analytical chemistry

Technical Note

pubs.acs.org/ac

A Solid-State pH Sensor for Nonaqueous Media Including Ionic Liquids

Brianna C. Thompson,*[,†,¶] Orawan Winther-Jensen,[†,‡] Bjorn Winther-Jensen,[§] and Douglas R. MacFarlane*[,†,‡]

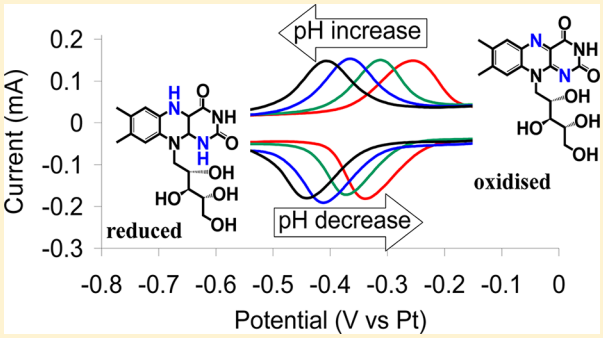
[†]School of Chemistry, Monash University, Clayton, VIC 3800, Australia

[‡]ARC Centre of Excellence for Electromaterials Science, Monash University, Clayton, VIC 3800, Australia

[§]Department of Materials Engineering, Monash University, Clayton, VIC 3800, Australia

Supporting Information

ABSTRACT: We describe a solid state electrode structure based on a biologically derived proton-active redox center, riboflavin (RFN). The redox reaction of RFN is a pH-dependent process that requires no water. The electrode was fabricated using our previously described 'stuffing' method to entrap RFN into vapor phase polymerized poly(3,4-ethylenedioxythiophene). The electrode is shown to be capable of measuring the proton activity in the form of an effective pH over a range of different water contents including nonaqueous systems and ionic liquids (ILs). This demonstrates that the entrapment of the redox center facilitates direct electron communication with the polymer. This work provides a miniaturizable system to determine pH (effective) in nonaqueous systems as well as in ionic liquids. The ability to measure pH (effective) is an important step toward the ability to customize ILs with suitable pH (effective) for catalytic reactions and biotechnology applications such as protein preservation.



Currently, measurement of proton activity (PA) (and thereby pH) in nonaqueous systems is hampered by lack of adequate and easily available measurement systems. Use of conventional glass-electrode pH probes, without considering the junction between the media and the water-based reference solution in the pH electrode, often produces unreliable or questionable measurements. In recent years, research in the field of ionic liquids (ILs) has been venturing further into biotechnological applications and a measure of the effective PA of the liquid is an important property in this context. This is particularly true for an understanding of phenomena such as the stabilization of proteins by some ILs, as well as the use of ILs more generally as solvents for pH-sensitive catalysis.[1−4] An ability to measure PA in ILs would not only help to control/study reactions where the PA is crucial, but also open up the route to tailor ILs with suitable PA to preserve therapeutic protein formulations.[5,6] Deviation from the protein preferred PA can affect its folding energy and stability, causing undesired behavior, such as aggregation and fibrillization.[5] Angell et al. have shown that varying PA can radically change normal folding processes of proteins.[6] Different proteins have different preferred PA ranges, e.g., lysozyme was stabilized in IL[7] with the PA around 4−6.[5] Recent work involving an IL stabilized form of the protein interleukin II, which is of interest in skin cancer treatment,[8] showed that it was necessary to control the PA in a narrow range in order to fully stabilize this protein. A buffer IL proved to be the means of doing so,[9] demonstrating that a means of directly measuring and adjusting PA in such hydrated IL mixtures is a vital issue.

