3D prints using OpenSCAD (openscad.org), a free computer aided design software program (Figure II.1.a). The standard cross channel MCE layout, with channels 45 μ m wide and 50 μ m tall, was adapted from one we published earlier [20].We added to this design two independent spiral channels for C⁴D, which were wrapped around the separation channel near the detection point. The coil radius is 100 μ m with an amplitude of 300 μ m and a pitch of 300 μ m (Figure II.1.b).



Figure II. 1. OpenSCAD design for C^4D spiral electrode device. (a) Top-down and (b) Orthogonal device view with electrophoresis reservoirs shown in purple, channels in green, and the C^4D electrodes with spirals in turquoise.

Devices were fabricated using a custom-built 3D printer we have described previously[21]. Briefly, this DLP stereolithography 3D printer uses 385 nm light to sequentially polymerize 10-µm-thick patterned resin layers. The resin consisted of 97%

PEGDA, 2% NPS UV absorber, and 1% Irgacure 819 photoinitiator. Once a device was polymerized, the channels were washed of excess resin under negative pressure and next rinsed with isopropyl alcohol. The microfluidic device was then exposed to a UV post-cure for 20 min to polymerize any excess resin (Figure II.2.a). After microchip preparation, gallium was melted by heating at about 35 °C and injected in the spiral channels by filling one channel end with gallium while negative pressure was applied to the other channel end using a plastic syringe to form metallic electrodes around the separation channel (Figure II.2.b). Copper wires were used for electrical contact to gallium electrodes and the detection circuitry (Figure II.2.c). Finally, the MCE-C⁴D is ready for applications (Figure II.2.d).



Figure II. 2. Scheme to assemble a fully microfluidic electrophoretic device. (a) device was exposed to a UV postcure; (b) Ga was melted and inserted into electrode channels by negative pressure using a plastic syringe; (c) copper wires were used for electrical contact; (d) device ready for measurements.

Briefly, the simple t-device had dimensions of 12 mm width and 23 mm long, with injection channel 5.3 mm length and separation channel 13 mm long, with an effective separation distance of 9.5 mm (Figure II.3.a). Concerning the two independent spiral channels for C⁴D (Figure II.3.b), each coil channel radius was 100 μ m, with a spiral amplitude of 300 μ m and pitch of 300 μ m, and producing an 11 mm total length of the gallium wire after filling (Figure II.3. c).



Figure II. 3. (a) Printed device showing the connections with the spiral electrodes and the microchip size. (b) Rendered CAD view of the microchip. (B1) Detail of the serpentine flow channel used to inject gallium. (B2) Inlet and outlet reservoirs connected to the serpentine channel for metal injection. (B3) Buffer waste reservoir. (B4) Sample reservoir. (B5) Buffer reservoirs. (c) 3D serpentine electrode channels surrounding the separation channel in a DLP-fabricated microfluidic device (A1) without melted gallium and (A2) filled with melted gallium to form spiral electrodes for C⁴D.

II.2.3. Instrumentation and electrophoretic separations

Electropherograms were obtained using a custom built C⁴D system described previously [9]. Platinum electrodes (1mm o.d.) connected with copper wires were placed to the reservoirs to apply the voltage for electrokinetic sample injection and CE separation. The MCE device and electric cables were arranged in an aluminium case to act as a Faraday cage, avoiding external electromagnetic interferences. One spiral electrode was connected to the signal generator whilst the other spiral electrode was connected to the input of the C⁴D circuitry by copper wires. This detector was used and operated with a sinusoidal excitation signal with frequency of 250 kHz and 3.5 V_p (Peak voltage). Amounts (10 μ L) of standard or buffer solutions were added to sample reservoir, sample waste, buffer reservoir, and buffer waste using a micropipette using gated injection protocol for inorganic cations and floating injection protocol for nitrate. The analytes present in the sample plug were then separated according to their mobilities and migrated toward the sensing electrodes under electrokinetic transport. The voltages for introduction and MCE separation were applied by a four-channel high voltage power supply ER430 (eDaq, Sydney, Australia). Data acquisition and electrokinetic control were performed using Labview and QuadSequencer (eDAQ), respectively.

II.3. Results and discussion

Achieving complex layouts, in this case spiral channels around a central separation channel, would be very complicated using conventional prototyping of microfluidic devices,

such as lithography and soft lithography owing to its subtractive characteristic. Also, most AM techniques lack the desired resolution needed to produce truly microfluidic devices. Thus, SLA and DLP are the most promising approach to use in this work to create complex microfluidic structures due to its high resolution. First, we tried to reproduce a classical tubular design of electrodes to perform C⁴D detection. However, for this electrode design, it was laborious to fill the channels with liquids in a homogeneous way due to trapped air. In this context, we created a spiral design, for which the performance can be comparable to a tubular electrode since it surrounds the channel as well. Furthermore, the electrode channels can be easily filled with liquid metals without compromising the electrode connection or even its integration.

II.3.1. Characterization and optimization of the electrode

In C⁴D, the excitation voltage and operating frequency are parameters that directly affect the analytical response. Thus, both parameters were optimized prior to maximizing the detector sensitivity. The C⁴D signal was investigated using frequency values ranging from 84 to 628 kHz keeping the excitation voltages constants at 3.0, 5.0 and 7.0 V_p values (Figure II.4) while the separation channel was filled with 5 mmol L⁻¹ MES/histidine BGE.



Figure II. 4. Frequency response for the C^4D -MCE device at different amplitudes of the excitation signal. The separation channel was filled with a buffer solution containing 5 mmol L⁻¹ of 2-(N-morpholino)ethanesulfonic acid and histidine.

According to our previous work [9] on C⁴D development, the best frequency of operation depends mostly on the geometric parameters of the detection cell, although the

conductivity of the BGE also exerts some influence. Moreover, the measurement of the baseline output voltage directly correlates with the peak area, being a more convenient method for frequency optimization [9]. As can see in Figure 1.5, by increasing the frequency of operation, the output voltage increases up to maximum value and from this point the output voltages decreases. This is due to the capacitances of the detection cell: at low frequencies, the wall channel capacitance dominates and blocks most of the excitation signal, while at high frequencies stray capacitances (including the input capacitance of the transimpedance amplifier) offer alternative paths for the signal. Usually, the frequency is set at this maximum for the best response. Moreover, this maximum is shifted to lower frequencies by increasing the amplitude of the signal. The maximum values measured at 3.0, 5.0, and 7.0 V_p were obtained for the MCE-C⁴D were 293, 238, and 207 kHz, respectively. In summary, for a given amplitude and BGE composition the frequency is scanned and set at the maximum for further experiments.

To study the analytical performance of the MCE-C⁴D as well as its use for monitoring cationic and anionic species in biological samples, the operating frequency and excitation voltage values were then fixed at 250 kHz and 3.5 Vp, respectively. We next sought to evaluate the operating frequency and excitation voltage values for the determination of the best separation conditions for K⁺, Na⁺ and Li⁺ concerning buffer concentration and the voltage applied. A standard mixture of these inorganic cations was selected as a model analyte system since they are commonly used ions to provide information about C⁴D system behavior, due to lack of complexity of the system [22,23]. Regarding the DLP-based microchips, the best results were achieved by using BGE composed of 20 mmol L⁻¹ MES/histidine (pH = 6.0) at 25 °C. The separation voltage of 800 V, injection voltage of 500 V, and injection time of 3 s were selected for the separation of K⁺, Na⁺ and Li⁺ with adequate efficiency and peak resolution (Figure II.5).



Figure II. 5. Electropherograms provided by the introduction of standard solutions containing the ions K⁺, Na⁺, and Li⁺ (25-200 μ mol L⁻¹). Respective calibration curves are also shown. Gated injection applying +500 V and + 800 V at sample and buffer reservoirs.

In addition, linearity of the method was evaluated using standard solutions of K⁺, Na⁺ and Li⁺ (25-200 μ mol L⁻¹). Adequate linearity (r > 0.996) was achieved for all calibration plots (Figure 2), which shows a suitable response of the detector to concentration of the analytes. This is better performance compared to other detectors fabricated by liquid metals and previously reported in the literature. For example, Thredgold et al. [7], reported a gallium electrode with an in-plane design for C⁴D. This electrode provided a linear correlation coefficient of 0.9729, 0.9794 and 0.9982 for K⁺, Na⁺ and Li⁺, respectively. Even though the electrodes were made of the same material, the electrode spiral geometry shown here offered a better linear response than in-plane electrode design.

II.3.2. Choice of background for determination of nitrate in saliva

Subsequently, nitrate determination was achieved under conditions previously reported [24]. In optimizing the detector, a buffer solution composed of lactic acid and histidine was prepared at different proportions to inspect its detection quality. The lactic acid concentration was fixed (30 mmol L^{-1}) and the histidine concentration varied in a range from 10 to 20 mmol L^{-1} (with 5 mmol L^{-1} increments). Despite all proportions successfully showing a signal for nitrate, the best performance considering analysis time, signal-to-noise ratio, ionic strength/buffer capacity and detectability was achieved using BGE 30 mmol L^{-1} lactic acid/15

mmol L⁻¹ histidine (pH 3.8) at 25 °C. Standard and sample solutions were introduced inside microchannels through a floating injection protocol. The separation voltage of -700 V, injection voltage -500 V and injection time 3 s were selected for determination of nitrate in saliva.

II.3.3. Analytical performance and application

Studies were carried out to systematically investigate the analytical performance of MCE-C⁴D for the determination of nitrate in biological samples. A common issue concerning the use of electrophoresis chips is electrokinetic injection, which can compromise the repeatability of sample injection. Therefore, we spiked a standard solution of nitrate to achieve a concentration of 100 μ mol L⁻¹ into the saliva sample and then we executed ten successive injections to investigate the injection repeatability (Figure II.6).

Intraday RSD of the peak area and migration time were 8.6 and 5.0%, respectively. Based on the statistical results it can be inferred that this MCE-C⁴D method provides suitable repeatability for ten successive injections. A summary of analytical features for this methodology involving MCE-C⁴D is shown in Table II.1.



