analytical chemistry

Article

pubs.acs.org/ac

Polyion Selective Polymeric Membrane-Based Pulstrode as a Detector in Flow-Injection Analysis

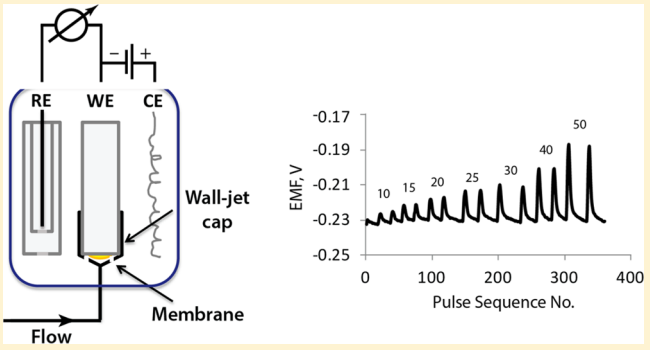
Andrea K. Bell-Vlasov,[†,∥] Joanna Zajda,[‡,∥] Ayman Eldourghamy,[§] Elzbieta Malinowska,[‡] and Mark E. Meyerhoff*[,†]

[†]Department of Chemistry, University of Michigan, 930 N. University, Ann Arbor, MI 48109-1055, United States

[‡]Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warsaw, Poland

[§]Faculty of Environmental Biotechnology Department, University of Minufiya, P.O. Box 79, Sadat City, Egypt

ABSTRACT: A method for the detection of polyions using fully reversible polyion selective polymeric membrane type pulstrodes as detectors in a flow-injection analysis (FIA) system is examined. The detection electrode consists of a plasticized polymeric membrane doped with 10 wt % of tridodecylmethylammonium-dinonylnaphthalene sulfonate (TDMA/DNNS) ion-exchanger salt. The pulse sequence used involves a short (1 s) galvanostatic pulse, an open-circuit pulse (0.5 s) during which the EMF of the cell is measured, and a longer (15 s) potentiostatic pulse to return the membrane to its original chemical composition. It is shown that total pulse sequence times can be optimized to yield reproducible real-time detection of injected samples of protamine and heparin at up to 20 samples/h. Further, it is shown that the same membrane detector can be employed for FIA detection of both polycations at levels ≥10 μg/mL and polyanions at levels of ≥40 μg/mL by changing the direction of the galvanostatic pulse. The methodology described may also be applicable in the detection of polyionic species at low levels in other flowing configurations, such as in liquid chromatography and capillary electrophoresis.



Polyanions such as glycosaminoglycans (GAGs) (e.g., the anticoagulant heparin and the anti-inflammatory agent chondroitin sulfate), sulfated polysaccharides (e.g., fucoidan, pentosan polysulfate, and carageenans), and polyphosphates, as well as many important polycationic species [e.g., arginine-rich polypeptides including protamine (the antidote of heparin), polymers of quaternary ammonium species, etc.] are difficult analytical targets for direct detection, owing to their lack of strong absorbance at wavelengths > 260 nm and no presence of electrochemically active domains. To address this problem, nonequilibrium polyion selective polymeric membrane electrodes for heparin and protamine (as representative biomedically important polyions) were first introduced by our group in the early 90s.[1−6] Such devices have been demonstrated to have many potential practical applications, including the measurement of heparin in whole blood,[2] the quantitation of carrageen in food products,[7] and the detection of enzyme activities that cleave polyions into smaller fragments not sensed by the electrodes and the detection of high charge density contaminants in commercially available heparins.[8,9] These sensors are prepared by doping polymeric membranes (e.g., plasticized PVC) with lipophilic anion-exchangers [tridodecylmethylammonium chloride (TDMAC)] for polyanions or lipophilic cation exchangers [e.g., dinonylnaphthalene sulfonic acid (DNNSH)] for polycations that can form strong cooperative ion pairs with the target polyion, causing polyion extraction into the organic membrane phase and, hence, a change in the phase boundary potential of the membrane.[3] Although such sensors are inexpensive and robust, they are irreversible and, hence, they are usually employed as single-use devices and are not adaptable to higher throughput flow-through analysis methods [e.g., flow-injection analysis (FIA)], where fully reversible potentiometric responses are required.

