Interfacial Electromigration for Accelerated Reactions and Small-Volume Analysis

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ABSTRACT: Microdroplets have recently emerged as a unique form of a chemical reactor that has gained significant attention due to the interfacial chemistry that produces dramatic reaction acceleration. In this work, we have developed a strategy that allows a thin film to be delivered directly to the interface by electromigration in a theta capillary. Additional acceleration was observed due to reactants being transferred to the interface in electro-oxidative C-H/N-H coupling of phenothiazine with *N, N'*-dimethylaniline, and short-lived intermediates were captured in the radical radical coupling of DMA. Importantly, we applied the combination of electromigration and interfacial microreactor for in-situ extraction of lipids from small-volume plasma samples and accelerated electro-epoxidation of unsaturated lipids to determine the double bond positional isomers in the small-volume serum. The unique feature of electromigration of thin film to the interface and accelerated interfacial reactions holds great potential in small-volume sample analysis for disease diagnosis and prevention.

Introduction

The emergence of microdroplets as tiny reactors has gained significant attention in recent years due to dramatic acceleration of reactions compared to the bulk counterpart. Microdroplets have since been utilized for chemical derivatization,[1] reaction mechanistic studies,[2] high-throughput reaction screening,[3-5] as well as rapid, small-scale, and sustainable synthesis.[6-8] Reaction acceleration in microdroplets is ascribed to the air-liquid interface.[9, 10] Partial solvation of reactants at the interface decreases the activation energy for interfacial reactions relative to bulk, producing accelerated reactions.[9, 11-13] Other interfacial factors that contribute to reaction acceleration in microdroplets include solvent evaporation which increases concentrations and extremizes pH,[14, 15] preferential orientation of reactants and shortened reactant distances which decrease the entropy and increase the free energy of the initial state,[9, 16] and strong electric field which lowers the reaction energy barrier by stabilizing the transition state or by activating the reactant.[17-19] Moreover, the interfacial effects cause spontaneous oxidation and reduction in aqueous microdroplets without adding reducing or oxidizing agents.[17, 20, 21] Reactive species attributed to spontaneous redox are still under debate, while data from different experiments suggest hydroxy radicals, hydrogen peroxide, water radical cations, and electrons formed at the microdroplet air-water interfaces are highly reactive oxidizing and reducing agents.[20, 21]

Microdroplets for reaction acceleration are often generated by electrospray, where sufficiently strong electric force is applied to the solution of a reaction mixture.[22] This results in a zone of high turbulence, known as the Taylor cone, which ejects a plume of charged droplets. Besides electrosprayed droplets, other spray methods including paper spray,[23, 24] sonic spray,[21, 25] thermospray,[26] as well as dropcast[9, 12] and various droplet levitation methods[27, 28] can also be used to form effective interfaces for reaction acceleration. Among these methods, reagents are often dissolved in the bulk solution and sprayed into microdroplets which contain the interfacial region and interior region. This motivates us to think whether reactants can be delivered directly to the interface for accelerated reactions.

Our group developed an interfacial microreactor formed at the meniscus using a large orifice emitter (75-139μm) when the voltage applied to the solution (2kV) is lower than the electrospray voltage (3kV). The meniscus provides a large air-liquid interface while maintaining the contact between reagents and the electrode, filling the gap of acceleration for electrochemical reactions which cannot be achieved in common formats of microdroplets.[29-31] However, the reactants were still premixed and were not introduced at the interface.

In this work, we have developed a strategy that delivers a thin layer of molecules to the interface via electromigration observed uniquely in a large orifice theta capillary (80μm) (Figure 1a). Theta capillaries have two barrels separated by a septum, thus keeping two solutions from mixing until they are sprayed by electrospray ionization (ESI).[32-35] We found a thin film could be electromigrated at the interface from one barrel to the other by applying a voltage just below the voltage to initiate ESI. It is worth noting that electromigration of a thin film is unique to large-orifice theta capillaries and not observed in traditional small ones (10 μm or below). We accurately delivered reactants to the interfacial microreactor by electromigration for further reaction acceleration and capture of surface intermediates invisible to electrosprayed microdroplets. Moreover, we take advantage of the thin-film migration for in-situ extraction of small-volume biological samples and apply accelerated lipid reactions in the interfacial microreactor for lipid isomer characterization at extremely low quantities. Molecular migration allows the directed delivery of molecules to the interface for dramatic reaction acceleration and enables extremely low-volume biological samples to be derivatized and analyzed at the isomer-level.