In previous studies, the PA of ILs have been estimated by potentiometric titration,[10−13] by using solvatochromic indicators (often using the Hammett method),[14−19] by NMR studies of tagged solvent molecules added to the IL[20] or by observation of proton chemical shift measurements in protic ILs (PILs) using NMR.[6] Byrne and Angell showed that the increase in the δ(N−H) shift of the PIL indicated an increase in the basic character of the IL.[6] Electrochemical methods have emerged recently; Bautista-Martinez et al. established an electrochemical method based on measuring the potential difference between the onset of the proton reduction and hydrogen oxidation reactions to measure PA.[21] Barhdadi et al. used two electrochemical approaches to determine pK_a of N-bases in several aprotic ILs.[10] Kanzaki and co-workers studied acid−base properties of *N*-methylimidazolium based PILs using potentiometric and calorimetric titrations. They established correlation between pK_s (the acid−base equilibrium constant) and pK_a and found that proton-donating ability of acids is different in the PILs.[22] However, these methods are usually based on the use of a bubbling H_2 electrode which limits their practical use. The dynamic determination of pK_a based on cyclic voltammetry[10]

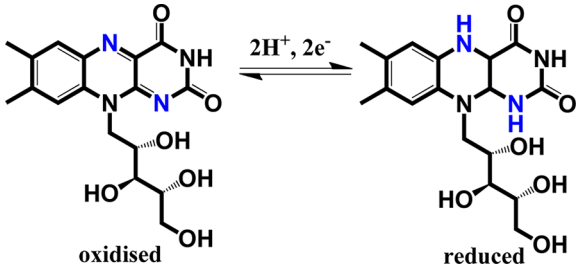
Received: November 19, 2012
Accepted: March 5, 2013

   dx.doi.org/10.1021/ac303354q | *Anal. Chem.* XXXX, XXX, XXX−XXX

has only been shown thus far to be applicable for measuring pK_a of acid–base systems dissolved in large amount of neutral aprotic IL, not for measuring the PA of the IL itself.

In this paper, we describe the fabrication of a miniaturizable solid-state electrode structure which is PA sensitive in nonaqueous liquids. By calibrating the sensor in aqueous buffer systems, it is possible to estimate equivalent PA in nonaqueous media and thereby obtain a measure of the apparent or effective pH (termed "pH (effective)") in these nonaqueous media. Given that a standard state for the proton has yet to be established in these media, this measurement cannot represent an absolute activity; nonetheless it provides an accessible method of comparing effective PA across a range of media. The sensor is based on the biologically derived, proton-active redox center, riboflavin (RFN). RFN is an essential water-soluble vitamin (vitamin B_2), which forms the electron shuttling center of flavin adenine dinucleotide (FAD), a coenzyme important in many enzymatic reactions. The oxidation and reduction of RFN (see Scheme 1) is a PA-dependent process that requires no water.[23,24] The reduction and oxidation of the RFN complex can be followed electrochemically. In this work, a PA sensitive electrode was constructed by incorporation of RFN into a conducting polymer, poly(3,4-ethylenedioxythiophene) (PEDOT), using the stuffing method previously shown to successfully establish the direct communication between the polymer and a biomolecule.[25,26] After calibration in various buffer-systems, the sensor capability is then demonstrated in a nonaqueous acid–base titration and to determine pH (effective) of a protic IL of particular interest in biotechnological applications, choline dihydrogenphosphate (choline dhp).

Scheme 1. Reduction and Oxidation of RFN Shows a Proton-Dependent Process Involving Two Electrons



METHODS

Buffer Preparation. To test the performance of the PEDOT/RFN electrode as a pH (effective) sensor in solutions with a range of ions, aqueous buffers were prepared across a pH range of 3–9. Details of the buffers used are as follows; Potassium hydrogen phthalate buffers ($C_8H_5KO_4$): pH 3.05, pH 4.00, pH 5.06. Sodium phosphate buffers (Na_2HPO_4/NaH_2PO_4): pH 4.4, pH 6.12, pH 7.00, pH 7.91, pH 8.55. The pH of these buffers was measured using a Mettler Toledo pH meter.

