Applications of Electrochemistry at Liquid/Liquid Interfaces for Ionizable Drug Molecule Sensing

Eunseo Goh, Hye Jin Lee*

Received September 2, 2016

Electrochemistry at an interface between two immiscible electrolyte solutions (ITIES) offers a great opportunity to be applied to electroanalytical, biological and energy related research fields. In particular, ion transfer processes across a polarized ITIES have been employed as powerful electrochemical ion sensing platforms. This review will highlight recent developments in addition to some challenges and future aspects on developing ion selective sensing platforms utilizing ITIES specific to ionizable pharmaceutical reagents (drugs).

Introduction

Charge transfer reactions across a polarized interface between two immiscible electrolyte solutions (ITIES) have been used for a wide range of applications including sensing platform development, energy research, and also biomimic studies. In particular, there have been extensive research efforts on developing ion selective sensors for inorganic, organic and biological ionized species in addition to theoretical approaches to elucidate charge transfer processes and develop new paradigms. There have been many recent review articles highlighting different aspects of ITIES including theory and applications on charge transfer process including drug ions. The purpose of this review is to provide some challenges and future aspects of a less highlighted research area on developing drug ion sensors using ITIES.

Since measurements of the current associated with a direct or assisted ion transfer reaction across a polarized ITIES can be used as an effective detection platform for non-redox ionic species, the ITIES based sensors hold a great promise for targeting drug ions. Due to a large library of ionophores selective towards metal ionic and anionic species, most ITIES sensors were focused on target ions for environmental and biological applications, but much fewer sensing applications have been reported for organic molecules including drugs. The majority of ITIES studies on drug molecules have focused on understanding the lipophilicities of interest of drugs for pharmacokinetics via voltammetric investigations of differently charged drug species partitioned at a polarised ITIES. Along with thermodynamic investigation of drugs at ITIES, these studies have provided important information and paved pathways for designing drug ion sensors. Therefore, this review will first introduce a short background of ion transfer reactions across a polarized ITIES followed by the construction of an interface between two immiscible electrolyte solutions, which is the foundation of sensing platform developments. The last section involving the application of ITIES for drug sensing will cover some representative examples developed recently by several groups.

Basic Principles of Ion Transfer Processes at the ITIES

There have been extensive research efforts to elucidate ion transfer processes across a polarized ITIES. The key aspect is that the potential difference across the interface between two immiscible electrolytes (water and oil) is correlated to the activity of an ionic species ($i$) distributed across the interface which can be
described in the below equation, which is analogous to the Nernst equation [eq (1)]
\[
\Delta_w \phi = \Delta_o \phi_i^0 + \frac{RT}{Z_iF} \ln \left( \frac{a_i^w}{a_i^o} \right)
\]
\[
= \Delta_w \phi_i^o + \frac{RT}{Z_iF} \ln \frac{c_i^w}{c_i^o}
\]  
(1)

where \(a_i\) and \(c_i\) are the activity and the concentration of the ion \((i)\), respectively, in the water \((w)\) and oil \((o)\) phases and \(\Delta_w \phi_i^0\) and \(\Delta_w \phi_i^o\) represent the standard and the formal transfer potentials respectively. From an analytical sensing perspective, individual ions transferring across the interface results in a current that increases proportionally as a function of increased ion concentration. Different equations depending upon the interface size that can be applied to describe the relationship between the current versus the concentration will be discussed in the next section.

**Construction of ITIES for Ionizable Drug Transfer Process**

An important analytical aspect on utilizing ion transfer reaction across the ITIES either directly or assisted by an ion selective ionophore present in the organic phase is that current measured due to the transfer process proportionally increases with respect to the target ion concentration. The current changes will in particular be influenced by the shape and size of the interface formed between two immiscible electrolyte solutions. The ITIES can be constructed in many different ways, though we would like to classify them into: (i) large scale ITIES, (ii) microITIES including microhole or micropipette or an array of microhole supported by polymeric or silicon substrates, (iii) nano-ITIES and (iv) non-conventional ITIES.