Results and Discussion

Delivery of a liquid thin film to the interface via electromigration in a theta capillary.

We have developed the strategy of delivering a liquid thin film to an air-liquid interface using the setup shown in Figure 1a. In a theta capillary with an orifice of 80µm barrel **a** was fitted with a Pt electrode immersed in a standard **A** solution (PC 16:0/18:1 at *m/z* 760, 50µM with 0.01% formic acid, and 1 µL) and barrel **b** was loaded with a standard **B** solution (PC 18:1/18:1 at *m/z* 786, 50µM with 0.01% formic acid, and 10 µL). The two molecules **A** and **B** do not react and were used to show the flow direction. We first formed a large air-liquid interface in barrel **a** by applying 2 kV to solution **A** (Figure 1b recorded by a microscope camera). When we increased the voltage to 2.5kV, interestingly, a thin film was observed moving from barrel **b** to **a**. The flow direction was determined by monitoring **A** and **B** using mass spectrometry (MS). Surprisingly, **B** was first shown in the mass spectrum through the Taylor cone formed at barrel **a** (Figure 1c-f), indicating the liquid flow was from **b** to **a** and the liquid transferred was restricted to the interface. Switching the standard solutions in the two barrels led to the same conclusion (Figure S1).

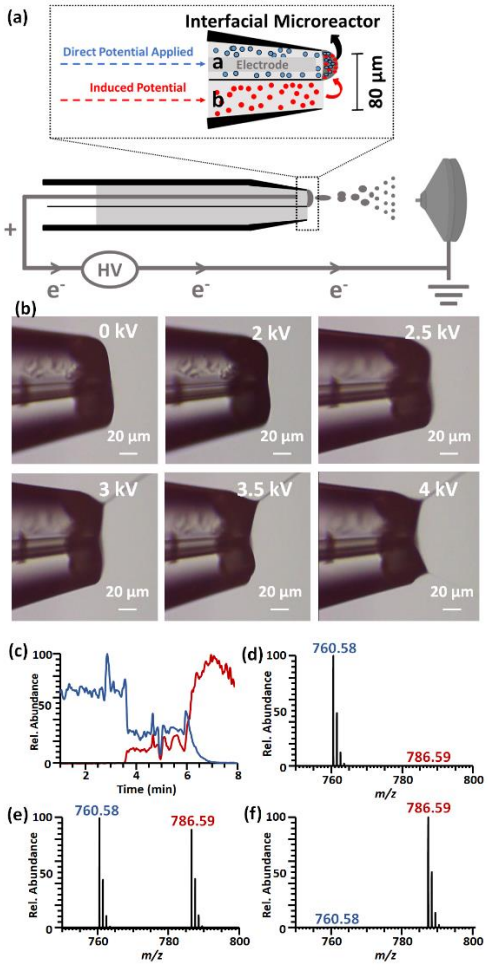


Figure 1. (a) Overview of electromigration in the theta tip-based interfacial microreactor. (b) Spraying modes achieved at various voltages with the electrode in the top barrel. (c) Selected ion chromatograms of the two standards: standard A (PC 16:0/18:1 at m/z 760, 50µM with 0.01% formic acid, and 1 µL) loaded into barrel **a** and standard B (PC 18:1/18:1 at m/z 786, 50µM with 0.01% formic acid, and 10 µL) loaded into barrel. Mass spectra shown at times (d) 1 minute, (e) after 5 minutes, and (f) after 8 minutes.

The electromigration of a thin film to the interface only occurs in large orifice theta glass capillaries using high voltages (40µm diameter for each barrel, optimally 2.5-3.2kV) and is different from electroosmosis[33] occuring in small orifice capillaries using low voltages (5-10µm diameter, 300-500V), which transfer the solution from barrel **a** to **b**. We observed the opposite liquid flows in these two types of capillaries using the zwitterion dye thioflavin S solution (see details in Supporting Information Figure S2). In addition, two Taylor cones were observed in the large orifice theta cappilary nESI when higher voltages (4kV) were applied (Figure 1b).

Electrochemical reaction acceleration and capture of short-lived intermediates by directed delivery of reactants to the interface.