PEDOT-RFN Electrode Preparation. The method of trapping the RFN in the network is based on the procedure described by Winther-Jensen et al.[26] and Thompson et al.[25] Vapor phase polymerized PEDOT film was prepared as previous reported[25,26] (see detail in Supporting Information). The unwashed film was soaked in a solution consisting of a saturated aqueous RFN solution (1.5 g/L) and washed several times with water. This led to removal of the excess reduced iron from the film, collapse of the expanded structure of the polymer network and trapping of the RFN inside the film. The composite material was dried at room temperature overnight before use. Typically, the required area of the working electrode was only ~2 × 3 mm.

PA Sensing. The PEDOT/RFN composite electrode was used as the working electrode in a 3-electrode electrochemical cell; this allows PA sensing of the electrolyte solution. The voltage shift of the redox couple of the entrapped RFN was observed using slow scan rate cyclic voltammetry. Platinum wire and platinum/cobaltocene were used as counter and pseudoreference electrode, respectively. As comparisons of peak potentials were to be made across different solvent systems, an internal standard redox couple was added into the electrolyte solution prior to measurement.[27,28] As the solubility of the commonly used ferrocene was insufficient in aqueous solutions, cobaltocene was used as reference redox couple in these solutions. The choice of cobaltocene or ferrocene as an internal reference in this work is designed to remove as far as possible any effect of the changing solvent environment on the redox potential of the reference system. Ten millimoles of cobaltocene (Sigma-Aldrich) was added to 1 mL of the electrolyte solution to be tested, and gently heated to 40 °C for up to an hour to assist dissolution.

Nitrogen gas was bubbled into the electrolyte for at least 30 min before CV scans were started. Scans were also performed in electrolyte systems without cobaltocene. At least 3 CV scans at 10 mV/s were made in each electrolyte, with the voltage range between −1.5 and −0.2 V vs platinum. The peak positions of the RFN and cobaltocene reduction and oxidation peaks were measured, and these peak positions were used to provide an estimate of the pH of the electrolyte solution (Figure 1).

pH Calculations. CV's from various pH buffers described in section Buffer Preparation, using PEDOT/RFN as the working electrode, were used to construct a calibration curve. The peak potentials (E_{ox}, E_{red}) or E_0 obtained from the mid point between E_{ox} and E_{red} of RFN were determined relative to the peak potential of cobaltocene, and plotted against the pH of the solution. Linear regressions of the data were performed to determine formulas and the standard error for estimations of pH.

To determine pH (effective) of the hydrated IL choline dihydrogen phosphate (choline dhp), the peak positions of RFN relative to the standard cobaltocene peaks were determined by CV scans of PEDOT/RFN in the mixtures of 10%, 20%, 40%, 60% and 80% (w/w) choline dhp in distilled water. The difference in E_0 voltages (RFN vs cobaltocene) and the formulas displayed in Figure 2 were used to determine the pH (effective) of the mixtures.

Acid–Base Titrations in Nonaqueous Media. PEDOT/RFN electrodes were used to monitor the pH (effective) change during the titration of triflic acid (Sigma) with n-butylamine (Sigma). Triflic acid forms a solid salt at the end point with butylamine; therefore, anhydrous propylene carbonate was used as a medium for the titration. One milliliter of triflic acid was mixed with 2 mL f propylene carbonate. Seven millimolar tetrabutylammonium hexafluorophosphate and 8 mM ferrocene were added as supporting electrolyte and as the internal standard, respectively. Ferrocene was used instead of cobaltocene in this experiment due to significant proton reduction at potentials higher than the redox reaction of

dx.doi.org/10.1021/ac303354q | Anal. Chem. XXXX, XXX, XXX–XXX

cobaltocene. CV scans in the range of +0.5 to −1 V (vs platinum) were performed. The pH (effective) was determined from the shift of the RFN reduction peak after conversion to the cobaltocene potential scale based on the Ferrocene/Ferricinium − $Co(C_5H_5)^{2+}/Co(C_5H_5)_2$ difference being −1.337 V.[29] The reduction peak potentials were used (see Supporting Information) in this case due to poor resolution of the oxidation peak potentials in highly acidic and highly basic conditions.