Figure II. 6. Electropherogram obtained for ten consecutive injections of a saliva sample solution containing 100 μ mol L⁻¹ of nitrate. BGE: 30/15 mmol L⁻¹ lactic acid/His (pH 3.8). Floating sample injection at -500 V for 3 s. Separation voltage was -700 V. C⁴D detector operating at 250 kHz and 3.5 Vp.

Parameter	Values
Migration time (s) ^a	17.8 ± 0.9
Peak Area ^b	0.040 ± 0.003
LOD (μ mol L ⁻¹)	2.0
$LOQ (\mu mol L^{-1})$	6.0
Linear range (μ mol L ⁻¹)	6-200
Analytical frequency (h ⁻¹)	105

Table II. 1. Analytical performance for the determination of nitrate using MCE-C⁴D methodology

^a (n=3); ^b average \pm standard deviation (n=10)

The method shows suitable analytical performance for determination of nitrate in saliva. Good correlation coefficient (r = 0.994), calibration parameters and wide linear range (Table II.1) were obtained in a linearity study using external calibration curves (Figure II.7.a), thereby enabling the device to perform quantitative analysis. We also characterized the detection limits for nitrate using an empirical approach, which involved successive measures of more dilute standard solutions until a signal-to-noise ratio of 3:1 was reached.

The LOD is suitable for the target analyte in saliva since the levels found in saliva of healthy and ill: for example, patients with periodontal disease can present levels of nitrate between 800 and 1500 μ mol L⁻¹ [25]. In addition, our LOD is lower or comparable to the values reported in existing protocols using MCE-C⁴D [24] and CE-UV [26].

Subsequently, the performance of the microchip and the optimized method was evaluated by the quantification of the target compound in biological sample. We spiked a standard solution of nitrate to achieve a concentration of 20 μ mol L⁻¹ in the saliva sample. In addition, we performed a specificity test, where we compared electropherograms from samples with and without the analyte (Figure II.7.b). These data demonstrate that our method has the ability to discriminate compounds commonly present in saliva from the target analyte.



Figure II. 7. (a) Calibration curve obtained from the introduction of standard solutions containing nitrate $(6 - 200 \ \mu \text{mol } \text{L}^{-1})$ using $\mu \text{CE-C}^4\text{D}$. BGE: 30 mM lactic acid/15 mM His (pH = 3.8). (b) Electropherograms obtained for the introduction of a sample containing 20 μ mol L⁻¹ of standard nitrate (–) and sample without nitrate (–). Floating sample injection at -500 V for 3 s. Separation voltage was -700 V. C⁴D Detector operating at 250 kHz and 3.5 V_{pp}.

The result obtained from our procedure is similar to the expected result (Table II.2), with a 5% relative difference. In a fast electrophoretic run, our microfluidic device allows obtaining information on the nitrate levels in a biological matrix

Table II. 2. Determination of nitrate in saliva by MCE- C⁴D.

Sample	Analyte	Expected (µmol L ⁻¹)	MCE-C ⁴ D (µmol L ⁻¹)	Relative Difference
Saliva	NO ₃ -	20	21 ± 2	-5%

II.4. Conclusion

In this work, we have demonstrated DLP-based 3D-printing of microfluidic devices with 40 µm channels for MCE analysis of reactive nitrogen species in biological samples. For the first time, we presented a DLP-based 3D-printed microfluidic device with C⁴D detection applied to screening nitrate in biological fluids with a high analysis frequency suitable for routine screening, and a method that does not require laborious sample preparation. We show the use of DLP 3D printing to make microfluidic devices for analysis of a medically relevant biomarker. These microfluidic devices have potential for use as a widely available diagnostic tool for nitrate.

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CHAPTER III: SLA BASED 3D-PRINTED MICROFLUIDIC DEVICE WITH INTEGRATED EC DETECTION

This chapter contains the results on the integration of the electrodes in a microfluidic device, presented in the article "Stereolithography based 3D-printed microfluidic device with integrated electrochemical detection" [1], along with subsequent results that are not yet published, on the final objective of coupling MCE with EC.

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Symbols and abbreviations

[Fe(CN) ₆] ^{3-/4-} hexacyanoferrate (III)/(II)	FDM fused deposition modelling	
$[\mathbf{Ru}(\mathbf{NH}_{2})]^{3+}$ hexaammineruthenium (III)	FTIR-ATR Fourier transform infrared	
	spectroscopy-attenuated total reflectance	
3D three dimensional	LOD limit of detection	
Ag/AgCl silver-silver chloride	LOQ limit of quantification	
AM additive manufacturing	MCE microchip electrophoresis	
BGE background electrolyte	PBS phosphate buffered saline	
C/resin graphite and resin	PDMS polydimethylsiloxane	
C ⁴ D capacitively coupled contactless	PMMA poly(methyl methacrylate)	
conductivity detection	- · · · · /	

CE counter-electrode	R _{ct} charge transfer resistance
CP carbon paste	RE reference electrode
CV cyclic voltammetry	RSD relative standard deviation
DLP digital light processing	SLA stereolithography
EIS electrochemical impedance	SWV square wave voltammetry
spectroscopy	Svv v square wave voltammetry
EMDs electrochemical microfluidic	WCA water contact angle
devices	
EOF electroosmotic flow	WE working electrode
FcMeOH ferrocene methanol	

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STEREOLITHOGRAPHY BASED 3D-PRINTED MICROFLUIDIC DEVICE WITH INTEGRATED EC DETECTION

Abstract

We demonstrate for the first time the conception of a reusable stereolithography (SLA) based 3D printed separation device with integrated electrodes for electrochemical (EC) detection. For this purpose, a new lab-made working electrode (WE) was developed by 3Dprinting a side wall microchannel along the separation microchannel and by inserting a graphite/ resin (C/resin) composite within this side wall microchannel. The 3D-printing conditions of the electrochemical microfluidic device (EMD) as well as the electrode composition, geometry, and integration were optimized for easy and low-cost fabrication (no need for a clean room) and suitable electrochemical response. The electrochemical characterization of the material formulation was first performed in bulk format (disk-shaped, 2 mm diameter) and then moved to the micrometric scale reaching electrode width of $172 \pm 12 \mu m$. The integrated electrodes were characterized by voltammetry and electrochemical impedance spectroscopy, and the response linearity of the transducer material was investigated by square wave voltammetry (SWV) using ferrocene methanol (FcMeOH). The C/resin electrode led to a good repeatability (RSD of $\pm 2\%$ (n=3) in the peak current values), linear response (R² = 0.995) in the 15 - 1000 μ mol L⁻¹ range, allowing for limits of detection and quantitation of 4.5 μ mol L⁻¹ and 15 μ mol L^{-1} , respectively. In order to develop a complex analytical device, the interest of this dedicated device was evidenced in a hydrodynamic state condition with [Ru(NH₃)₆]³⁺ as a proof-ofconcept, ranging from 0 to 55 μ L min⁻¹ mimicking the electroosmotic flow conditions, which is a bulk liquid motion very common in electrically driven separation techniques. In addition, the feasibility of coupling microchip electrophoresis (MCE) with EC was tested with an endchannel chronoamperometric detection of $[Ru(NH_3)_6]^{3+}$, achieving LOD and LOQ of 4.5 µmol L^{-1} and 15 µmol L^{-1} , respectively, linear range (100 to 700 µmol L^{-1}) and good correlation coefficient ($R^2 = 0.996$).

Keywords: 3D-printing, microfluidic device, electrochemical detection, carbon-based electrodes.

III.1. Introduction

Outstanding works microfluidics and electrochemistry as emerging tandem for analytical microsystems have been reported by several groups [2–4]. However, the manufacturing of electrochemical microfluidic devices (EMDs) must solve certain challenges. The classical fabrication processes to assemble devices with microfluidic channels require clean room facilities and the alignment and positioning of the electrodes can induce some issues in the analysis reproducibility. The electrode material must be compatible with the material of the device to avoid leakage and promote a robust experimental platform. Although miniaturized ready-to-use devices with electrochemical detection are commercially available, they are coupled with microelectrodes made of precious metals, such as gold or platinum, turning the devices very expensive [5]. Metal electrodes are usually fabricated by depositing a nanometric layer of conductive material via evaporating or sputtering the metal onto the substrate to form the electrode. These methods generally require an adhesion layer to get adherent metallic coatings and dedicate expensive instrumentation and laborious processes [6]. Moreover, metal electrodes have a relatively short lifetime and tendency for fouling when dedicated to the detection of organic compounds [7].

Conversely, carbon-based electrodes are made of less expensive materials and present a reduced fouling, and large working range for detection when compared to metal electrodes [8]. The carbon-based electrodes are usually produced by several approaches, including pencil drawing [9,10], embedding [11], and screen-printing [12]. In addition, the implementation in EMDs can be made by mixing a polymer or a resin-based material with the carbon and inserting in a previously created channel or zone. The electrodes are manufactured by building a groove in polydimethylsiloxane (PDMS), poly(methyl methacrylate) (PMMA) or glass, where a mixture of a carbon-based material (graphite, graphene, carbon nanotubes and so on) is placed with a binding agent to produce a working electrode (WE). The binding agent commonly used for integration of carbon paste (CP) electrodes is mineral oil, but some studies have described the use of the same polymer material for microchip fabrication and electrodes preparation to improve mechanical ruggedness and substrate compatibility. Regel et al. [13] developed integrated graphite/ PMMA composite electrodes with 100 µm width into a PMMA substrate. More recently, Gouyon et al. [14] described the development of screen-printed graphite/ PDMS microband electrodes of 30 µm width into a PDMS substrate for amperometric detection. Therefore, new alternatives to produce EMDs that reduce manufacturing steps, facilitate the integration of components, and increase the throughput are being explored.

Three-dimensional (3D) printing technology, or additive manufacturing, is a versatile way to assemble complex pieces in simple and automated steps. Nowadays, Stereolithography (SLA) and Digital Light Processing (DLP) are the most promising approaches for routine creation of MD due to their high resolution [15]. Whereas those commercial resins are not specifically formulated to attend the requirements of microfluidics, several groups have reported custom printers and materials to successfully leverage to conceive microfluidic designs with elements of less than 50 µm dimensions [16]. Conversely, even with advanced resins and printers the fabrication process is still limited by the laser spot size for SLA and uncured resin which is laborious to be washed from fully enclosed channels [15]. An alternative fabrication method which can deal perfectly with these issues mentioned before is to print open-faced channels and seal the 3D-printed part to a secondary substrate, for instance PDMS [17,18], adhesive tape [19] and PMMA [20]. This way, open-faced channels speed the procedure of resin cleaning and can lead to smaller channel dimensions.