Electro-oxidative C-H/N-H coupling of phenothiazine (PTZ, **1**) with *N,N'*-Dimethylaniline (DMA, **2**) was chosen as a reaction system for study as it forms C-N bond under the mild condition and a series intermediates are formed during the reaction. In this experiment, DMA and LiOTf were loaded into barrel **a** with the Pt electrode and PTZ was loaded into barrel **b**. Once a voltage of 2.8-3.2 kV was applied to the Pt electrode, the interfacial microreactor (meniscus) was formed at barrel **a** and a thin film of PTZ was delivered to the interfacial microreactor. The electro-oxidative coupling product was formed immediately (Figure 2). The acceleration enabled by interfacial eletromigration allows the additional acceleration of 2.42 times compared to pre-mixing reagents in the bulk followed by interfacial reaction which showed an apparent acceleration factor (AFF) of 67 (See Supporting Information for calculation).

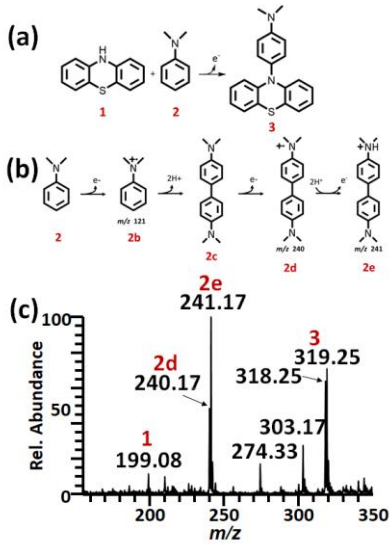


Figure 2. (a) C-H/N-H electro-oxidative cross-coupling reaction between PTZ (1) and DMA (2); (b) DMA homocoupling reaction; (c) mass spectrum

of the reaction observed using electro-migration coupled with interfacial microreactor for reaction acceleration.

The combination of electromigration in a theta capillary with the interfacial microreactor allows us to capture the competative product formation and short-lived intermediates. In junction with the electro-oxidative C-H/N-H coupling product formation (**3**), which was confirmed by tandem mass spectrometry, the in-situ electrochemical radical radical coupling of the reactant DMA (Figure 2b) also occrred. DMA oxidized to generate its corresponding radical cation (**2b**). This product then underwent homocoupling of the radical cation to form the dimer (**2d**) and further protonation to form the protonated dimer (**2e**).[38]

Electromigration for an in-situ lipid extraction from human plasma.

Biofluids are often employed for effective disease diagnosis. Small-volume detection is tantalizing as it offers remarkable advantages such as portability, inexpensiveness, capacity for mass production, and unique applicability of analyzing body fluids that have limited volumes including those from the eye, blisters, and the cerebrospinal area. Traditional extraction and derivatization often cannot be achieved in these samples due to the limited volumes.

Lipids play a significant role in the cellular functions including biological membrane structural support, energy storage, and cell signaling. Dysregulated lipid metabolism has been reported in many diseases,[39] therefore lipids can be important biomarkers for diagnosing disease, monitoring disease progressions, and evaluating treatment effectiveness. In this work, we initiated to demonstrate the application of electromigration to achieve in-situ extraction and profiling of lipids from small-volume human plasma (<0.1µL), avoiding using traditional lipid extraction such as Bligh & Dyer,[40] Folch,[41] Matyash,[42] which are step-heavy, requring large volume samples and timely processes. Human plasma was chosen for analysis as it is known to contain lipids that are biomarkers for disease diagnostics and readily available.[45-47]

In a theta capillary, we loaded human plasma (<0.1µL) into barrel **b** and the spray solvent into barrel **a**. After applying a voltage of 2.5kV, the meniscus of air-liquid interface formed. Then a thin film of minimal human plasma was electromigrated from its barrel directly onto the meniscus at 3kV. This allowed a small amount of mixing between the sample and solvent to occur only at the interface where the lipid extraction took place. Following the in-situ extraction, lipids were then detected via MS as a very fine plume of charged droplets were released from the meniscus. The lipid profile and identified lipids from the human plasma were shown in Figure 4 and Figure S3. Lipids of 25 classes have been tentatively identified including polar and nonpolar lipids. Glycerophospholipids such as PC, PA, PE, and PI, and triglycerides (TG) dominated the profile. A modified Matyash solvent which contained MTBE/ACN/H2O (v/v=40:4:1) with 10mM of NH_4Cl and 1mM HCl was used as the optimal extraction/spray solvent after comparing to the traditional Matyash solvent (Figure 3) and other systems (Table S1). The addition of NH_4Cl was helpful in identifying nonpolar lipids such as CE, DG and TG. Additionally, electromigration allows the lipid to be observed in MS with a limit of detection of 10fM (Figure S4).