The Bakker group first pioneered a reversible polyion-selective sensor in the early 2000s and termed the device a pulstrode.[10,11] Since then, these pulstrodes have been used to detect both polyanions and polycations.[11,12] The pulstrode is a three-electrode system involving working, counter, and reference electrodes, where the polymeric membrane within the working electrode is doped with a lipophilic ion-exchanger salt with no intrinsic ion-exchange properties. Although the membrane does not have ion-exchange properties initially, a galvanostatic pulse between the working and counter electrode for a short time period (1 s) can polarize the lipophilic salt species in the membrane phase and induce localized ion-exchange at the membrane/sample interface, which provides the basis for subsequent potentiometric measurement during a period when no current pulse is applied. A typical measurement

Received: February 10, 2014
Accepted: March 20, 2014
Published: March 20, 2014

   dx.doi.org/10.1021/ac500567g | *Anal. Chem.* 2014, 86, 4041−4046

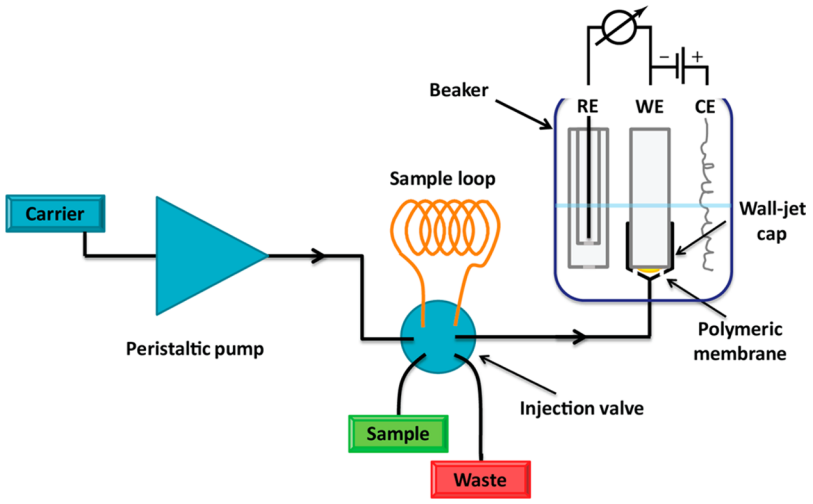


Figure 1. Schematic diagram of the flow-injection system coupled with polyion selective polymeric membrane-based pulstrode used for the experiments reported.

consists of a three-pulse sequence: the galvanostatic pulse, an open-circuit period, and then a potentiostatic pulse at a given applied potential. The principles of this method and the function of each of these steps have been provided in prior work reported by Bakker and co-workers[10,11] with respect to polycation sensing and by Gemene et al.[12] for polyanion sensing.

In principle, the pulstrode sensor, unlike the earlier single-use polyion selective membrane electrodes, should be useful as a detector in flow-through analytical systems. Herein, we explore for the first time this avenue by demonstrating the utility of a plasticized PVC membrane-based electrode containing the lipophilic salt of (TDMA/DNNS), as both a polycation and polyanion sensitive detector in a simple flow-injection analysis (FIA) arrangement. Both protamine and heparin are used as model analytes, and it is shown that the pulse sequence for detection can be optimized to achieve sample throughputs of up to 20 samples/h with detection limits of 10 μg/mL protamine and 40 μg/mL heparin, respectively, when a 200 μL injection loop is employed.

EXPERIMENTAL SECTION

Reagents. High molecular weight poly(vinyl chloride) (PVC), 2-nitrophenyloctyl ether (*o*-NPOE), tridodecylmethylammonium chloride (TDMAC), tetrahydrofuran (THF), heparin sodium salt, protamine sulfate salt from salmon, and all buffer salts were purchased from Sigma-Aldrich (St. Louis, MO). Dinonylnaphthalene sulfonic acid (DNNSH) as a 49% solution in xylenes was a gift from King Industries (Norwalk, CT).

Preparation of Ion-Exchanger Salt. The lipophilic ion-exchanger salt was prepared by metathesis in benzene in a 1:1 molar ratio of DNNSH and TDMAC. The benzene-salt solution was washed several times with deionized water until the aqueous solution was neutral. The benzene phase was then dried, and the residual salt was redissolved in THF and then dried again for use in preparation of the polyion selective membranes.