Herein, a simple protocol for the creation of an EMD using SLA-based 3D printer was developed and optimized to build a low-cost reusable microsystem of analysis. This method uses an inexpensive and commercially available printer and resin to create the devices [21]. In this work, the manufacture of microfluidic channels as small as $100 \times 200 \,\mu\text{m}$ was optimized with an SLA-based 3D printer, and a one electrode channel was created into the microdevice at the side wall of the main separation channel, to overcome the complication linked to the alignment. The electrode channel was then filled by an optimized carbon-based lab-made material as electrodes, using graphite as conductivity charge and resin as binding agent, to ensure the compatibility of the electrode material with the printing platform. In addition, we coupled an electrokinetic separation technique in this EMD platform to reach a MCE-EC system. The electrochemical properties of the integrated electrode was investigated using model electroactive hexacyanoferrate(III)/(II) species, ferrocene methanol (FcMeOH), [Fe(CN)₆]^{3-/4-} and hexaammineruthenium (III) ([Ru(NH₃)₆]³⁺), as proof-of-concept.

III.2. Materials and methods

III.2.1. Reagents and materials

All the reagents utilized here are of analytical grade. The solutions were prepared with deionized water of 18.2 M Ω ·cm (EMD Millipore, MA, USA). Graphite powder noted as C (2 – 15 µm microcrystal grade 99.9995%, Alfa Aesar, Germany), resin Formlabs Clear Resin (V4) (Formlabs, Somerville, USA) were utilized to fabricate the device and the electrode.

Ferrocene methanol (noted as FcMeOH, 97%), hexaamineruthenium (III) chloride (98%), phosphate buffered saline tablets (10 mM), potassium hexacyanoferrate (II), potassium hexacyanoferrate (III), potassium nitrate, sodium citrate and Eosin (99%) were used as received (Sigma Aldrich, Darmstadt, Germany).

III.2.2. Instrumentation and electrochemical measurements

A commercial 3D printer Formlabs 2 SLA 3D (Formlabs, Somerville, USA) was used throughout this work. The width of the channels was measured using an inverted microscope Axio Observer A1 (Zeiss, Germany) equipped with a HAL 100 light source and with a filter set 43 (excitation 550/25 nm; emission 605/70 nm) for fluorescence with the purpose to evaluate the geometry of the electrode.

Infrared spectra were obtained using a Fourier Transform Infrared Spectrometer (Cary 630 Spectrometer, Agilent) with an Attenuated Total Reflection (ATR) sampling module. The FTIR-ATR spectra were collected from 4000 to 600 cm⁻¹, at a resolution of 4 cm⁻¹, 128 scans were made for each measurement. Static ultrapure water contact angle done using a DSA100-Drop Shape Analyser from Krűss (Germany). Images of droplet formation captures using a high -resolution camera were evaluated with the Advance Image Analysis Software (Krűss, Germany). Measured contact angle values were an average of at least three measurements at several location points on the device.

Electrochemical characterizations of C/resin electrodes were performed by cyclic voltammetry (CV), square wave voltammetry (SWV) and electrochemical impedance spectroscopy (EIS). A Model 263 a Potentiostat/Galvanostat coupled with the Powersuite software (Princeton Applied Research, USA) was employed for CV and SWV measurements. The characterization of the paste in disk-shaped electrode was made with a 3-electrode setup (platinum wire as counter-electrode (CE) and silver-silver chloride wire as a pseudo-reference electrode (RE). The characterization of the integrated microband electrodes and their analytical performances were performed with a 2-electrode setup where the pseudo-RE and the CE were short-circuited ($172 \pm 12 \mu m$ C/resin microband as WE, and silver-silver chloride wire as CE and pseudo-RE placed in the outlet reservoir).

EIS was performed with Autolab PGSTAT128N potentiostat (Metrohm, Herisau, Switzerland) equipped with the FRA32M module. The applied potential was 0.7 V for $[Fe(CN)_6]^{3-/4-}$ system and -0.2 V for $[Ru(NH_3)_6]^{3+}$. The frequencies were scanned from 1 to 100,000 Hz and 10 to 80,000 Hz, respectively, with a signal amplitude of 10 mV at ten

necessary points according to frequencies per decade. The NOVA[®] 2.1.4 software (Metrohm, Herisau, Switzerland) was used for measurements and for calculation of the charge transfer resistance (R_{ct}). The R_{ct} values were acquired *via* non-linear regression of the semicircle portion of the Nyquist plot.

III.2.3. Fabrication and design of electrochemical microfluidic device (EMD)

In order to study the 3D printer resolution, a microfluidic device with seven straight channels of varying widths was printed with dimensions of 200 μ m (depth) and from 50 to 300 μ m (width). The devices were printed in eight different positions with angle inclination of 15, 60, and 85° in Y and X axes each, 45° both XY axes and perpendicular to Z axis.

Microfluidic devices for 3D printing were designed using Fusion 360 (Autodesk, San Rafael, USA) design software. The device for characterization and analyses consists in a single channel of 20 mm length, $95 \pm 6 \mu m$ width and $180 \pm 11 \mu m$ height and a triangle-shape electrode channel with a rectangular surface with dimensions of $172 \pm 12 \mu m$ width and $180 \pm 11 \mu m$ height touching the separation channel (Figure III.1). The open-face channel device was printed using the optimized parameters.



Figure III. 1. (a) Sketch of the designed device using Fusion 360 design software. (b) Inset showing the connection between the electrode channel and the separation channel (interface dimension width) and the distance from the outlet reservoir and the electrode channel.

III.2.4. Fabrication and design of microchip electrophoresis with electrochemical detection

We manufactured a MCE device by the optimized printing conditions previously used to assemble the EMD. We achieved channels of $100 \,\mu m$ width by printing $150 \,\mu m$ width designed.
Furthermore, we maintained the electrode design and the lab-made conductive material containing 9/8 ratio of carbon and resin, respectively. The EMD and MCE embraced several differences considering the design, such as: EMD had a single separation channel with a WE electrode positioned inside the microchannel 1.5 mm away from the outlet reservoir, while the MCE device carried standard cross channel layout with a WE electrode placed inside the microchannel with 100 μ m away from the outlet reservoir (Figure III.2).



Figure III. 2 (a) Sketch of the microchip capillary electrophoresis designed using Fusion 360 design software. (b) Components of the microfluidic device designed: sample reservoir (S), sample waste reservoir (SW), buffer reservoir (B) and buffer waste reservoir (BW).

The final device for electrophoretic separation and detection consists in a simple t-device with an injection channel of 16.5 mm length and a separation channel of 38 mm length, 100 μ m width and 300 μ m height, with an effective separation distance of 29.5 mm (Figure 3.2). The electrode channel has a triangle-shape with a rectangular surface with dimensions of 547 μ m width and 300 μ m height touching the separation channel.

III.2.5. III.1.5. Electrode preparation

A new working electrode design was created, one triangular channel for electrochemical detection with in-channel configuration. A new electrode composition has been investigated. A mix of graphite powder and Clear resin (C/ resin) were prepared as a conductive paste in several w/w ratios (4/8; 5/8; 6/8; 7/8; 8/8; 9/8), respectively. The paste was made in a plastic flask by

properly mixing both components by hand with a spatula until complete integration of graphite powder in a homogeneous paste.

III.2.6. III.1.6 Electrode integration into EMD

The electrode channel (Figure III.3.a) was filled with the C/resin paste using a syringe (Figure III.3.b) and subsequently exposed to UV radiation (at 365 nm) for 4 h at 50 mJ \times cm⁻² dose (UV Biolink BLX, Vilber Lourmat, France) until complete reticulation of the paste (Figure III.3.c). Next, the excess of paste was removed carefully by polishing the surface with wet sandpaper (3M, USA) (Figure III.3.d). Subsequently, the device was sealed using a silicone tape (ThermalSeal RTS TM, Sigma Aldrich, Darmstadt, Germany) (Figure III.3.e). To remove bubbles and ensure the horizontality of the setup, a gentle pressure was applied onto the silicone tape for a suitable adhesion. Finally, the EMD is ready for applications (Figure III.3.f).



Figure III. 3. Scheme to assemble a fully electrochemical microfluidic device: (a) 3D-printed device with open channels; (b) the electrode channel is filled with C/ resin paste; (c) 3D-printed device containing C/ resin paste is exposed in UV radiation; (d) the excess of the paste is removed by wet sandpaper; (e) EMD is sealed with silicone tape; (f) EMD is ready for applications.

III.2.7. Electrode integration into MCE device

First, the 3D-printed device was sealed with a silicone tape (ThermalSeal RTS TM), then the electrode channel was filled with the optimized conductive paste using a syringe. Subsequently, the full device was exposed to UV radiation at 365 nm for 4 h at a dose of 50 mJ \times cm⁻² (UV Biolink BLX, Vilber Lourmat, France) until complete reticulation of the conductive paste (Figure III.4).



Figure III. 4. Scheme to assemble a fully microfluidic electrophoretic device.

III.2.8. Electrode cleaning

We performed a CV in large potential window from 0 V to 1 V (*vs.* Ag/AgCl) in 0.1 M sulfuric acid solution by performing 100 cycles at a rate of 1 V/s, 50 cycles at a rate of 0.5 V/s, 25 cycles at a rate of 0.25 V/s, and 10 cycles at a rate of 0.1 V/s.

III.3. Results and Discussion

III.3.1. Printing Optimization

The commercial resin Formlabs Clear Resin (V4) (Formlabs, Somerville, EUA) was utilized to print the devices and integrated the electrodes channels. SLA-based 3D printers have the resolution limited by laser spot size of 140 μ m (XY-resolution) and 25 μ m thick layer (Z-resolution) as well, which was the limiting factor in achieving small channels. To define the minimum feasible channel size, a microfluidic device with seven straight channels of varying widths was printed (Figure III.5.a). Each channel was designed to have a depth of 200 μ m and widths from 300 to 50 μ m. The devices were printed in eight different positions (Figure III.5.b), with angle inclination of 15, 60, and 85° in Y and X axis each, 45° both XY axis and perpendicular (XY = 0°) (Figure III.5.c).