Safety Considerations. Triflic acid fumes when expose to moisture in the air and has to be handled in a fume hood. Acid−base titration of triflic acid and butylamine has to be carried out in a fume hood as the reaction can be vigorous. Personal protective equipment (gloves, apron and goggles) has to be worn.

RESULTS AND DISCUSSION

Aqueous Buffer Measurements. The PEDOT-RFN electrode was first tested in aqueous buffers to confirm the linearity of the RFN peak potential shifts across a range of pH ranges between 3 and 9 with a variety of buffer compositions. Typical cyclic voltammograms (CVs) are shown in Figure 1.

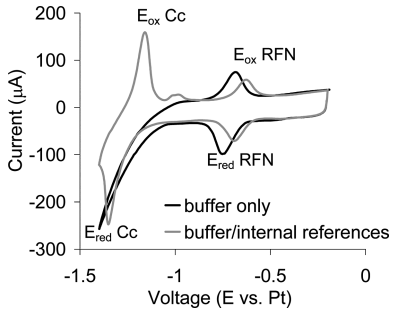


Figure 1. CVs of PEDOT/RFN in aqueous pH 7.0 phosphate buffer with a cobaltocene (Cc) internal reference. The peaks used in the pH calculations are labeled.

The RFN peak positions relative to the cobaltocene peak shifted linearly with pH, with higher voltages observed in solutions with lower pHs, as shown in Figure 2. Beyond the pH

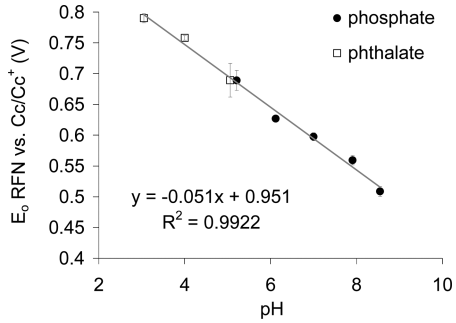


Figure 2. E_0 (RFN) in the PEDOT/RFN electrode versus a cobaltocene (Cc) reference redox pair in a range of buffer solutions.

range 3−9, the peak positions become less responsive to pH. The R^2 value of the linear fit in Figure 2 is 0.99, indicating good linearity of the data. The calculated standard errors associated with estimates of pH based on the linear regression were ±0.02. Thus, these studies in aqueous solution demonstrate that the PEDOT-RFN electrode is capable of producing a signal that shifts linearly with pH, and can be used to yield pH estimates in a small volume of electrolyte. A slope of 0.059 V per decade would be expected for a 1 $e^-/1$ H^+ or 2 $e^-/2$ H^+ process and the observed slope in the pH range 3−9 is close to this value. However, as shown recently,[30] the process is somewhat more complex and involves several intermediate steps, the result of which could produce a lower slope overall.

pH (Effective) Estimates in a Nonaqueous Acid−Base Titration. To demonstrate the use of the PEDOT-RFN electrode in a nonaqueous context, the pH (effective) was monitored during addition of butylamine to triflic acid as concentrated solutions (33% v/v) in propylene carbonate. Protic ionic liquids based on the triflate anion have been explored extensively by Watanabe's group in recent years and their potentiometric measurements provide some insight into the effective proton activity in these media under various conditions of temperature and concentration.[31] The titration began from pure triflic acid and butylamine was gradually added. A CV was measured after each addition of butylamine and the reduction potential differences between RFN and ferrocene were obtained. The pH (effective) estimates were calculated as described in the Methods section and the resulting titration curve is shown in Figure 3. Triflic acid is a strong acid