Membrane Preparation. All polyion selective membrane films contained 10 wt % of TDMA/DNNS salt and were ~200 μm thick. The sensing membranes were all formulated to contain a 1:2 by weight ratio of PVC and *o*-NPOE plasticizer. All membrane components were dissolved in THF, and the membrane was prepared by solvent casting this solution into glass rings on glass plates and letting the solutions dry overnight (in fume hood).

Electrodes. Membranes were cut with a cork borer (8 mm diameter) from the parent membrane and placed into an electrode body (Oesch Sensor Technology, Sargans, Switzerland). The actual membrane area was 20 mm^2. All sensors were conditioned overnight in a 10 mM phosphate buffer, pH 7.4, with 10 mM NaCl added, and the inner-filling solution in contact with the inner Ag/AgCl reference was the same as the outer conditioning solution. The external reference electrode was a double-junction Ag/AgCl electrode with 3 M KCl as the inner filling solution and 1 M LiOAc as a bridge electrolyte. A coiled Pt-wire was used as a counter electrode for all pulsed chronopotentiometric measurements (see Figure 1).

Measurements. A conventional three-electrode setup was used for the pulstrode measurements with the TDMA/DNNS-based polymeric membrane electrode serving as the working electrode with the flowing sample passing over the outer surface of the membrane in a wall-jet configuration (see Figure 1). The solution outlet holes of the plastic syringe that provided the housing for the wall-jet assembly entered into a small beaker into which the reference and counter electrodes were placed. The electrochemical measurements were conducted with an AFCBI bipotentiostat (Pine Instruments, Grove City, PA) controlled by a NI-DAQPad 6015 interface board and LabVIEW 8.6 data acquisition software (National Instruments, Austin, TX) on a PC computer. Initially, an uptake (galvanostatic pulse) time of 1 s (magnitude and direction changed depending on the target analyte), a zero-current pulse of 0.5 s, and a potentiostatic pulse of 0 V vs Ag/AgCl (stripping potential) of 15 s were used. Eventually, to enhance throughput, the potentiostatic pulse period was varied to examine the shortest period that still provided a full return to baseline potential. For measurements involving heparin, a 20 μA current was employed during the galvanostatic pulse, and for protamine, a −12 μA current pulse was applied. These current magnitudes have been previously optimized for largest EMF responses during the zero current pulse period under static conditions (in absence of flow) for heparin[12] and protamine.[11] The potentials were sampled as the averaged data

dx.doi.org/10.1021/ac500567g | *Anal. Chem.* 2014, 86, 4041−4046

during the last 10% time period of the 0.5 s zero current pulse. To assess the composition changes in the membranes of the working electrode as a function of use time in the FIA system, classical potentiometric measurements were conducted with the working electrodes using a 16-channel, high-impedance interface (Lawson Laboratories Inc., Malvern, PA) vs the same double junction reference electrode used in the pulsed chronopotentiometric FIA measurements. All experiments were carried out at room temperature (21−23 °C).

FIA System. A schematic representation of the flow injection analysis system used throughout the experiments reported here is shown in Figure 1. A peristaltic pump (MINIPULS3, Gilson, Middleton, WI) was used to induce carrier stream (buffer) flow and a 6-port manual injection valve (VICI, Houston, TX), equipped with a 750 μL sample loop (or 200 μL sample loop for higher throughput rate only), was employed to introduce samples into the system. A polyion selective membrane electrode, serving as a detector, was mounted in a wall-jet mode using a custom-made adapter (plastic syringe housing). The entire flow-through system was assembled using Teflon tubing (0.8 mm i.d.).