Figure III. 5. Microfluidic devices (a) design with seven straight channels of varying widths (300; 200; 150; 125; 100; 75; 50 µm); (b) after printing step with supports. (c) disposed in several positions in the build platform for printing.

After printing the channels, we measured the channel width channels by optical microscopy. The devices printed with inclination angle of 85°, 60°, 15° in X axis accomplished suitable results, on the other words we attained grooves in these conditions. The device printed with angle of 85° in X axis achieved well defined open-channels in the designed widths between 300-150 µm. However, the printed dimensions were significantly different from the designed dimensions. All the channels are considerably shallower than the designed width, decreasing until a specified width of 50 µm where no channel is visible. The discrepancies between designed and actual dimensions in channels were due to the 140 µm laser spot size and were consistent with other 3D printed microfluidic channels [22]. Indeed, the laser spot effect can be minimized increasing the angle of inclination. The device printed with inclination angle of 45° had an impact of the laser spot in both channel depth and width. This way the smaller channels were clogged. Lastly, the devices orientation in the printer with angle of 85°, 60°, 15° in Y and perpendicular $(XY = 0^{\circ})$ did not print any channel, due to the high impact of the laser spot size in the channel's width. Notably, we printed the same device with fully enclosed channels with the same dimensions and angles as those in Figure 3.5.a, all the channels were clogged with cured resin and not even the largest channel $(300 \times 200 \,\mu\text{m})$ could hold solution. Thereby, we used the device with printing orientation of 85° inclination angle in X axis. The

smallest channels achieved were $95 \pm 6 \ \mu m$ width and $180 \pm 11 \ \mu m$ height (n = 10) with a designed system of 150 μm width and 200 μm height.

III.3.2. Electrode design

The design of the microband electrodes has been developed to avoid any leaking upon the sealing of the entire device. For this purpose, the electrode material must avoid spreading through the separation channel accessing the interface part. Therefore, several electrode geometries and the distances between the electrode and the separation channel were evaluated to provide a suitable configuration. Devices with electrode channels holding two geometries (circle and triangle) and different gaps (configurations 1 to 6 corresponding to gaps of 60, 40, 20, 10, 0 and -10 μ m, respectively) between them and the separation channel were designed and then printed (Figure III.6).



Figure III. 6. Open-channels microfluidic device containing several electrodes with two geometries (circle and triangle) in different gaps from 1 to 6, (60, 40, 20, 10, 0 and -10 µm, respectively).

The connection between the electrode channel and the separation channel (interface width, corresponding to the accessible electrode in the separation channel) was measured (n = 10) by optical microscopy and then plotted versus the gaps, for both geometries (Figure III.7). Circle and triangle channels achieved a linear behavior. The triangle geometry produced electrode widths smaller than 400 μ m, suitable to fabricate microband electrodes. The circle geometry produced larger electrodes (from 400 μ m to 1280 μ m). Evaluating the slope of the curve, the gaps of the circular geometries (m = -12.5) affected enormously the electrode width

when compared with the triangle geometries (m = -3.23), creating larger interfaces in circular geometries.



Figure III. 7. Corresponding plot of the interface dimensions of the electrode (width) as a function of the theoretical distance between the electrode channel and the separation channel (gap).

To assess the geometry of the electrode (circular and triangle), the electrode channels were sealed and filled using a syringe with an aqueous solution 10% (w/v%) of Eosin to monitor by fluorescence imagery the profile of liquids when inserted in the electrode channel. It can be observed in Figure III.8 that the electrodes with circle geometry promoted a leak of the eosin into the microchannel, because the interface dimension is larger for circle than for triangular configuration. On the other hand, the electrodes with triangle geometry allowed maintaining the liquid into the electrode channels when the liquid was added at low pressure conditions, despite the connection with the separation channel. Therefore, the C/resin can be efficiently hold into the detection triangle channel (configurations 1 to 3), which promoted smaller widths due to higher gaps between channels.



Figure III. 8. Images from fluorescence microscopy of the electrode channels (triangle and circle geometries) filled with a fluorescent dye. Circular and triangle geometry, the electrode channels were sealed and filled using a syringe with an aqueous solution 10% (w/v%) of Eosin.

Regarding configurations 1, 2 and 3, the connection between the electrode channel, which will hold the conductive material and limit the surface area of the electrode, and the separation channel was scrutinized for a proof of concept. The detection triangle channel in all three configurations and the separation channel were printed in a new design (Figure III.9.a). Both separation and electrode channels were sealed with a silicone tape and a voltage (10 - 400 V) was applied in a 50 mmol L⁻¹ sodium citrate electrolyte (pH = 6.0). For this purpose, two Pt wire electrodes were positioned at the inlet of the separation channel, placed at 10 mm of the desired channel electrode, to verify the efficient contact between these channels, and to calculate the resistance of the interface.



Figure III. 9. (a) Open-channels microfluidic device containing electrodes with triangle geometry in different gaps from 1 to 3, (60, 40 and 20, respectively). (b) Current versus electric field (ohmic curve) obtained from 3D-printed device with electrode channels (1) triangle 1; (2) triangle 2; and (3) triangle 3. Conditions: 50 mmol L^{-1} citrate electrolyte (pH = 6.0).

A current response with a linear behaviour in relation to the applied potential was achieved ($R^2 \ge 0.993$), indicating that these electrodes had an efficient connection with the separation channel (Figure III.9.b). The resistance of the interface and the separation microchannel were calculated as the inverse of the slope of the curve. The resistance of printed the separation microchannel was determined (1.14 M Ω), enabling the calculation of the interface resistance values at configuration 1, 2 and 3, which are 3.60 M Ω , 2.50 M Ω and 2.43 M Ω , respectively. The electrode with triangle geometry 2 demonstrated a resistance value similar with the configurations 1 and 3. On the other hand, this configuration revealed a better connection with the separation channel in terms of interface width (172 ± 12 µm). Despite its larger width than the configuration 1 (123 ± 10 µm), the configuration 2 still provides the microelectrode properties.

III.3.3. Optimization of microelectrode material

The proposed new material conceived for microelectrode preparation is composed by two compounds. The first is the graphite powder with particles size inferior to 15 μ m. The addition in of an insulator material allows the formation of electrical percolating networks inside the material, leading to increase electrochemical properties of the material in a several

orders of magnitude. The second is the Formlabs clear resin, the same material used to manufacture the devices, ensuring the compatibility of the electrode with the device substrate. We performed introductory experiments with several mixtures of graphite powder and resin at different weight ratios to optimize material in terms of gain in conductivity, simplest fabrication, and mechanical stability. We evaluated graphite and resin (C/resin) mixtures with w/w% ratios of 4/8, 5/8, 6/8, 7/8, 8/8 and 9/8, respectively. Lower ratios are not suitable since the graphite particles are too dispersed compromising the conductive network between the particles. We achieved a viscous paste with mixtures of 4/8, 5/8, 6/8 and 7/8, easy add into glass capillary tubes to fabricate larger electrodes (CV experiments) and to incorporate into the electrode channel before curing. Regarding ratios larger than 7/8, the viscous paste was denser but the viscosity was still compatible with an integration into the electrode channel. The solidification of the electrode was ensured by curing process using a UV light. The curing process of the Clear resin usually needs around 1 h at dose of 50 mJ× cm-2 for complete reticulation. Adding the graphite powder into the resin leads to affect the kinetics of the resin reticulation, especially when the C/ resin ratio is the high (7/8, 8/8 and 9/8) this way the curing process of the new material has been optimized for 4 h at a dose of 50 mJ× cm⁻² to ensure the complete solidification of the material.

The electrical conductivity of the electrode materials was evaluated using the 2-point probe resistance method (Table III.1) and compared to other reported conductive polymeric electrode materials in the literature (Table III.2).

	• •	· · · ·
w/w ratio	Resistivity	Conductivity
	Ω.cm	S cm ⁻¹
4/8	65.79	1.52×10^{-2}
5/8	11.92	8.39 × 10 ⁻²
6/8	3.88	$2.58 imes 10^{-1}$
7/8	2.06	4.84×10^{-1}
8/8	1.20	8.31×10^{-1}
9/8	1.17	8.57 ×10 ⁻¹

Table III. 1 Resistivity and conductivity of the custom resin (n = 3).

The C/resin ratios of 4/8 and 5/8 were discarded since the graphite particles were proved too dispersed compromising the integrity of the conductive network. For the other ratios, the

conductivity was measured in the 2.5 to 8.6×10^{-1} S cm⁻¹ range. Only two materials based on silver nanoparticles (Table III.2) demonstrated a better conductivity than the C/resin 9/8 one [23,24]. This can be attributed to the fact that silver ink is intrinsically more conductive than the carbon. On the other hand, silver has a notably limited upper potential range in which it can be utilized before being oxidized. Carbon is a less expensive material and combines important benefits in the behaviour of the electrodes, for instance reduced fouling, and large working range for detection when compared to metal electrodes.

Conductive polymeric material	Conductivity (S cm ⁻¹)	Ref.
UDMA/(PPy/Ag)	6.00 × 10 ⁻³	[25]
Polyacrylate resin/ (PANI NF/graphene)	4.00 × 10 ⁻⁹	[26]
yttria-stabilized zirconia	2.10 × 10 ⁻²	[27]
PEGDA/PEDOT	5.00 × 10 ⁻²	[28]
Polyacrylate resin/CNTs	2.00×10^{-2}	[29]
PEGDA/ AgNPs	6.7×10^{-6}	[30]
PEGDA/ AgNPs	2.4×10^{-6}	[31]
2-hydroxyethyl acrylate/Ag	8.70 × 10 ⁻⁶	[32]
Acrylamide/ SiO ₂ ⁻ SO ₃ ⁻ Na ⁺	2.90 × 10 ⁻²	[33]
phototherme/Silver ink	3.70 × 10 ⁴	[23]
DSM/silver ink	2.00×10^{5}	[24]
HDDA/ CNTs	3.33 × 10 ⁻³	[34]

Table III. 2. Conductivity of resin-based materials

UDMA: Urethane dimethacrylate; PPy: polypyrrole; PANI NF: polyaniline nanofibers; PEDOT: Poly(3,4ethylenedioxythiophene); PEGDA: poly(ethylene glycol) diacrylate); HDDA: Hexanediol diacrylate

It is generally known that decreasing insulator content in the carbon paste mixture may cause a desirable increase in the conductance of the material, however this process can be saturated in determined concentration ratio. This phenomenon can be observed in the exponential curve showing the dependence of conductivity with the C/resin ratio (Figure III.10). When the electrically conductive particles are in maximal permanent multiple contact, the full conductivity of the C/resin material is thus attained. In this situation, the resin denotes only a filler of free space between the graphite particles and has basically no effect on the overall conductivity, saturating the process [35].