Figures 1–3 shows schematics of the electrochemical set-up for different types of ITIES alongside cyclic voltammograms (CVs) for representative drug ion transfer processes across the ITIES. For a large scale ITIES (Figure 1a) with an interfacial area in the cm\(^2\) range, the interface is regarded as planar and ion interfacial transfer will be governed by a semi-infinite diffusion flux which results in a peak shaped voltammogram (see CV shown in Figure 1b for 0.1 mM timolol drug ion transfer reaction across ITIES). The Randles–Sevcik equation (2)\(^{15}\) for a redox reaction at a solid electrode can thus be rewritten and used to determine the concentration of the transferring ion \((i)\) with respect to the measured concentration when the ionic species \((i)\) transfers across the ITIES:

\[
I_{p}^{w-o} = 0.4463z_iAFc_i^w \sqrt{\frac{RT}{F}} \sqrt{D_i^w} 
\]  
(2)

where \(I_{p}^{w-o}\) is the maximum forward peak current, \(z_i\) is the ion \((i)\) charge, \(A\) is the interfacial area, \(F\) is the Faraday constant, \(c_i^w\) and \(D_i^w\) are the aqueous bulk concentration and the diffusion coefficient of ionic species \(i\),

![Figure 1](image_url)
respectively and \( \nu \) is the rate of potential sweep. The large scale ITIES in conjunction with cyclic voltammetry has often been used for lipophilicity studies of target drug ions, but have not been widely used for sensing purposes due to the mechanical instability of two liquids in addition to the inconvenience of requiring a four-electrode configuration.

The miniaturization of ITIES down to micro or nanoscale in conjunction with gelifying the organic phase could help design ITIES-based drug sensors by improving the mass transport of ion species resulting in enhanced detection performance and also better mechanical stability of ITIES.\(^\text{11}\) The microscale ITIES can be classified into (a) micropipette supported ITIES, (b) microhole-supported ITIES in a single or array format based on polymer composite or silicon membranes, and micro-cavity-supported ITIES formed in polymer substrates. As can be seen in Figure 2a, for the transfer of ionic species \((i)\) at a micropipette tip supported ITIES, the linear diffusion flux dominates when the ion transfer from the water to the organic phase resulting in a peak shaped voltammogram. When the ion transfers back from the organic to water phase, the hemispherical diffusion flux governs leading to a steady-state voltammogram. The steady state current \((I_{ss})\) increases proportionally as a function of the ion \((i)\) concentration, according to eq (3)\(^\text{16}\):

\[
I_{ss} = Kz_iFD_i^W c_i^W r
\]

where \(K\) is a geometric constant dependent on the pipette tip size, \(r\) is the radius of the tip, and the other parameters were defined in eq (2). As can be seen in Figure 2b, the \(I_{ss}\) change

Figure 2. Schemes showing electrochemical cell set-ups for (a) a micropipet and (c) a single microhole on a thin polymer film supported ITIES. (b and d) CVs for various concentrations of (R) propranolol ranging from 0.0375 (light gray) to 0.10 mM (black) with 0.050 mM of α1-acid-glycoprotein in solution (b) and for different concentrations of protonated topotecan (HTOPO\(^+\)) changed from (i) 0 to (v) 200 \(\mu\)M (d). Water/1,2-DCE and water/PVC-NPOE gel interfaces were used. Reprinted with permission from refs. 31 for (a) and 17 for (d) (Copyright 2012 and 2015, American Chemical Society).
associated with the transfer of (R) propranolol across the micro-ITIES increased as the (R) propranolol concentration increased which can be used as a drug-sensing platform.

In addition to a micropipette supported ITIES, a single or an array of microhole(s) fabricated in a thin polymeric or silicon membrane have been used (see Figure 2c). A hemispherical diffusion flux will govern when the target ion transfers across the microhole-ITIES resulting in steady-state voltammograms. Equation (4) can then be used to correlate the steady-state current and the target ion concentration transferring across the ITIES\(^1\):

\[
I_{ss} = 4n z_i F D_i^{w} c_i^{w} r
\]

where \(r\) and \(n\) are the radius and the number of microholes, respectively, and the other parameters were defined in eq \(2\). For an array of microholes, eq \(4\) can also be used when the microhole interfaces are diffusionally independent of each other.