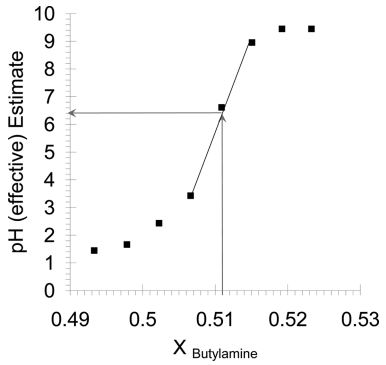


Figure 3. pH (effective) estimate during an acid−base titration of triflic acid with butylamine as concentrated solutions in propylene carbonate. The pH (effective) error estimates from the calibration curve (±0.02) are within the size of the data points.

and thereby understood to be an active source of protons. At the beginning of the titration, a strong proton reduction wave appeared and, only after a small amount of butylamine ($X_{butylamine}$ = 0.008) had been added, was the riboflavin reduction peak distinguishable from the proton reduction wave. Upon increasing the amount of butylamine, the pH (effective) increased and a clear step was observed between pH 3 and pH 8. From an estimate of the point of maximum slope in Figure 3, the end point was estimated to be pH (effective) 6.4 where the mole fraction of butylamine ($X_{butylamine}$) was 0.511 ± 0.006. The end point is quite clearly not $X_{butylamine}$ = 0.5 from Figure 3 and this is thought to be the result of small amounts of impurities in the butylamine (including water and CO_2 absorbed during the procedure). This illustrates the usefulness of the electrode in revealing such impurity issues. In the context of protic ILs, which are often prepared in a similar fashion, a 1:1 mol ratio used to produce such an ionic liquid would actually be slightly acid-rich, potentially producing a distinctly acidic IL.

pH (Effective) Estimates in a Hydrated IL across a Range of Water Activities. The PEDOT/RFN electrode

dx.doi.org/10.1021/ac303354q | Anal. Chem. XXXX, XXX, XXX−XXX

material was used in choline dhp with a range of water contents to provide an insight as to how pH (effective) is affected by the water activity. Choline dhp is typical of the hydrated ILs used in biological applications. The estimates of pH (effective) based on shifts in E_0 are shown in Figure 4 below.

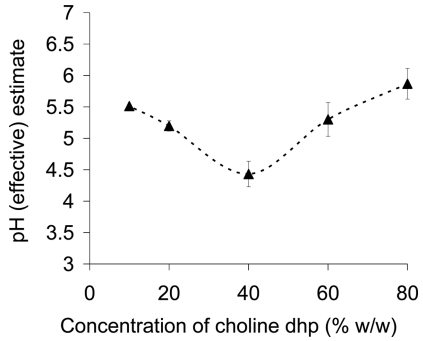


Figure 4. pH (effective) estimates for choline dhp across a range of water contents.

These results clearly indicate a somewhat unexpected set of trends in pH (effective) as water content is varied in the choline dhp-water system. Beginning with the dilute aqueous solution, the pH (effective) initially drops below that of the dilute aqueous value (~5.5) with increasing choline dhp concentration. This reflects the impact of the increasing dhp content on ion activity in an electrolyte solution such as this. At higher salt concentrations, the trend reverses and the pH (effective) begins to rise with increasing salt content due to the impact of the decreasing water activity on the dhp dissociation reaction (eq 1); the free energy of formation of the H_3O^+ species represents an important contributing factor in the overall free energy change in this process.

$$H_2PO_4^- + H_2O = HPO_4^{2-} + H_3O^+ \quad (1)$$

Solvation of the dhp anions by one another in complex-ion species of the type discussed by Johannsson et al.[32] may also be a factor in this increasing pH (effective) as this type of solvation stabilizes the labile proton in the complex. Further studies to confirm this behavior will be reported elsewhere.