Figure III. 10. Dependence of the conductivity on C/resin composition for several ratios of graphite and resin, respectively.

Subsequently, we evaluated the influence of each ratio composition on the electrochemical behavior by CV (Figure 3.11) using FcMeOH as a probe. We conducted this study using disk-shaped electrode using glass capillary tube as a body of the electrode, with a 2 mm diameter. We used a 3-electrodes system in classical electrochemical cell (Figure III.11.a). It is remarkable that for lower graphite content in the composite (6/8; 7/8), a waveshaped anodic current is observed related to oxidation of Fe (II) to Fe(III). The surface may form a network of micrometric conductive parts and could thus be assimilated as a network of separated microelectrodes with a radial diffusion and no overlapping of the diffusion layer. As the C/resin ratio increased (8/8; 9/8), the shape of the cyclic voltammograms gradually moved from a wave-shaped signal to a peak-shaped one (Figure III.11.b). Concerning the C/ resin ratio of 9/8, the voltammogram displays anodic peak for FcMeOH with highest peak intensity of $5.36 \pm 0.06 \,\mu\text{A}$. The ΔE (higher than 57 mV) of the probe must be attributed to the slow transfer kinetics of the charge characterized by peak-to-peak separation of 228 ± 5.77 mV, which is in accordance with earlier reports for graphite [36]. In addition, the ΔE increases according to the increase of the scan rate, such a high deviation suggests the quasi-reversible electron transfer process at higher scan rates [37].



Figure III. 11. (a) 3-electrode apparatus used to perform the electrochemical characterization of the lab-made conductive paste; WE = C/resin (6/8 - 9/8) ratio, circular (diameter 2 mm), RE = Ag-AgCl wire, CE = Pt wire. (b) Cyclic voltammetry on C/resin electrodes of 1 mmol L⁻¹ FcMeOH. Electrolyte: 10 mmol L⁻¹ phosphate buffer. Scan rate 25mV s⁻¹. (c) Scan rate of 1 mmol L⁻¹ FcMeOH from 10 mV s⁻¹ to 360 mV s⁻¹.

Using the electrode material with C/resin ratio of 9/8, peak current values versus square root of the scan rate (\sqrt{v}) were plotted to estimate the electrode area (using Randles-Sevcik equation) (Figure III.11.c). The obtained values are 90-97% of the theoretical geometric area of a 2 mm diameter disk. Furthermore, a small intercept value of the linear variation of I_p vs \sqrt{v} was observed, which can be associated to the non-faradaic current generated due to the relative dielectric nature of the resin-based electrode as compared to conventional noble metal electrodes [38]. C/resin 9/8 was thus chosen for further experiments due to its acceptable conductive and mechanical properties suitable to prepare the integrated electrodes.

Additional characterization of the C/ resin 9/8 electrode was performed by EIS and CV using redox species that are more surface dependent. Figures III.12. a-b display Nyquist plots for $[Fe(CN)_6]^{3-/4-}$ and $[Ru(NH_3)_6]^{3+/2+}$, where the fitted electrochemical data allows the determination of a charge transfer resistance (R_{ct}) of 4489 Ω and 834 Ω , respectively. This confirms that the $[Fe(CN)_6]^{3-/4-}$ redox process is sensitive to the nature of the surface of the electrode. The cyclic voltammograms of $[Fe(CN)_6]^{3-/4-}$ and $[Ru(NH_3)_6]^{3+}$ (Figure III.12 c-d) clearly show the ill-reversibility of the $[Fe(CN)_6]^{3-/4-}$ redox process and are in accordance with the EIS data.



Figure III. 12. EIS spectra of (a) 5 mmol L⁻¹ $[Fe(CN)_6]^{3-/4-}$ in 100 mmol L⁻¹ M KNO₃ electrolyte and (b) 5 mmol L⁻¹ $[Ru(NH_3)_6]^{3+}$ in 10 mmol L⁻¹ PBS electrolyte. CV of (c) 5 mmol L⁻¹ $[Fe(CN)_6]^{3-/4-}$ in 100 mmol L⁻¹ KNO₃ electrolyte and (d) 5 mmol L⁻¹ $[Ru(NH_3)_6]^{3+}$ in 10 mmol L⁻¹ PBS electrolyte. WE = (9/8) C/resin, disk electrode (diameter 3 mm); CE: = Pt wire; RE = Ag/AgCl (KCl sat.). EIS experimental conditions: Frequency range: 1 to 100,000 Hz and 10 to 80,000 Hz, potential applied: 0.7 V and -0.2 V for $[Fe(CN)_6]^{3-/4-}$ and $[Ru(NH_3)_6]^{3+}$, respectively, with a signal amplitude of 10 mV with 10 data points per frequency decade. CV experimental conditions: Scan rate 50 mV s⁻¹; step potential: 5 mV.

III.3.4. Coupling electrochemical detection into microfluidic device

III.3.4.1. Integrating the conductive paste in the microfluidic device

We integrated the electrode in the device by filling the electrode channel with the conductive lab-made paste with the optimized composition. The fully device was exposed in the UV chamber for the post-curing process of the printed device and for reticulation of the electrode paste for 4 h at dose of 50 mJ×cm⁻². It is remarkable that the post-curing process is a

required step to finalize the device fabrication, so we took advantage of this process to obtain the integrated electrodes in the same step, minimizing the number of processes to achieve a complete device. In addition, the electrode connection with silver ink or conductive tape is not necessary, since the wires can be connected directly in the conductive paste inside the open electrode channel, and it is fixed during the post curing process. After that, we polished the device aiming to remove the excess of composite, and to smooth the surface of the device for the sealing process using a silicone tape (ThermalSeal RTSTM). Finally, we accomplished a sidewall integrated microband electrode to perform as a WE at in-channel detection mode (Figure III.13).



Figure III. 13. Schematic of the proposed EMD with an inset showing the lateral integrated microband electrode.

III.3.4.2. Electrochemical characterization

The electrochemical characterization of the microband electrode has been performed using FcMeOH as redox probe. A 2-electrodes setup, pseudo-RE and CE in short circuit (C/resin versus silver/ silver chloride), has been used to perform the all the follow experiments. The WE electrode is positioned inside the microchannel (Figure III.14.a) while the pseudo-RE electrode is positioned in the outlet reservoir, which is placed 1.5 mm of the electrode channel. First, we filled the channel with the redox probe solution to execute CV acquisition. Between each acquisition, the channel was washed with water and buffer before renewing the solution to certify the repeatability of the measurements, accomplishing a relative standard deviation of $\pm 2\%$ in the peak current values (n = 3) (Figure III.14.b).



Figure III. 14. (a) Triangle integrated electrode C/resin into the device sealed with silicone tape. (b) Linear voltammetry of 1 mmol L⁻¹ FcMeOH on this electrode integrated in a 3D-printed device (n = 3). Electrolyte: PBS 0.01 mol L⁻¹ (pH = 6.7). WE: C/resin 9/8 ratio, RE/CE = Ag-AgCl wire. Scan rate 25 mV s⁻¹

The CV of FcMeOH demonstrated features with a half-wave oxidation potential at 260 \pm 16 mV and wave intensity of 77.0 \pm 0.8 nA (Figure III.15.a) We plotted the peak current values against square root of the scan rate (\sqrt{v}) (Figure III.15.b) and we used the slope value to calculate the dynamic surface area (0.027 mm²) of the sensing platform by Randles-Sevcik equation. The experimental current density is more pronounced on the microelectrode format than on the millimetric format, increasing from 1.74 μ A × mm² to 2.84 μ A × mm² for FcMeOH with a scan rate of 50 mV s⁻¹, strengthening the motivations for electrode miniaturization.



Figure III. 15. Cyclic voltammetry on C/resin electrodes of (-) 1 mmol L⁻¹ FcMeOH in PBS 10 mmol L⁻¹ and (-) blank solution. WE = C/resin 9/8 ratio, RE/CE = Ag-AgCl wire. Scan rate 50 mV s⁻¹. (b) Anodic peak current versus the square root of scan rate plots of integrated C/ resin electrode (9/8).

After setting up a simple protocol for the creation of EMD using SLA-based 3D printer, we demonstraded the electrochemical detection employing the 2-electrodes setup to study the response linearity of the transducer material by square wave voltammetry (SWV). Figure III.16 demonstrated a anodic peak responses against different concentrations of FcMeOH, ranging from 15 μ mol L⁻¹ to 1000 μ mol L⁻¹ on integrated electrode. Symmetrical, sharp, and well-defined peaks at around 0.15 V were observed. The sensing electrode has a linear response in the range of concentrations explored with a coefficient of regression of 0.995. The LOD and LOQ values were 4.5 μ mol L⁻¹ and 15 μ mol L⁻¹, respectively, which were in accordance with the literature [39].



Figure III. 16. (a) SWV at C/resin (9/8) microelectrode integrated in a 3D-printed device in 10 mmol L⁻¹ PBS containing 1 mmol L⁻¹ FcMeOH (from 15 to 1000 μ mol L⁻¹) under static conditions. (b) Corresponding calibration plot of the SWV peak current as a function of the probe concentrations. Parameters: $\Delta E = 10 \text{ mV}$; f = 30 Hz; E_{step}: 6 mV.