In addition to miniaturizing the interface, the mechanical stability of ITIES has been improved by gelifying the organic phase for expediting the sensing applications. Plasticizer such as polyvinylchloride (PVC) dissolved in organic solvent hot cast onto a single or an array of microhole(s) supported in a thin membrane were developed for a wide range of ion sensing applications recently including topotecan drug sensing.\(^1\) Like the case of micropipette supported ITIES, current changes due to protonated topotecan (HTOPO\(^+\)) ion transfer from the water to gel phase linearly increased with respect to the topotecan concentration (see Figure 2d). The sensing platform is amenable to miniaturization and can also be transformed as a portable and field-applicable disposable chip device. Note that due to the viscous organic gel layer filled in the hole, when the ion transfers back from the gel to water phase, it is usually limited by a linear flux resulting in a peak shaped voltammetric response (see Figure 2d).

Further enhanced mass transport alongside a lowered IR drop can be achieved by reducing the ITIES down to nanoscale. For example, Arrigan et al. demonstrated that nanoscale-ITIES array based ion sensors could be 50 times more sensitive than those using microscale-ITIES arrays and 1000 times more sensitive than a single macro-ITIES.\(^1\) A single (or dual) nanopipette prepared by pulling a quartz capillary or placing nanoporous materials containing geometrically irregular or regular arrays of pores can be utilized for creating nanoscale-ITIES (see the scheme in Figure 3a). A regular array of nano-ITIES featuring silicon nitride membrane.

**Figure 3.** (a) Scheme of an electrochemical cell set-up for an array of nanohole ITIES supported in a silicon nitride membrane and (b) CVs for protonated propranolol transfer across nanoscale-ITIES featuring silicon nitride nanoporous membranes with 400 pores (\(d = 100\) nm) in a hexagonal arrangement. Propranolol concentrations were varied from 0 (black), 20 (red), 40 (blue), 60 (green), 80 (pink), to 100 \(\mu\)M (orange). Scan rate: 5 mV s\(^{-1}\). Reprinted with permission from ref. 18 (Copyright 2015, American Chemical Society).
nitrile nanoporous membranes with 400 pores in a hexagonal arrangement, with either 50 or 17 nm radius was successfully used to monitor different concentrations of propranolol. Similar to the microhole supported ITIES, the hemispherical diffusion flux governs when the ion transfer occurs across the nanoscale interface resulting in steady-state voltammograms.

Sensing platforms based on ITIES can be modified in different ways for a particular purpose. For instance, Girault’s group developed a new experimental set-up for studying the partitioning of ionizable drugs at the ITIES fabricated on 96-well microfilter plates with microporous filters to support 96 organic liquid membranes. This offers a rapid and efficient tool for determining the partition coefficient (log $P$) of many different ionized drugs. Another new way to create ITIES is the use of a microneedle array with an embedded micro-ITIES platform for detecting propranolol. Future designs featuring different ITIES configurations with specific functionality or purpose will definitely accelerate the ITIES research field for sensing applications including drug molecules.

**Applications of ITIES for Drug Sensing**

High performance liquid chromatography (HPLC) and LC-mass spectrometry have been most commonly used for the analysis of drug molecule concentrations in addition to optical or electrochemical sensing methods. As mentioned in the introduction, the use of ion transfer processes across ITIES is promising for drug sensing because most drugs are ionizable, no labeling process with redox detection probes is required and also it can be easily miniaturized with portability. However, most studies on drug molecules at the ITIES have been focused on electrochemical investigations for the partition of an ionizable drug across ITIES in order to elucidate the lipophilicity of drugs and the resulting partition coefficient ($P$) and the ratio of the activity of a solute in both phases in equilibrium, which is a common measure of lipophilicity. Thorough review articles on evaluating $P$ of an ionizable drug experimentally using electrochemical techniques such as cyclic voltammetry, differential pulse voltammetry and square wave voltammetry can be found in the literature.

Such electrochemical lipophilicity investigations on drug ion transfer processes across the ITIES can provide an important insight for developing drug ion sensors. Nevertheless, only few reports are available for particularly ionizable drugs compared to the vast research efforts made on the use of ITIES for sensing of inorganic and also small organic ions. Table 1 provides a summary of drug ion studies at ITIES particularly aiming at developing sensing platforms. Some of the references cover the detection limit of the drug sensing performance that is a valuable parameter.

Initial research efforts on using ITIES for analytical drug sensing were focused on solving the mechanical instability of the liquid/liquid interface. For instance, Ortuno et al. investigated a method to gelate the organic phase with polyvinylchloride–nitrophenyloctylether (PVC–NPOE) membrane which was then incorporated into flow-injection analysis for determining verapamil and imipramine in human urine and human serum, respectively. A linear relationship between current peak height and drug concentration was reported with negligible interference effects of some common ions and pharmaceutical excipients on the verapamil and imipramine.