This work has demonstrated that electrodes constructed from PEDOT combined with the PA-sensitive biological redox mediator provide a simple way to electrochemically estimate the pH of very low water content media. By being able to measure the pH (effective) of media across a range of water activities and neat acid/base compositions of the type used in protic ionic liquids, a better understanding and control of proton activity can be realized. The reference redox couple (ferrocene or cobaltocene) has been dissolved in the mixture in this work; however, it could equally be isolated in a separate fritted reference electrode assembly as is common in ion selective electrodes.

CONCLUSION

In summary, we have demonstrated a system to measure pH (effective) trends in aqueous and nonaqueous solutions covering pH 3–9 using a bioderived redox couple incorporated in PEDOT. The system is suitable for miniaturization as only a few square millimeters of electrode area is needed and there is no need for H_2 bubbling reference electrode. The established calibration curve was then used to estimate pH (effective) in choline dhp water mixtures and in a nonaqueous acid–base titration. The general approach we describe here of the use of a conducting polymer to host a biomolecule/redox center as a chemical sensing species has wider applicability to biotechnological applications, particularly for applications involving proteins or biochemical processes, for example, biosensors and biocatalytic electrodes.

ASSOCIATED CONTENT

Supporting Information

Vapor phase polymerized (VPP) PEDOT film preparation and Standard curve for E_{red} of RFN vs cobaltocene. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*D.R.M.: address, School of Chemistry Monash University, Clayton, VIC 3800 Australia; e-mail, douglas.macfarlane@monash.edu; fax, +61 3 9905 4597; tel, 61 3 9905 4540. B.C.T.: e-mail, brianna@uow.edu.au; fax, +61 2 4298 1477; tel, 61 2 4298 1905.

Present Address

¶B.C.T.: Intelligent Polymer Research Institute, University of Wollongong, AIIM Facility, Innovation Campus, University of Wollongong, NSW 2522, Australia.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

D.R.M., B.W.-J. and O.W.-J. gratefully acknowledge the Australian Research Council for fellowships.

REFERENCES

(1) Adams, C. J.; Earle, M. J.; Roberts, G.; Seddon, K. R. *Chem. Commun.* **1998**, 2097–2098.
(2) Adams, C. J.; Earle, M. J.; Seddon, K. R. *Green Chem.* **2000**, *2*, 21–23.
(3) Forsyth, S. A.; MacFarlane, D. R.; Thomson, R. J.; Von Itzstein, M. *Chem. Commun.* **2002**, 714–715.
(4) Welton, T. *Coord. Chem. Rev.* **2004**, *248*, 2459–2477.
(5) Angell, C. A.; Byrne, N.; Belieres, J. P. *Acc. Chem. Res.* **2007**, *40*, 1228–1236.
(6) Byrne, N.; Angell, C. A. *J. Mol. Biol.* **2008**, *378*, 707–714.
(7) Byrne, N.; Wang, L. M.; Belieres, J. P.; Angell, C. A. *Chem. Commun.* **2007**, 2714–2716.
(8) Foureau, D. M.; Jones, C. P.; Weaverz, K.; Vrikkis, R. M.; MacFarlane, D. R.; Mckillop, I. H.; Salow, J. C.; Elliott, G. D. *J. Immunother.* **2010**, *33*, 896.
(9) MacFarlane, D. R.; Vijayaraghavan, R.; Ha, H. N.; Izgorodin, A.; Weaver, K. D.; Elliott, G. D. *Chem. Commun.* **2010**, *46*, 7703–7705.
(10) Barhdadi, R.; Troupel, M.; Comminges, C.; Laurent, M.; Doherty, A. *J. Phys. Chem. B* **2012**, *116*, 277–282.
(11) Geng, W. G.; Li, X. H.; Wang, L. F.; Duan, H. L.; Pan, W. P. *Acta Phys.-Chim. Sin.* **2006**, *22*, 230–233.
(12) Kanzaki, R.; Uchida, K.; Song, X.; Umebayashi, Y.; Ishiguro, S. I. *Anal. Sci.* **2008**, *24*, 1347–1349.
(13) Malham, I. B.; Letellier, P.; Turmine, M. *Talanta* **2008**, *77*, 48–52.
(14) Thomazeau, C.; Olivier-Bourbigou, H.; Magna, L.; Luts, S.; Gilbert, B. *J. Am. Chem. Soc.* **2003**, *125*, 5264–5265.
(15) Chu, Y.; Deng, H.; Cheng, J. P. *J. Org. Chem.* **2007**, *72*, 7790–7793.
(16) Xing, H. B.; Wang, T.; Zhou, Z. H.; Dai, Y. Y. *J. Mol. Catal. A-Chem.* **2007**, *264*, 53–59.