RESULTS AND DISCUSSION

In this work, a polyion selective pulstrode is employed as a universal polyion detector in a FIA system to detect both polycations and polyanions with the same membrane composition. Initial preliminary experiments were conducted using a carrier stream of 10 mM phosphate buffer, pH 7.4, with 10 mM NaCl at a flow rate of 0.6 mL/min and a 750 μL sample loop. The large sample volume employed was to ensure that an adequate number of data points (EMF measurements) could be obtained in the presence of the sample to define a full sample peak, given that a voltage signal is only recorded every 16.5 s (see parameters for pulses in Experimental Section). The polymer membrane of the working electrode is doped with a 1:1 molar ratio of tridodecylmethylammonium ($TDMA^+$) and dinonylnaphthalene sulfonate ($DNNS^-$), where $TDMA^+$ provides selectivity to polyanions (heparin)[12] and $DNNS^-$ to polycations (protamine)[11] when the membrane is polarized. During a typical pulse sequence, the galvanostatic pulse is applied through the working sensor and counter electrode, during which the membrane is polarized, with a concomitant ion-flux of complementarily charged ions migrating from the sample toward the polymeric membrane (see Figure 2). Under an anodic current pulse, the lipophilic ion-exchanger salt ions are redistributed in the membrane, with $TDMA^+$ migrating toward the sample/membrane interface and $DNNS^-$ moving toward the inner interface in contact with the fixed internal electrolyte solution. Thus, the outer interface becomes an anion-exchange interface and the inner solution/membrane a cation-exchange interface (see Figure 2). An analogous process occurs when an initial cathodic current pulse is applied, with $DNNS^-$ moving toward the sample/membrane interface for selective detection of polycations. During the subsequent open circuit portion of the sequence, the previously extracted background ions are exchanged with the target polyions from the sample to provide a phase boundary potential change that relates to the concentration of polyions present. The measured EMF during this period is void of undesired *i*R drop, making it analogous to classical zero current potentiometric measurements. During the potentiostatic pulse, the membrane is depolarized, and all ions that are initially extracted are stripped out to restore the membrane to its original composition, making the sensor fully reversible and suitable for FIA measurements.

The typical dynamic potentiometric responses of the pulstrode detector in the FIA mode (using preliminary pulse parameters) toward injected samples of protamine and heparin samples are shown in Figure 3. All signals, obtained for differing concentrations of both protamine and heparin, exhibit good reproducibility for duplicate injections and reversibility. However, it should be noted that these measurements are only used to demonstrate the detectable concentration range

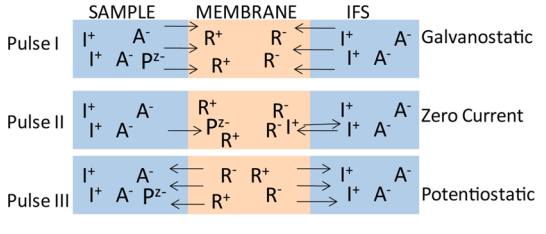


Figure 2. Movement of salt and ions within a membrane and at interfaces during pulse sequence employed for detection of polyanions using anodic current pulse in FIA arrangement. The polyion is represented by P^{z-}, the interfering ions by I^+ and A^-, and R^+ and R^- are the $TMDA^+$ and $DNNS^-$ ions of the lipophilic ion-exchanger salt within the membrane phase. It should be noted that smaller anions (A^-) also enter the outer surface of the sensing membrane during pulse 1 period but are eventually outcompeted for serving as the counteranions to R^+ sites by the polyanions (P^{z-}).