The most widespread species of targeted drug studied using ITIES is propranolol, which is an adrenergic α-receptor blocking drug. The Arrigan group has also studied the detection of propranolol in artificial saliva based on ion-transfer voltammetry across arrays of micro-ITIES. Differential pulse stripping voltammetry (DPSV) measurements along with preconcentrating the drug, which is performed via holding the potential for a certain time, has been used to improve sensitivity. The drug transfer occurred from the water to the organic phase via arrays of microscale-ITIES. The DPSV peak current response increased with respect to the drug concentration ranging from 0.05 to 1 µM in the saliva matrix with a detection limit of 0.02 µM. This demonstrated that the ITIES based sensor in conjunction with DPSV could be used for drug concentration analysis in biological matrixes.

The Arrigan group has also studied the interfering effect of bovine serum albumin (BSA), which is a serum protein, on propranolol sensing in the artificial serum matrix when using a microhole array supported ITIES with a voltammetric technique. Again using DPSV, 0.05 µM propranolol could be detected even in the presence of BSA. Voltammetric sensing of protonated propranolol using arrays of nanoscale
ITIES were further investigated. Nanoporous silicon nitride membranes having 400 pores \((r = 17 \text{ or } 50 \text{ nm})\) in a hexagonal configuration were used to create nano arrays supported ITIES. It was shown that the current density measured for propranolol sensing was at least 6 times higher for the nanoITIES with 17 nm radius pores than that of using 50 nm radius pore array, which can be explained by the increased ion flux due to the convergent diffusion to the smaller interfacial area. This work successfully demonstrated that ion transfer processes at a nanoscale ITIES could powerfully be employed for pharmaceutical analysis applications.

Another highlight work on the detection of propranolol is the use of a water/organic gel interface supported in a hollow microneedle platform for the in vivo study of drugs. Such microneedle supported ITIES could prevent fluid extraction, which maybe arises from the potential difference set at the interface when the ionizable drug transfer occurs across the interface, with the benefit of using microscale interface offering fast diffusion of ionic species resulting in the enhanced detection performance. The microneedle based ITIES sensor was characterized using tetraethyl ammonium ion transfer processes with cyclic voltammetry (CV) technique. Using DPSV, the microneedle based sensor showed a linear dynamic range between 50 – 200 nM for propranolol with a limit of detection of 50 nM.

In addition to propranolol, other drug molecules including anticancer drugs have been studied; for instance, Pereira et al., demonstrated that the transfer processes of the lipophilic anticancer drug daunorubicin (DNR) could be monitored across the large scale water/1,6-dichlorohexane (1,6-DCH) interface using differential pulse voltammetry (DPV). The lipophilicity studies of DNR at the ITIES were presented in the form of an ionic partition diagram and the partition coefficients of both neutral and ionic forms of the drug were determined. Also an ITIES based sensor for DNR was developed with a detection limit of 0.80 µM and a dynamic range of 12 – 82 µM when using DPV. The same group further investigated the interaction between DNR and double stranded DNA at a water/1,6-DCH interface using DPV and evaluated the binding constant as \(1.7 \times 10^4 \text{ M}^{-1}\).

| Drugs       | ITIES type                                                                 | Detection method | Sensitivity (nA µM\(^{-1}\)) | LOD (µM) | Ref |
|-------------|---------------------------------------------------------------------------|-----------------|------------------------------|----------|-----|
| Propranolol | Microhole array supported silicon membrane with PVC-NPOE gel              | DPV             | 1.34                         | 0.1      | 24  |
|             |                                                                           | DPSV            | −8.13                        | 0.02     |     |
|             | Microhole array supported silicon membrane with PVC-1,6-DCH gel           | DPV             | 0.07                         | 4        | 25  |
|             |                                                                           | DPSV            | −0.82                        | 0.05     |     |
|             | Hollow silicon microneedle array with PVC-1,6-DCH gel                     | DPSV            | 0.0018 \((d = 34 \text{ nm})\) | 0.008    | 20  |
|             |                                                                           | CV              | \(0.0011 \((d = 100 \text{ nm})\)\) |          |     |
| Daunorubicin| Microhole array supported PET film with 1,6-DCH                           | DPV             | 0.019                        | 0.80     | 26  |
| Imipramine  | PVC plasticized membrane with NPOE                                       | Flow-injection pulse | –                      | 1        | 23  |
| Tenofovir   | Cellulose membrane with 1,2-DCE                                           | CV              | 7.09                         | 5        | 29  |
| Ractopamine | Microhole array supported silicon membrane with PVC-1,6-DCH gel           | LSSV            | 0.272                        | 0.1      | 28  |
| Topotecan   | A microhole supported PET film with PVC-NPOE gel                           | DPSV            | 0.012                        | 0.1      | 17  |
| Verapamil   | PVC plasticized membrane with NPOE                                        | Flow-injection pulse | –                      | 5        | 22  |