III.3.4.3. Renewal of integrated electrode

One of the goals of this work was to attain a reusable device, this way we developed strategies to renew the electrode surface after a several and exhaustive experiments. Since the

electrodes are placed inside the device with an irreversible sealing, is not possible renew the surface area by classical polishing. Furthermore, microchip electrodes may not be cleaned using the aggressive methods commonly applied to disk electrodes because several chemical cleaning procedures, for example the use of piranha solution, hot nitric acid and high concentrations of sulfuric acid are not compatible with the substrate material of the microchips [40]. To overcome this issue, we developed a strategy to renew the electrode area, inside the channel. This electrode can be renewed using a simpler protocol (which is described in material and methods) - instead of aggressive solutions, we used a sulfuric acid in low concentration (0.1 mol L⁻¹) and cyclic voltammograms for cleaning the electrode surface. The electrode was reused up to 30 times until its current signal start to decrease by passivation (Figure III.17). A better reversibility (smaller peak-to-peak separation) and an enhanced voltammetric peak intensities for the FcMeOH redox couple was observed, confirming the cleanliness of the electrode surface.



Figure III. 17. CV behaviour of FcMeOH 1 mmol L⁻¹ in PBS at C/resin (9/8) microelectrode integrated in a 3Dprinted device (a) after reuse of the electrode for 30 times (n =3) and (b) after electrode electrochemical treatment of the WE using sulfuric acid solution (0.1 mol L⁻¹) (n =3), see the text for details. Scan rate: 25 mV s^{-1} .

III.3.4.4. Application of 3D-printed EMD

So as to further prove the usesfullness of such a cheap and simple EMD created with a SLA-based 3D printer, the electrochemical response of a probe, injected continuously and hydrodynamically in the separation channel, was measured on the integrated lab-made detection channel previously optimized. For this purpose, a $[Ru(NH_3)_6]^{3+}$ solution (5 mmol L⁻¹) was continuously injected in a flow rate ranging from 0 (no flow) to 55 µL min⁻¹ and analyzed by cyclic voltammetry (Figure III.18.a). These flow values were chosen to mimic the electroosmotic flow inside the microchannel, which is promoted by electrically driven separation techniques.



Figure III. 18. (a) Influence of the flow rate on the CV (forward scan) at C/resin 9/8 WE in a solution of $[Ru(NH_3)_6]^{3+}$ 5 mmol L⁻¹ in PBS. Flow rate ranging from 5 to 55 µL min⁻¹. Scan rate 50 mV s⁻¹. (b) Dependence of the current on the flow rate inside the microchannel.

As evidenced in Figure III.18.b, the increase in the flow generated hydrodynamically in the separation channel increases the reduction current of $[Ru(NH_3)_6]^{3+}$. This is indeed due to the narrowing of the diffusion layer, which increases the concentration gradient, and the convection of the solution ensured by the continuous flow, renewing the solution and thus the analyte. The hydrodynamic flow conditions inside the channel offer benefits when compared with static mode, such as the evolution of the peak current and around 4-fold improvement in LOD and LOQ values.

III.3.4.5. Coupling amperometric detector with electrophoresis in a microdevice

We have created a microchip electrophoretic device with a detection channel filled with the optimized lab-made conductive paste and sealed with the silicone tape (ThermalSeal RTSTM), (Figure III.19).



Figure III. 19. Bottom and top view of the proposed device.

III.3.4.6. Electroosmotic flow (EOF) characterization

Further characterizations of the surface of the 3D-printed material have been developed in order to evaluate the electroosmotic flow generated inside such 3D printed microchannel of the MCE system. The surfaces were characterized by water contact angle (WCA) and Fourier transform infrared spectroscopy – attenuated total reflectance (FTIR–ATR). WCA measurements permitted to characterize the wettability of the device substrate (Figure III.20), while FTIR-ATR spectra indicated that the absorbance of the bands attributed to the groups presented in the microdevice substrate (Figure III.21).



Figure III. 20. Water contact angle measurements of proposed device surface (Formlabs Clear resin).

For 3D-printed material, the WCA value was equal to $78.7\pm0.3^{\circ}$ (n = 3), this value is 33% lower than the value for PDMS (WCA = 117°) [41]. These results indicate that 3D-printed material have a more hydrophilic behaviour than PDMS, material commonly used to fabricate MCE devices. This detail is potentially interesting for microchip fabrication since it is an indication of better wettability on 3D-printed device when using aqueous solutions, supporting high electroosmotic flow (EOF) rates. Certainly, it would be not necessary to use a surfactant to improve better wettability in the subsequent experiments.

Figure III.21 displays FTIR-ATR spectrum from the uncured resin (before printing) and cured resin (3D-printed material). Both spectra from cured and uncured resin presented similar absorption bands located at 2952 and 2868 cm⁻¹ which are assigned to carbon/ hydrogen stretching vibration modes of $-CH_2$ and $-CH_3$ groups from the polymer backbone. The band around 1450 cm⁻¹ is provided by the wagging mode of $-CH_3$ groups. The band located at 1704 cm⁻¹ is assigned to carbonyl group, and the band located at 3373 cm⁻¹ is assigned to hydroxyl group (stretching mode). As it can be noted, the spectrum presented an absorption of intense O—H axial deformation with a large band between 3300 — 3500 cm⁻¹, this phenomenon is explained due to the presence of carboxylic acid dimers in both cured and uncured resin. The presence of carboxylic groups promotes the creation of high EOF rates inside the channel. The band located at 1639 cm⁻¹ related to C=C group (stretching mode) is present only in the uncured

resin, this was expected since the chain reaction polymerization started breaking the C=C double bonds of the monomers.



Figure III. 21. FTIR-ATR spectrum of uncured (-) and cured (-) FormLabs resin.

The electroosmotic flow of a single rectangular 3D-printed microchannel, 100 μ m width, 300 μ m height and 38 mm length, was determined with the neutral marker method. The neutral marker method involves measuring the time required for a neutral marker molecule to migrate from the separation channel inlet to the detection system under a electric field. The electroosmotic mobility was determined with 1 mmol L⁻¹ FcMeOH prepared various BGEs of pH ranging from 2 to 12 : phosphate (pH 2, 6, 12), carbonate (pH 10) and borate (pH 8) buffers, at 20 mmol L⁻¹ (Figure III.22).



Figure III. 22. pH dependence of μ_{eo} in microchannels device for a pH range from 2 to 12. μ_{eo} monitored with the neutral marker method. Neutral marker: Ferrocene methanol; BGE: 20 mmol L⁻¹ phosphate and borate.

For pH 8, 10 and 12 the electroosmotic mobility was evaluated between 5.7 and 8.6 × 10^{-5} cm² V⁻¹ s⁻¹ (n=3), evidencing a negative charge on 3D-printed microchannel. This may probably be due to the presence of carboxylic acid groups evidenced in FTIR-ATR spectrum of the 3D-printed device. Certainly, it is a lower value of electroosmotic mobility when compared with other materials of microdevices, such as silica (around 5.0×10^{-4} cm² V⁻¹ s⁻¹) and PDMS (around 2.0×10^{-4} cm² V⁻¹ s⁻¹)[42].

III.3.4.7. Electrophoretic separation channel

The joule heating effect on the 3D-printed device was evaluated by monitoring the electric current in several values of the applied potential (Figure III.23). We calculated the electrical resistance (1.14 M Ω cm⁻¹) using the 1/slope of the curve current versus voltage in the linear region (from 0 to 210 V cm⁻¹).



Figure III. 23. Ohmic curve (current vs electric field) provided by the 3D-printed microfluidic device in 50 mmol L^{-1} phosphate buffer solution.

We observed a linear behavior till an electric field of 210 V cm⁻¹, whereas the Joule heating effect is more pronounced at higher electric field values,, causing a deviation from linearity. The dissipation of the Joule heating is less efficient when compared with other MCE material nature, for instance silica or glass [43]. This drawback can be overcome by using more diluted BGE (lower ionic strength, the Joule heating can be minimized but the buffer capacity

can be compromised. Therefore, we used the separation potential of 700 V (184 V cm⁻¹), and we decreased the buffer concentration from 50 mmol L^{-1} to 10 mmol L^{-1} of phosphate buffer, the ionic strength of 10 mmol L^{-1} is then suitable to attain a good agreement between Joule effect and buffer capacity.

III.3.4.8. Electrode Characterization

The electrochemical characterization of the microband electrode has been performed using $[Ru(NH_3)_6]^{3+}$ as redox probe under static conditions. A 2-electrodes setup, pseudo-RE and CE in short circuit (silver/ silver chloride), has been used to perform all the follow experiments. The WE electrode is positioned inside the microchannel while the pseudo-RE electrode is positioned in the outlet reservoir, which is placed 100 µm from the working electrode. We calculated the active surface area of the electrode by plotting the peak reduction values of the probe $[Ru(NH_3)_6]^{3+}$ against the square root of the scan rate (Figure III.24.a). In this scan rate range, a clear linearity was found ($R^2 = 0.998$). The slope value allowed to calculate the dynamic surface area (0.30 mm²) of the sensing platform by Randles-Sevcik equation.



Figure III. 24. (a) CV on C/resin electrodes of 5 mmol L⁻¹ $[Ru(NH_3)_6]^{3+}$ in 10 mmol L⁻¹ phosphate buffer in several scan rates (10-250 mV/s) and cathodic peak current vs $(V/s)^{1/2}$. (b) SWV of $[Ru(NH_3)_6]^{3+}$ (from 15 to 1000 µmol L⁻¹) and calibration plot between the SWV peak current and the values of probe concentrations. WE = 9/8 microband (547 µm width), RE/CE = Ag-AgCl wire.

We also performed a SWV under static conditions for different $[Ru(NH_3)_6]^{3+}$ concentrations. A linear regression was obtained between the peak current intensity and the analyte concentration ranging from 15 to 1000 µmol L⁻¹ (Figure III.24.b). The LOD and LOQ were 4.5 µmol L⁻¹ and 15 µmol L⁻¹, respectively, which are in accordance with the values found in the literature [39].

In electrophoretic separations, the application of a difference of potential is required to drag the ions under an electric field through the microchannel. Coupling microchip electrophoresis with electrochemical detection can cause challenges due to interferences that can occur between the electric fields generated by both techniques. This electric field may have a strong impact on the electrochemical detector placed inside in the separation channel. Concerning amperometric detection, the electric current produced by the separation potential (10-30 kV) inside the channel can be six or more orders of magnitude greater than the electrochemical currents measured at the amperometric detector [44]. This effect was studied by running cyclic voltammetry [Ru(NH₃)₆]³⁺ in PBS (phosphate buffered saline) 10 mmol L⁻¹ (pH = 7.4) placed in a microchannel, with or without the application of a separation voltage for electrophoresis and using a C/resin electrode at the end channel configuration.