dx.doi.org/10.1021/ac303354q | *Anal. Chem.* XXXX, XXX, XXX–XXX

(17) D'Anna, F.; La Marca, S.; Noto, R. *J. Org. Chem.* **2009**, *74*, 1952−1956.
(18) Robert, T.; Magna, L.; Olivier-Bourbigou, H.; Gilbert, B. *J. Electrochem. Soc.* **2009**, *156*, F115−F121.
(19) Zhao, Y. W.; Long, J. X.; Deng, F. G.; Liu, X. F.; Li, Z.; Xia, C. G.; Peng, J. J. *Catal. Commun.* **2009**, *10*, 732−736.
(20) Angueira, E. J.; White, M. G. *J. Mol. Catal. A: Chem.* **2007**, *277*, 164−170.
(21) Bautista-Martinez, J. A.; Tang, L.; Belieres, J. P.; Zeller, R.; Angell, C. A.; Friesen, C. *J. Phys. Chem. C* **2009**, *113*, 12586−12593.
(22) Kanzaki, R.; Doi, H.; Song, X.; Hara, S.; Ishiguro, S. I.; Umebayashi, Y. *J. Phys. Chem. B* **2012**, *116*, 14146−14152.
(23) Sawyer, D. T.; Gerber, J. N.; Amos, L. W.; De Hayes, L. J. *J. Less-Common Met.* **1974**, *36*, 487−499.
(24) Tatwawadi, S. V.; Santhanam, K. S. V.; Bard, A. J. *J. Electroanal. Chem. Interfacial Electrochem.* **1968**, *17*, 411−420.
(25) Thompson, B. C.; Winther-Jensen, O.; Vongsvivut, J.; Winther-Jensen, B.; MacFarlane, D. R. *Macromol. Rapid Commun.* **2010**, *31*, 1293−1297.
(26) Winther-Jensen, B.; Chen, J.; West, K.; Wallace, G. *Polymer* **2005**, *46*, 4664−4669.
(27) Hultgren, V. M.; Mariotti, A. W. A.; Bond, A. M.; Wedd, A. G. *Anal. Chem.* **2002**, *74*, 3151−3156.
(28) Silvester, D. S.; Ward, K. R.; Aldous, L.; Hardacre, C.; Compton, R. G. *J. Electroanal. Chem.* **2008**, *618*, 53−60.
(29) De Vreese, P.; Haerens, K.; Matthijs, E.; Binnemans, K. *Electrochim. Acta* **2012**, *76*, 242−248.
(30) Tan, S. L. J.; Webster, R. D. *J. Am. Chem. Soc.* **2012**, *134*, 5954−5964.
(31) Lee, S. Y.; Ogawa, A.; Kanno, M.; Nakamoto, H.; Yasuda, T.; Watanabe, M. *J. Am. Chem. Soc.* **2010**, *132*, 9764−9773.
(32) Johansson, K. M.; Izgorodina, E. I.; Forsyth, M.; MacFarlane, D. R.; Seddon, K. R. *Phys. Chem. Chem. Phys.* **2008**, *10*, 2972−2978.