Table 1. Summary of different ITIES ion detection platforms for various drug molecules.
Some other examples of drug molecule investigated includes a protonated racopamine (RacH\(^+\)) analysis in artificial serum based on RacH\(^+\) transfer across a microhole supported water/1,6-DCH interface using linear sweep stripping voltammetry (LSSV) with preconcentration of RacH\(^+\) in the gel via applying the drug transfer potential.\(^{28}\) As well as detecting RacH\(^+\), the interfering effect of serum protein on the detection signal was investigated, which should be considered when dealing with any biological fluid analysis.\(^{25}\) The detection of tenofovir using the membrane stabilized water/1,2-dichloroethane (1,2-DCE) interface was also studied where dibenzo-18-crown-6 in the organic phase was used as a ligand to facilitate the tenofovir transfer reaction.\(^{29}\) Recently, our group reported a microscale-ITIES sensor for determining the anticancer drug topotecan concentration in biological samples, the voltammetric responses of the topotecan transfer reaction across the interface at different aqueous pH values were first studied to address the lipophilicity of the drug, which provided an well-defined voltammetric response of topotecan transfer processes at pH 4.\(^{17}\) This condition was finally used to design topotecan sensitive sensors featuring a microhole supported-water/PVC-NPOE gel interface with a DPSV technique where a 1 V (vs. Ag/AgCl) was applied for 30 s to preconcentrate protonated topotecan (HTOPO\(^+\)) in the gel layer prior to fast stripping of the ion. HTOPO\(^+\) spiked in diluted serum samples ranging from 2.5 to 100 µM could be analyzed (see Figure 4).

**Summary and Outlook**

Current changes associated with target drug ion transfer processes across a polarized ITIES, also being a linear function of the drug concentration, can be powerfully used for developing drug sensitive or selective sensors. The efforts of using supportive membranes or substrates with micro-interface features, as well as gelating one of the liquid phases, have enabled great advances in transforming ITIES to a field potentially applicable for instrument incorporated sensing devices for determining a wide range of charged inorganic, organic, drug and even protein species. However, there are still many challenges including selectivity and sensitivity to tackle when using ITIES for developing drug ion sensors. The selectivity of ITIES sensors relying on a direct drug ion transfer reaction can be improved by employing an ionophore or ligand that can selectively interact with a target drug with a high association constant. Unlike metal ionic and small anionic species where there have been extensive efforts made for exploring targets using specific organic ionophores, there are only a few reports targeting drug ions of which most are biological molecules. Considering that the majority of drugs have complicated ring structures, which are often difficult to dissolve in the water phase, there are great opportunities for organic chemists to design specific ionophores for drug sensing technologies.

Another challenge is to improve the detectable target concentration range of ITIES sensors, which is usually in the micromolar concentration range of target when using voltammetric methods. The use of differential pulse stripping voltammetry combined with preconcentrating the target in the gel layer followed by fast stripping can enhance the detectable target concentration about ten-fold or slightly more than that of linear sweep or cyclic voltammetry. In addition to the use of different electrochemical techniques, increasing the number of micro- or nano-holes in a micro or nano-ITIES supported polymeric or silicon
substrates could increase the sensitivity or detection limit. More dramatic enhancement in sensitivity is needed when dealing with trace amount of target in real samples. We envision all these efforts will promote the development of the electrochemistry of ion transfer processes across the ITIES for further applications in drug sensing.

Acknowledgement
This research was supported by the National Research Foundation (NRF) of Korea funded by the Ministry of Science, ICT, and Future Planning (grant number: NRF-2015R1A2A1A15052198).