We used a 2-electrodes setup with pseudo-RE and CE (Ag-AgCl) in short circuit placed in the outlet reservoir. The cyclic voltammetry was performed under separation voltage at different values inside the channel (Figure III.25)



Figure III. 25. Influence of separation voltage on the cyclic voltammograms of 5 mmol L⁻¹ [Ru(NH₃)₆]³⁺. BGE 10 mmol L⁻¹ PBS (pH = 7.4) in the end-channel configuration. WE: 9/8 microband (547 μ m width), RE/CE = Ag-AgCl wire. Separation voltage: 0 to +700 V (inlet). Scan rate: 50 mV s⁻¹.

The potential of reduction of the probe is shifted to more positive values when the separation voltage increases. In the absence of the separation voltage, the probe was reduced at a potential value of -0.37 V *vs*. Ag-AgCl; with the separation voltage of 700 V (inlet side) the potential of reduction shift to -0.2 V *vs*. Ag-AgCl. The shift in the potential can be explained by the interaction between the electric field for the separation and the one for detection. Furthermore, we obtained a signal without higher noise even under higher electric fields. This can be attributed to the fact that end-channel alignment explores the strategy of placing the WE at the exit of the separation channel, $5-100 \mu m$ away from the outlet end. By doing so, the influence of the separation electric field on the electrochemical detection is avoided. In this context, the 2-electrodes set-up in an end-channel configuration is efficient for the detection of the redox probe at detection potential -0.2V/ Ag-AgCl.

The end-channel can suffer from another drawback that we need to check out: since the WE is placed outside the microchannel, the sample undergoes a dilution in the reservoir which can impact on the analytical performances [3]. In this context, we studied the end-channel configuration, where the working electrode is positioned within the separation microchannel to avoid the sample dilution. In addition, we tested the current stability under several electric field values (Figure III.26). For this configuration, we used a wireless potentiostat for chronoamperometric measurements since the use of classical potentiostat can be damaged by the application of the separation voltage during the electrophoretic separation without grounding. We performed chronoamperometric measurements to study of any influence of the electric field on a 2-electrode setup in a cross-shaped system at a potential reduction of -0.2V. The set-up consisted in a C/resin electrode of 547 μ m width placed just before the end of the separation channel (around 100 μ m of distance) and a pseudo-RE/CE made of an Ag-AgCl wire immersed in the nearest well.



Figure III. 26. Influence of the separation electric field on the chronoamperometric current of the BGE 10 mmol L^{-1} phosphate buffer (pH = 7.4) in an in-channel configuration. WE: 9/8 microband (547 µm width), RE/CE = Ag-AgCl wire. Separation voltage: 0 to +700 V (inlet). Potential of detection: -0.2V.

In the absence of the separation voltage, we have a lower current value. Applying a separation voltage, the current increased in negative values and the noise increased proportionally when the separation voltage is rising. This can be attributed to higher joule effect under higher electric field value (100 V cm⁻¹). In addition, this behaviour can be related to the electrical interference in the WE electrode. The WE potential is noticeably increasing with the increase of the electric field, which can cause side effects in the WE, for instance generation of gases in the electrode due to oxidation of H₂O.

Despite the increased noise achieved by applying high voltages (700 V), we still can have a good signal-to-noise S/N ratio (S/N \geq 1).

III.3.4.9. Application of 3D-printed MCE-EC

We employed the 2-electrode setup for the separation and end-channel chronoamperometric detection of $[Ru(NH_3)_6]^{3+}$ (100 to 700 µmol L⁻¹) in the microfluidic device. The BGE was composed of 10 mmol L⁻¹ phosphate buffer (pH 7.4) since this media has been used for all the studies concerning this device. For this purpose, we used a "floating"

injection mode (500V for 5s), followed by an electrophoretic separation (700V for 750 s) and chronoamperometric detection at -0.2V/ Ag-AgCl. The electropherogram is presented in Figure III.27.



Figure III. 27. Electropherograms of BGE 10 mmol L⁻¹ of phosphate buffer (pH = 7.4), 500 μ mol L⁻¹ [Ru(NH₃)₆] ³⁺ in the end-channel configuration and calibration plot between the peak areas and the values of probe concentrations (100 to 700 μ mol L⁻¹). WE: 9/8 microband (547 μ m width), RE/CE = Ag-AgCl wire. Injection potential: +500V (sample inlet), separation potential: +700 V (buffer inlet) and detection potential: -0.2V/ Ag-AgCl.

According with Figure III.27, we achieved a suitable signal-to-noise S/N ratio relation despite the noise from separation voltage. The reduction peak provided by the introduction of the probe has migration time of 114 s. Since the electroosmotic flow mobility in pH= 7.4 is around 5.0×10^{-5} cm² V⁻¹ s⁻¹, the electrophoretic mobility of [Ru(NH₃)₆]³⁺ was estimate from its migration time minus electroosmotic flow mobility, equal to 9.0×10^{-5} cm² V⁻¹ s⁻¹. The device provided suitable analytical performance for determination of [Ru(NH₃)₆]³⁺. Good correlation coefficient (R² = 0.996), calibration parameters and wide linear range (100 to 700 µmol L⁻¹) were obtained in a linearity study using external calibration curve (Figure III.27). Therefore, C/resin-based electrode was proved suitable for electrophoretic separation coupled with chronoamperometric detection.

III.4. Conclusion and future perspectives

We demonstrated the used of an SLA-based 3D printer to fabricate two kinds of device, EMD and MCE coupled with amperometric detection. We proved the concept of SLA-based 3D-printing for microfluidic devices with easy electrode integration design and new electrode formulation for EC detection. We characterized this new electrode material by electrochemistry using FcMeOH and $[Fe(CN)_6]^{3-/4-}$, providing a good reproducibility and linear response. The analytical method led to a LOD and LOQ of 4.5 µmol L⁻¹ and 15 µmol L⁻¹, for $[Ru(NH_3)_6]^{3+}$ respectively. The use for chronoamperometry coupled with electrophoretic separation has been demonstrated with the quantitation of $[Ru(NH_3)_6]^{3+}$ in PBS, by coupling a high separation voltage with a wireless potentiostat for amperometric detection. This analytical method led to 3 min. The proposed devices are reusable with an electrode surface which can be electrochemically renovate without the necessity to open the device, promoting robustness in the system.

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GENERAL CONCLUSION AND FUTURE PERSPECTIVES

µTAS has emerged in recent years aiming at outstripping the performance of conventional analytical devices, as they deal with very small sample and reagent volumes, low fabrication cost, and decrease of the physical size of the portable devices that would be used in any part of the world without technical expertise or specific experimental conditions [1].

The bibliography study at the beginning of the project demonstrated the challenges still remaining for miniaturization in chemical and biomedical analyses. First, the microdevice materials, geometry and fabrication processes should be further improved so as to gain efficiency, simplicity, as well as to provide low cost and high throughput mass production. Then, implementing more sensitive detection methods would lead to a robust analytical device for a short-time analysis of targeted compounds. Finally the integrated coupling between separation and detection within a microdevice to improve the analytical performances still faces some technological issues. According to this state of the art and so as to answer these three challenges, this work focused on the development of 3D- printing technology to design and fabricate μ TAS that will provide an efficient separation by coupling electrokinetic methods and a powerful detection by electrochemical modes.

According to the literature about additive manufacturing and 3D printing photopolymerization, the fabrication of microfluidics using 3D printing is still in the early stages. Recently, Quero et al. [2] published a systematic study to improve the potential of Fused Deposition Modeling (FDM) 3D printers for the fabrication microfluidic devices with channels size of $58 \times 65 \,\mu$ m. To the best of our knowledge, this was the first time that a FDM based 3D printer reached a microfluidic scale with reproducibility. Conversely, they still cannot reach channels as small as $18 \times 20 \,\mu$ m, dimensions provided for a custom DLP 3D printer [3]. As indicated in Chapter I, vat photopolymerization (VP) printing is the most appropriate technology to fabricate microfluidic platforms with a suitable reproducibility. The first aim of this work was then to develop 3D printing technologies based on VP to manufacture sensing platforms. The second challenge was to integrate electrodes in the microdevice for detection purpose, with the electrode cells integration during the 3D process. This work presents two strategies and technologies for integrating electrodes in different configurations (spiral

electrodes, triangle-shaped electrodes), made of different materials (gallium or a home-made paste composed of a resin and carbon) in the 3D-printing process, so as to perform either contactless conductivity or amperometric detection. The optimization of the electrode design, fabrication process within the microdevice, the materials nature and the reproducibility of production were performed. The sensing platforms were then characterized in terms of electrochemical performances. Finally, the coupling of these electrochemical detection with electrokinetic separation was implemented in these two configurations.

Table 1 summarizes the different strategies developed in this work, which proved their great interest for both CE or MCE-EC in 3D-printed microdevices. According to the employed 3D-printer, different microfabrication protocols have been proposed.

Device	Technology	Printer	Resin	Channel size width × height (µm)	Electrode material	Separation	Detection
1	DLP	Acrea 3D	PEGDA	45×50	Galium	CE	C ⁴ D
2	SLA	FormLabs	Clear FormLabs	95 ×180	C/ resin	-	Amperometry
3	SLA	FormLabs	Clear FormLabs	100×300	C/ resin	CE	Amperometry
4	DLP	Solus Pro 3D printer	Versapic 1147 Clear	103× 200	-	CE	Amperometry

Table 7. The main features of the devices developed in this project.