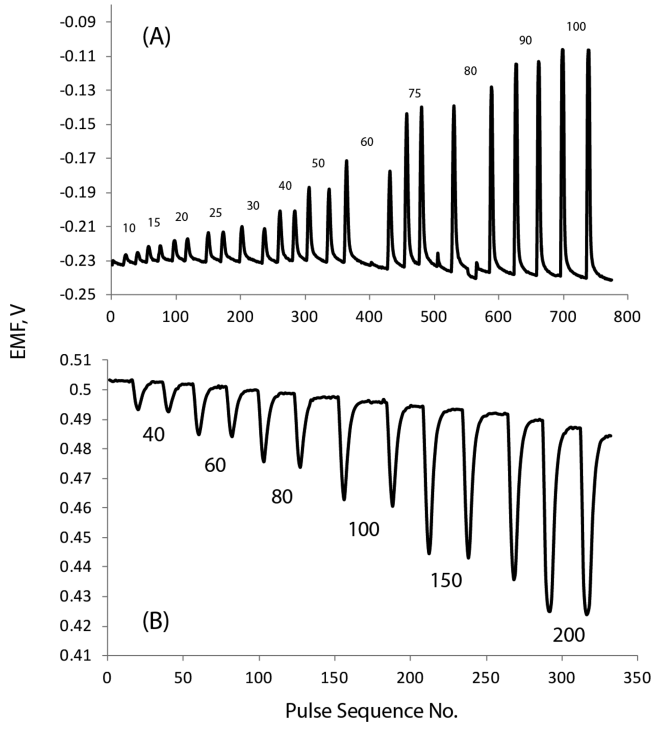


Figure 3. Dynamic potentiometric response of polyion pulstrode to (A) protamine; duplicate sequential injections of standards in the range of 10−100 μg/mL; and (B) heparin; duplicate sequential injections of standards in the range of 40−200 μg/mL in 10 mM phosphate buffer, pH 7.4, with 10 mM NaCl present. The diluent stream was the same buffer solution, and flow rates were set at 0.6 mL/min. Numbers above and below peaks represent concentrations in micrograms per milliliters. Every 100 pulses is ∼30 min.

dx.doi.org/10.1021/ac500567g | *Anal. Chem.* 2014, 86, 4041−4046

and not employed to demonstrate true precision and stability of the sensors (see Figures 5 and 6, below, for precision data). In the polyanion sensing mode that employs an anodic current pulse (i.e., heparin as the target analyte), a slight negative baseline shift is observed, which may be due to very small amounts of higher MW heparin remaining inside the membrane during the potentiostatic pulse due to the very strong cooperative ion pair it makes with $TDMA^+$.[4] However, the overall potential changes from baseline for a given concentration of heparin remain relatively constant and reproducible. The linear response range for protamine injections is 10−100 μg/mL, whereas for heparin, the range is from 40 to 200 μg/mL (equivalent to 7−36 units/mL).

Since the measurements are conducted under flowing conditions, it is essential to determine the stability of the sensors. Two different pulstrode sensors with the same membrane composition were used for calibrations toward protamine and heparin, respectively. Each sensor was calibrated twice a day and placed in a stagnant conditioning buffer solution between measurements. The FIA response toward protamine was found to be stable for 3 d, while responses toward heparin were stable over a 5 d period, with stability defined as potential change values of less than 5% in peak heights when compared to those on day 1 of operation for a particular sensor. After these times, the overall EMF responses decreased significantly. The reason for the longer stability toward heparin may be related to the lipophilicities of each portion of the ion-exchanger salt within the membrane phase. Values of organic-aqueous phase partition coefficients for $DNNS^-$ and $TDMA^+$ were determined using Virtual Computational Chemistry Laboratory.[14] The results show that $DNNS^-$ has ca. 2 orders of magnitude smaller partition coefficient compared to that of $TDMA^+$ (log K^{part} = 7.71 vs 9.75).

To confirm that $DNNS^-$ was more quickly washed out from the membrane phase, studies were conducted using classical potentiometry using KBr as the analyte species. For freshly prepared membranes, the ion-exchanger salt in the membrane has no intrinsic ion-exchange properties and, therefore, does not respond to any ions present in the sample solution in classical zero-current potentiometry (see Figure 4A). However,

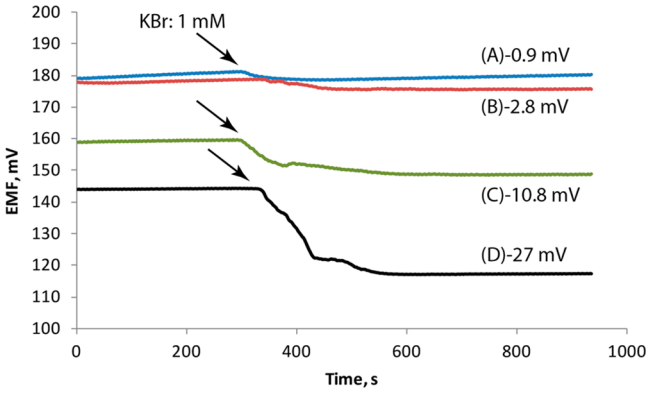


Figure 4. Open circuit potentiometric response of polymeric membrane electrode to 1 mM KBr (A) before pulstrode measurements, (B) after 500 pulse sequences in flow mode, where anodic current was applied during galvanostatic pulse, (C) after 3 d in the flow system with no galvanostatic pulsing, and (D) after 500 pulse sequences in the flow mode with cathodic current applied during the galvanostatic pulse. All measurements were conducted in a 10 mM phosphate buffer, pH 7.4, containing 10 mM NaCl.