References
1) H. J. Lee, D. W. M. Arrigan, M. N. Karim, H. R. Kim, "Electrochemical Strategies in Detection Science", ed. D. W. M. Arrigan, 2015, Chap. 9, Royal Society of Chemistry.
2) P. Peljo, H. H. Girault, "Encyclopedia of Analytical Chemistry", 2012, John Wiley & Sons, New York.
3) D. W. M. Arrigan, G. Herzog, M. D. Scanlon, J. Strutwolf, "Electroanalytical Chemistry: A Series of Advances", Bard, A. J.; Zoski, C. G., Eds. 2013, Vol. 25, p 105, CRC Press, New York.
4) Z. Samec, Electrochim. Acta, 84, 21 (2012).
5) H. H. Girault, in "Electroanalytical Chemistry", Bard, A. J.; Zoski, C. G., Eds. 2010, Vol. 23, p 1, CRC Press, New York.
6) R. A. W. Dryfe, in "Advances in Chemical Physics", ed. S. A. Rice, 2009, Vol. 141, p 153, John Wiley & Sons, New York.
7) S. Liu, Q. Li, Y. Shao, Chem. Soc. Rev., 40, 2236 (2011).
8) Z. Samec, Chem. Rev., 88, 617 (1988).
9) G. Herzog, Analyst, 140, 3888 (2015).
10) M. Velicky, A. N. J. Rodgers, R. A. W. Dryfe, K. Tam, ADMET & DMPK, 2, 143 (2014).
11) D. W. Arrigan, Anal. Lett., 41, 3233 (2008).
12) R. Gulaboski, F. Borges, C. M. Pereira, M. N. D. S. Cordeiro, J. Garrido, A. F. Silva, Combinatorial Chemistry & High Throughput Screening, 10, 514 (2007).
13) H. Alemu, Pure Appl. Chem., 76, 697 (2004).
14) G. Bouchard, P. A. Carrupt, B. Testa, V. Gobry, H. H. Girault, Chem. Eur. J., 8, 3478 (2002).
15) A. J. Bard, L. R. Faulkner, "Electrochemical Methods: Fundamentals and Applications", 1980, John Wiley and Sons, New York.
16) B. Liu, M. A. Mirkin, Electroanalysis, 12, 1433 (2000).
17) H. R. Kim, C. M. Pereira, H. Y. Han, H. J. Lee, Anal. Chem., 87, 5356 (2015).
18) Y. Liu, J. Strutwolf, D. W. Arrigan, Anal. Chem., 87, 4487 (2015).
19) S. M. Ulmeanu, H. Jensen, G. Bouchard, P. A. Carrupt, H. H. Girault, Pharm. Res., 20, 1317 (2003).
20) P. Vazquez, G. Herzog, C. O. Mahony, J. O. Brien, J. Scully, A. Blake, C. O. Mathuna, P. Galvin, Sens. Actuators B, 201, 572 (2014).
21) S. Nussbaumer, P. Bonnabry, J. Veuthey, S. F. Souverain, Talanta, 85, 2265 (2011).
22) J. A. Ortuno, C. Sanchez-Pedreno, A. Gil, Anal. Chimi. Acta, 554, 172 (2005).
23) J. A. Ortuno, A. Gil, C. Sanchez-Pedreno, Sens. Actuators B, 122, 369 (2007).
24) C. J. Collins, D. W. Arrigan, Anal. Chem., 81, 2344 (2009).
25) C. J. Collins, C. Lyons, J. Strutwolf, D. W. Arrigan, Talanta, 80, 1993 (2010).
26) J. A. Ribeiro, F. Silva, C. M. Pereira, Anal. Chem., 85, 1582 (2013).
27) J. A. Ribeiro, C. M. Pereira, F. Silva, Electrochim. Acta, 180, 687 (2015).
28) M. Sairia, D. W. Arrigan, Talanta, 132, 205 (2015).
29) S. H. I. Hamid. "Liquid-Liquid Interface Ion-Transfer Amperometric Sensors for Tenofovir as a Model Nucleoside/Nucleotide Anti-Retroviral Drug", MSc Thesis, University of the Western Cape, 2014.
30) C. J. Collins, A. Berduque, D. W. Arrigan, Anal. Chem., 80, 8102 (2008).
31) P. Lopes, R. Kataky, Anal. Chem., 84, 2299 (2012).