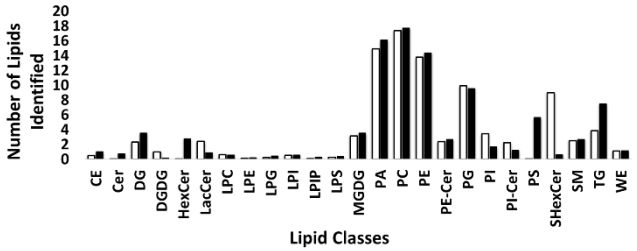
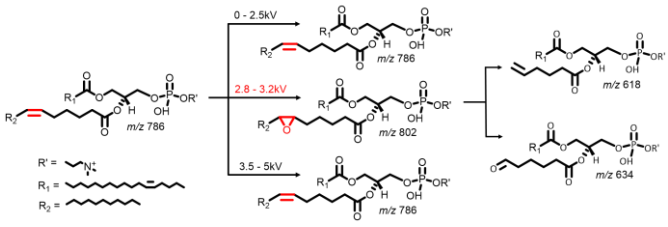


Figure 3. Small-volume human plasma lipid profiling via in-situ extraction by electromigration in a theta capillary coupled with MS analysis. Lipid profile comparison was generated between the use of Matyash solvents (white bar) and the modified Matyash solvents (black bar).

Electromigration combined with the electrochemical interfacial microreactor for in-situ extraction and characterization of fatty acids from mouse serum at the isomer level.

Lipid characterization is challenging due to lipid structural diversity and isomeric forms. Lipid derivatization is an attractive strategy for isomer identification when coupled with tandem MS as it involves minimal instrument modification and reagents are often easy to access. Our group developed voltage-controlled electro-epoxidation of lipid double bonds (Scheme 1) to characterize the double-bond positions in unsaturated lipids.[37, 43] After loading lipids in ACN and water in the presence of HCl into the interfacial microreactor, the chloride in an acidic environment was oxidized to hypochlorite followed by epoxidizing the lipid double bond into an epoxide.[30] In this work, we combined electromigration and electrochemical interfacial microreactor to derivatize lipids extracted in-situ from small-volme serum samples for lipid characterization at the isomer level.

We started the experiment with a lipid standard PC(18:1/18:1) (50µM) loaded into one barrel of the theta capillary and the solvent system (ACN/water, v/v=4:1, and 10mM HCl) into the other barrel with the Pt electrode. When a relatively low voltage was applied (2.5-2.7kV), PC(18:1/18:1) at *m/z* 786 along with its corresponding sodium and potassium salt adducts at *m/z* 808 and *m/z* 824 were detected (Figure 4a). After applying the voltage between 2.8-3.2kV, the interfacial microreactor was formed and electromigration of the lipid standard occurred. The formation of the mono-epoxide at *m/z* 802 and the di-epoxide at *m/z* 818 was observed (Figure 4b). Further, when we applied a relatively high voltage (3.5kV or greater), the interfacial microreactor was no longer formed and the only species detected was the protonated lipid (Figure 4c). This can be explained by the surface tension of the meniscus (interfacial microreactor) being overcome by coulombic forces and the rounded meniscus becoming inverted into a jet of liquid.[13] The formation of the epoxide at the lipid double-bond allows for the use of collision-induced dissociation (CID) to differentiate diagnostic ions for characterization. The diagnostic ions at *m/z* 634 and *m/z* 618 indicate the double bond was at Δ 6 in PC(18:1/18:1) (Figure 4d).



Scheme 1. Interfacial voltage-controlled electroepoxidation of lipid PC(18:1/18:1) coupled with tandem MS to generate diagnostic fragments at *m/z* 618 and 634 for lipid double-bond position determination.