when sensors are placed in a continuous flow arrangement (exposed to flowing 10 mM phosphate buffer, pH 7.4, with 10 mM NaCl) without pulsing for 3 days and then tested on the bench using conventional potentiometry, they exhibit a more significant negative voltage response toward additions of standard 1 mM KBr compared to freshly made working electrodes that were not exposed to flowing conditions for 3 d (see Figure 4C). This anionic response to the bromide ion suggests that some excess $TDMA^+$ was present in the polymeric membrane phase following the extended exposure to the flowing buffer.

When a fresh sensor is placed under flowing conditions with the polyanion pulse sequence but only for 500 pulses (during the first day), only a very small negative potential change is observed in the presence of KBr, similar to the sensor that has not been exposed to flowing conditions. However, when the sensor is polarized in the flow mode for 3 d using a cathodic current pulse (as would be employed for polycation detection), it exhibits a much larger negative potential change of approximately −30 mV in response to 1 mM KBr in the static potentiometric beaker experiment (see Figure 4D). This is likely because as the cathodic current is applied during the galvanostatic pulse, $DNNS^-$ moves toward the sample/membrane interface and concentrates in this region, allowing it to be more efficiently washed out from the membrane phase owing to its decreased lipophilicity. In contrast, when the anodic current pulse is applied for polyanion sensing, the $DNNS^-$ moves toward the membrane/inner filling solution interface, where the solution is stagnant and $DNNS^-$ can partition back into the membrane, while $TDMA^+$ ions move to the front side of the membrane. Since $TDMA^+$ is more lipophilic than $DNNS^-$, much less $DNNS^-$ is washed out from the membrane by the flowing conditions. Therefore, the 1:1 molar ratio of $DNNS^-$ to $TDMA^+$ within the sensing membrane remains constant for a longer period of time when using the polyanion sensing mode of operation. As expected, this "extraction" effect is much less pronounced in static measurements with polyion sensors and likely also with pulstrodes used in similar nonflowing conditions.

The most widely used polyions in pharmaceutical applications are heparin and protamine.[15−20] Protamine is an arginine-rich polypeptide used as an antidote to the anticoagulant heparin in medical procedures where it binds strongly to heparin forming a neutral complex.[17] Since large heparin doses can lead to bleeding complications, and protamine overdose is known to cause complement activation and other toxic effects in patients, monitoring of their concentration in blood is potentially quite useful.[16−20] In consideration that the pulstrode response is also governed by the presence of sodium or chloride ions, experiments were carried out to determine both heparin and protamine in a FIA mode in which a physiological level of NaCl background is present. Experiments using samples made in a 10 mM phosphate buffer, pH 7.4, with 100 mM NaCl and using this same buffer as the carrier stream were therefore performed. Significant potentiometric response to protamine is still observed (Figure 5A) when sensing polycations, while indiscernible FIA signals for different heparin concentrations are observed in the polyanion sensing mode (data not shown). This is consistent with the low responses toward heparin obtained in static (no flow) measurements using the pulstrode method under similar high NaCl conditions[12] and may relate to much slower kinetics of ion-exchange at the membrane/sample