To the best of our knowledge, the device 1 was the first microchip electrophoresis device with electrochemical detection fabricated using 3D printing technology. This microdevice contains cross-shaped channels and spiral electrodes around the separation channel for microchip electrophoresis and capacitively coupled contactless conductivity detection (C⁴D). The whole device was prepared in a digital light processing based 3D-printer in poly(ethylene glycol) diacrylate resin. Outstanding X-Y resolution of the customized 3D printer ensured the fabrication of 40 μ m cross-section channels. The spiral channels were filled with melted gallium to form conductive electrodes around the separation channel. We optimize the device and the methodology, which is simple, is faster than other methods in the literature with 40 s total separation time for nitrate, and has low environmental impact, including minimal waste generation. The determination of nitrate was achieved with a background electrolyte composed of 30 mmol L⁻¹ lactic acid and 15 mmol L⁻¹ histidine (pH 3.8). The limit of detection for nitrate was found to be 2 μ mol L⁻¹. The device was employed in the analysis of nitrate in saliva samples, a significant biomarker in clinical diagnosis related to inflammatory diseases. The integrated electrokinetic injection and separation modes as well as the C⁴D detection make this platform suitable for sensing small inorganic and organic ions which lack native fluorescence, strong chromophores, or functional groups for derivatization. Therefore, this device 1 provides a universal detection mode, for charged species.

We demonstrated as well for the first time the conception of a reusable stereolithography (SLA) based 3D printed separation device with integrated electrodes for electrochemical (EC) detection (devices 2 and 3). This platform can perform square wave voltammetry (SWV) and amperometric detection, which are sensitivity detection techniques when compared with C⁴D detection. The electroactive species can be detected under pressure driven or electrically driven flows. For this purpose, a new lab-made working electrode (WE) was developed by 3D-printing a side wall microchannel along the separation microchannel and by inserting a graphite/ resin (C/resin) composite within this side wall microchannel. The 3Dprinting conditions of the electrochemical microfluidic device (EMD) as well as the electrode composition, geometry, and integration were optimized for easy and low-cost fabrication (no need for a clean room) and suitable electrochemical response. The electrochemical characterization of the material formulation was first performed in bulk format (disk-shaped, 2 mm diameter) and then moved to the micrometric scale reaching electrode width of 172 ± 12 μm. The integrated electrodes were characterized by voltammetry, and the response linearity of the transducer material was investigated by square wave voltammetry (SWV) using ferrocene methanol (FcMeOH). The C/resin electrode led to a good repeatability (RSD of $\pm 2\%$ (n=3) in the peak current values), linear response ($R^2 = 0.995$) in the 15 – 1000 µmol L⁻¹ range, allowing for limits of detection and quantitation of 4.5 μ mol L⁻¹ and 15 μ mol L⁻¹, respectively. In order to develop a complex analytical device, the interest of this dedicated device was evidenced in a hydrodynamic state condition with $[Ru(NH_3)_6]^{3+}$ as a proof-of-concept, ranging from 0 to 55 μ L min⁻¹ mimicking the electroosmotic flow conditions, which is a bulk liquid motion very common in electrically driven separation techniques. Finally the electrokinetic separation step was implemented in the device. For this purpose an optimization of the position of the respective electrodes for separation and detection was performed, and a deep study allowed to determine the configuration in which the lower interference between separation and detection electric field and the best detection performance occurs. The proof of concept of this MCE-EC was validated with the separation and quantitation of $Ru(NH_3)_6]^{3+}$. This analytical method led to with an analysis time inferior to 3 min. The proposed devices are reusable with an electrode surface which can be electrochemically renovate without the necessity to open the device, promoting robustness in the system. This device is then suitable to determine small and large redox species in samples with complex matrices, such as iron and serotonin in serum.

Each device proved to have strong and weak points, that will be depending on the application. The device 1 contains a universal detector integrated suitable for for sensing small inorganic and organic ions. In addition, it contains an injection, separation and detection methods integrated. On the other hand, The Acrea 3D printer has small build area the size (around 19.5 mm²), which limits the size of the device and consequently the separation channel length as well. Thus, the separation efficiency is compromised. This limitation can be improved by modifying the design of the separation channel. Instead of design a straight separation channel length in the same limited area of the device. The device 2 performs sensitive detection techniques when compared with C⁴D detection. Electroactive species can be detected under hydrodynamic flow and electrically driven flow. However, we still have to insert the platinum electrodes to apply potential. The manipulation of these electrodes can be a laborious step. For further fabrication designs, electrodes to apply potential can be integrated into the microfabrication step. Furthermore, the reference electrode can be also directly fabricated into the platform, facilitating the fabrication process.

Concerning the microfabrication improvement, the two main points are the channel size and a simpler process. Thanks to the improvement in 3D printers precision, future works will be able to lower these dimensions. As for the process simplification, we performed preliminary experiments (device 4). Indeed, the developed device 3 implied a subsequent
sealing step its design and microfabrication were further optimized so as to avoid this step and achieve a 3D-printing in one step. A summary is provided below.

Digital light processing based 3D-printed microchip electrophoresis device with integrated electrochemical detection

The Chapter III demonstrated the development of a microfluidic device with electrochemical detection. In the manufacture procedure, a sealing step was necessary (which is commonly used in the microfabrication process) due to the lack of resolution of the 3D printer. In order to decrease the manufacturing steps, we utilized a digital lighting processing (DLP) 3D printer with a higher number of parameters that can be optimized (XY resolution, layer thickness, exposure time, transition decrement, anti-aliasing) compared with the printer used in the Chapter III. The transition decrement is a parameter which controls the rate of decay of exposure time from exposure time value used to create the base of the device until the exposure time value to create the device parts with details and needs high resolution (channels, cover part and reservoirs).

Herein, we demonstrate the beginning of the conception of a reusable based 3D printed separation device with integrated electrodes for electrochemical (EC) detection. For this purpose, a lab-made working electrode (WE) was developed by 3D-printing a side wall microchannel for the electrode, along the separation microchannel and by inserting a graphite/ resin (C/resin) composite within this side wall microchannel. We used a commercial resin and DLP based 3D printer without the sealing process. We started to optimize the parameters to manufacture close microfluidic channels.

1. Microchips Design and Fabrication

A Solus Pro 3D printer with SprintRay projector and a Versapic 1147 Clear resin was used throughout this work. MD for 3D printing (Fig. 4.1.a) was designed using Fusion 360 (Autodesk, San Rafael, USA) design software. The device for characterization and analyses consists in a single channel of 38 mm length, $103 \pm 7 \mu m$ width (Fig. 4.1.b) and a triangle-shape electrode channel with a rectangular surface with dimensions of $352 \pm 6 \mu m$ width height touching the separation channel (Fig. 4.1.c).



Figure 1. (a) Full 3D-printed MCE device. (b) Top view of close channel of the device. (c) Side-wall triangleshape electrode channel touching the separation channel.

The closed channel device was printed using the resin Versapic 1147 Clear resin. The printer specifications were optimized in layer thickness 10 μ m, resolution 12 μ m, exposure time of 30 s for base layers and 2 s to build channels, cover part and reservoirs, with transition decrement of 5 s.

2. Printing optimization

DLP-based 3D printers have the resolution limited by several parameters, pixel size of the projector (xy-resolution), exposure time and thick layer (z-resolution) as well, which were the limiting factors in achieving small channels. To define the minimum feasible channel size, we studied several exposure times and transition decrements to assemble MD. The exposure time was fixed at 30 s (base layers) / 1s (others) with 3 s of transition decrement, achieving channels of $127 \pm 12 \mu m$ (Fig. 4.2.a). Thus, the exposure time and transition decrement were increased, 30 s (base layers) / 2 s (others) with 5 s of transition decrement, to reach a higher polymerization leading smaller channels. As a result, channels with $103 \pm 7 \mu m$ were accomplished (Fig. 4.2.b).



Figure 2. Images from optical microscopy of 3D-printed channels with around (a) 127 µm and (b) 103 µm width.

As future perspectives, electrodes will be integrated into the full-printed MCE device. Then, the new device will be characterized and optimized to perform as a platform for screening serotonin and iron in biological samples.

3. Bibliography

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RÉSUMÉ

Dans le domaine de la conception de µTAS pour l'analyse de composés à l'état de traces ou d'ultra-traces, nous avons développé des stratégies innovantes employant l'électrophorèse sur micropuce (MCE) couplée à des détections électrochimiques (EC). À cette fin, nous avons conçu et développé des méthodologies d'impression 3D intégrant ces deux étapes analytiques pour une production simple, à faible côut et à haut débit. Deux matériaux d'électrode (gallium ou pâte de résine/carbone) et deux configurations ont été développés pour une integration efficace d'électrodes, en surmontant la nécessité d'alignement des électrodes pour la détection de la CE couplée à la MCE, et en évitant l'interférence des deux champs électriques. Leurs performances électrochimiques et leur couplage efficace avec les séparations électrophorétiques ont été démontrés avec des microdispositifs imprimés en 3D. La configuration des électrodes en spirale s'est avérée efficace pour coupler la séparation électrophorétique avec la détection par conductivité à couplage capacitif sans contact du nitrate dans la salive avec une limite de détection de 2 mM. La configuration des électrodes en forme de triangle a démontré son efficacité pour la quantification par MCE-EC des molécules redox avec de bonnes limites de détection. Ces innovations soulignent le grand intérêt de l'impression 3D pour le développement de MCE-EC pour une large gamme d'applications biomédicales ou environnementales.

MOTS CLÉS

détection électrochimique; impression 3D; électrodes intégrées; microfluidique; electrophorèse capillaire

ABSTRACT

In the context of designing powerful µTAS for the analysis of trace of ultra-trace compounds, we developed innovative strategies employing microchip electrophoresis (MCE) coupled to electrochemical (EC) detections. For this purpose, we designed and developed 3D-printing methodologies integrating these two analytical steps for costeffective and easy high-through-put production. Two electrode materials (gallium or homemade resin/carbon paste) and configurations were developed for an easy EC integration in the MCE, overcoming the alignment necessity of the electrodes for EC detection coupled to MCE, and avoiding the two electric fields interference. Their electrochemical performances and their efficient coupling coupling with electrophoretic separations were demonstrated with 3D-printed microdevices. The spiral electrodes configuration was proved powerful for coupling electrophoretic separation with capacitively coupled contactless conductivity detection of nitrate in saliva with a 2 mM limit of detection. The triangle-shaped electrode configuration demonstrated its efficiency for the MCE-EC quantitation of redox molecules with good limits of detection. These innovation improvements highlight the great interest of 3D printing for MCE-EC developments for a wide range of biomedical or environmental applications.

KEYWORDS

capillary electrophoresis; electrochemical detection; 3D printing; integrated electrodes; microfluidic.