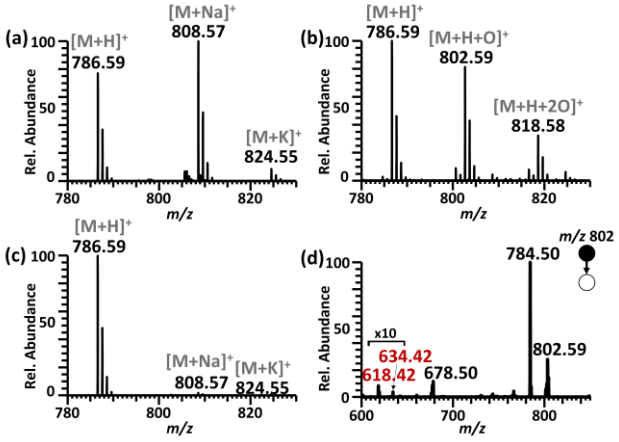


Figure 4. Mass spectra of lipid standard PC(18:1/18:1) obtained at a voltage of (a) 2.5-2.7 kV; (b) 2.8-3.2 kV; and (c) 3.5 kV and (d) tandem mass spectrum of electro-epoxidation product ions at *m/z* 802 via CID. The diagnostic ions to locate double bond positon in PC(18:1/18:1) are shown in red.

After demonstrating the feasibility of coupling electromigration and the interfacial microreactor in the theta capillary, we began the analysis of lipids from small-volume mouse serum at the double-bond position isomer level. Fatty acids (FA) were focused in this study in order to show the method applicable to negatively charged lipids besides positively charged lipids. In order to form electro-epoxidation products of FA by applying positive potential to the electode and detect FAs and their electro-epoxidation products in the negative ion mode, alternating current (AC) voltage was used to replace the DC voltage, so hyperchloride was formed by anodic oxidation to enable the epoxidation of FAs in positive mode.[43] The AC voltage was applied using a rapid polarity switch to observe the FAs and derivatives in negative mode. A solution of EtOAc with NH_4Cl and HCl[44] was chosen for the in-situ extraction and derivatization of FA from small-volume mouse serum as EtOAc was efficient for in-situ FA extraction, and the addition of the NH_4Cl and HCl formed the source to initiate epoxidation. Electroepoxidation products were observed for FAs after in-situ extraction from the mouse serum and were shown in Figure 5a. The electro-epoxidation products of FAs including lauric acid (*m/z* 199), myristoleic acid (*m/z* 225), myristic acid (*m/z* 227), palmitoleic acid (*m/z* 253), palmitic acid (*m/z* 255), oleic acid (*m/z* 281), and steric acid (*m/z* 283), were clearly shown in the spectrum via the in-situ FA extraction and epoxidation. These ions were then isolated and fragmented via CID, producing the diagnostic ions to determine the double-bond positions (Figure S6). In total, 21 lipid double-bond positional isomers were identified after in-situ extraction of small-volume mouse serum followed by electro-epoxidation in the interfacial microreactor and tandem MS analysis. The distribution of double-bond positional isomers were shown in FA 18:1, 16:1 and 14:1 (Figure 5b).

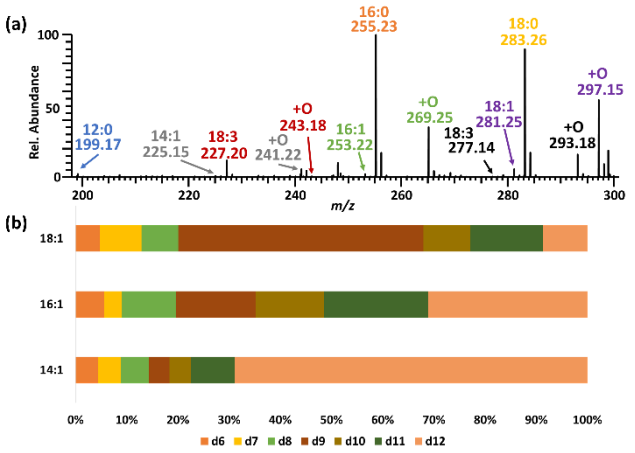


Figure 5. Mass spectrum of lipids from small-volume mouse serum after in-situ extraction by electromigration followed by electro-epoxidation in the interfacial microreactor; and (b) double-bond positonal isomer distribution in FA 18:1, FA 16:1 and FA 14:1.

Conclusions

In this study, we have developed a strategy of delivering a thin film to an inteface using a voltage-controlled electromigration in a theta capillary. Electromigration has been characterized by tracking the liquid flow using lipid standards and dye. The electromigration coupled with interfacial microreactor has been demonstrated for reaction acceleration at the interface, gaining extra acceleration by directed delivering reactants to the interface. We also demonstrated its powerful application in small volume in-situ extraction and derivatization for analyzing lipids at the isomer level using a small quantity of biological samples.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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