dx.doi.org/10.1021/ac500567g | Anal. Chem. 2014, 86, 4041−4046

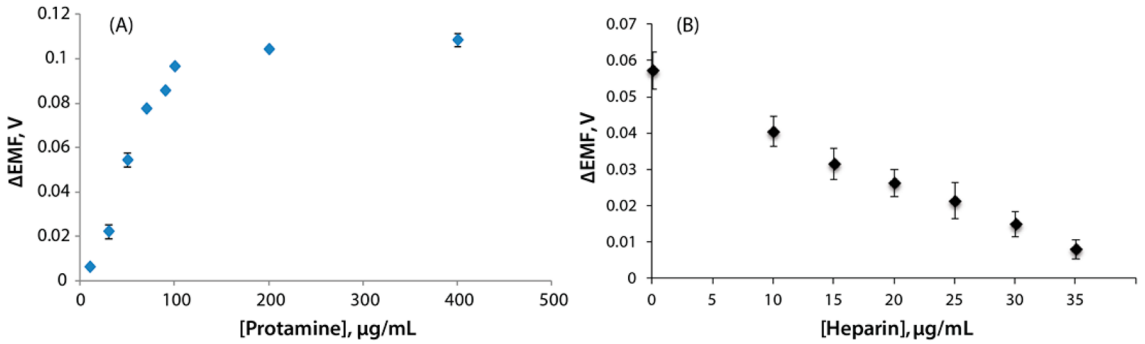


Figure 5. (A) Calibration of ΔEMF response (peak voltage − baseline voltage) to protamine in the flow-injection mode in 10 mM phosphate buffer, pH 7.4, containing 100 mM NaCl. It should be noted that the standard deviation for $n = 3$ injections at each concentration is so small that it cannot be observed for some data points. (B) Response to increasing concentrations of heparin with a constant concentration of 60 μg/mL protamine present in the test sample in the 10 mM phosphate buffer, pH 7.4, containing 100 mM NaCl. Data in (B) represents testing with three different sensors with the same membrane composition in the FIA system, where standard deviation was calculated using data from all three sensors. Flow rates for both A and B were set at 0.6 mL/min.

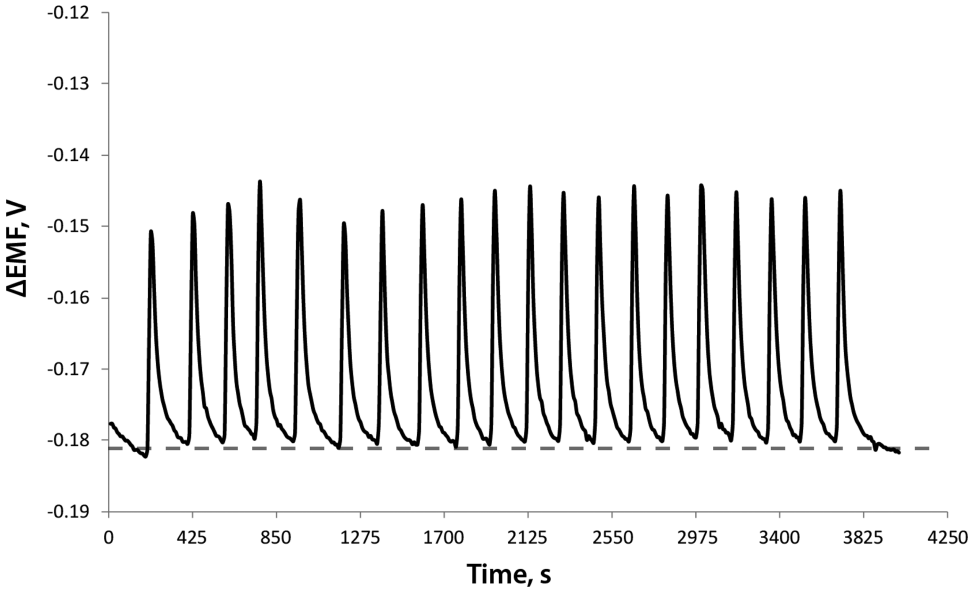


Figure 6. Dynamic potentiometric response to repeated injections ($n = 20$) of 60 μg/mL protamine in 10 mM phosphate buffer, pH 7.4, containing 100 mM NaCl. A sampling rate of approximately 20 samples per hour has been achieved. For data shown, the average ΔEMF = 33.7 mV ± 1.42 mV ($n = 20$). Flow rate was set at 0.93 mL/min.

interface for heparin compared to protamine, given that the average MW of heparin is in the range of 15000 Da (compared to protamine with MW = 5100).[21] Since the period where heparin can effectively be extracted into the membrane is only during the galvanostatic and open circuit periods (1.5 s), it is likely that significantly more protamine can be extracted during this short period than heparin and, thus, the ability to outcompete the smaller counterions in the sample and membrane phases during and after the polarization period (Na^+ in polycation sensing mode and Cl^- in the polyanion sensing mode) is greater in the case of protamine sensing.

To detect heparin under physiological conditions in the FIA mode, a constant concentration of protamine (60 μg/mL) was then mixed with standard amounts of heparin and the resulting mixture was injected into the FIA system operated in the polycation-sensing mode. As shown in Figure 5B, as the concentration of heparin increases, a decrease in the EMF response to protamine is observed. The linear range of heparin concentrations that can be detected is from 10−35 μg/mL, which corresponds to 2−6 units/mL, lying within the range of heparin concentrations used in certain clinical procedures, like open-heart surgery, extracorporeal membrane oxygenation, etc.[16−19]

It should also be noted that preliminary experiments using protamine spiked into blood plasma were also attempted with the new FIA arrangement. Such experiments showed much larger positive EMF responses than for the same concentration of protamine alone in the buffer. This data is at odds with what was previously reported by Bakker and co-workers,[11] using pulstrode type protamine sensors in the static mode, where the presence of blood components did not perturb the ability to detect protamine. The only difference in the sensor employed in that earlier work versus the present device was the anion exchanger species of the lipophilic salt used within the membrane, which was a tetradodecylammonium species, compared to tridodecylmethylammonium employed in this work. However, the cationic species is moved toward the inner interface during the cathodic current pulse for polycation detection and, hence, more research is needed to understand why some components in the blood that was tested in this work

dx.doi.org/10.1021/ac500567g | Anal. Chem. 2014, 86, 4041−4046

led to such a larger positive interference in detection of protamine.

Flow-injection analysis normally allows for high sample throughput, and therefore, flow rate and potentiostatic pulse duration times were further optimized to increase the number of samples analyzed per hour via the arrangement shown in Figure 1. To achieve higher throughput, we examined shortening the potentiostatic pulse segment of the sequence. Experiments were carried out to determine how short the potentiostatic pulse could be without compromising reproducibility and maintaining a stable baseline. In prior studies with pulstrodes, it was recommended to apply a 10−30 times longer potentiostatic pulse than galvanostatic one to restore the membrane to its initial state.[11] However, in the optimized polyion sensing FIA system, it was found that decreasing the pulse to 6 s and thereby collecting data points every 8 s could be implemented without decreasing reproducibility. In order to further improve the number of samples measured per hour, experiments were carried out to optimize sample loop size and flow rate. Ultimately, a much smaller sample loop volume of 200 μL (vs 750 μL originally) and increased flow rate up to 0.93 mL/min were employed. Under these conditions, a sampling rate of 20 samples/h can be achieved with a relative standard deviation of ±4.2% for the ΔEMF peak values when 60 μg/mL protamine is monitored (see Figure 6).

Typically, 30−300 samples per hour can be handled in modern FIA systems, and even up to 1000 have been reported in specially designed microsystems with optical detection.[13] Thus, the obtained sample throughput in this work may not appear to be significant. However, given the high molecular weights of the species detected and considering that use of the traditional pulstrode method in a static mode requires manually changing sample and background solutions as well as the further manual rinsing of all electrodes between samples, detecting polyions at low levels with a throughput of 20 samples per hour is still a step forward. By using more highly plasticized membranes in the working electrode, or even fully organic liquid membranes, it may be possible to increase the rate of polyion extraction during and after the galvanic pulse. This might also lead to faster membrane recovery times during the potentiostatic pulse. Taken together, this approach might enable the use of smaller sample volumes and shorter pulse sequences that may lead to even higher throughputs.

CONCLUSION

We have demonstrated, for the first time, that polyion selective pulstrodes can be used as detectors in an FIA measurement configuration. Within such a flow-through system, this sensing mode provides relatively stable and reproducible signals that are proportional to both polycation and polyanion concentrations using a single working membrane electrode. While demonstrated here for use in detecting heparin and protamine as model analytes, it is envisioned that this method could prove useful for quality control monitoring of a wide range of products/supplements that utilize polyions as active ingredients. Further, the methodology described here should also be applicable in developing flow-through methods to detect specific enzyme activities that cleave polyions into smaller fragments that are not sensed by the pulstrode. Finally, the use of polyion selective pulstrodes as universal detectors in preparative LC separation methods employed to isolate or fractionate polyion species, as well as their use as detectors in modern microfluidic devices, should also be possible.

AUTHOR INFORMATION

Corresponding Author

*E-mail: mmeyerho@umich.edu. Tel: 734-763-5916. Fax: 734-647-4865.

Author Contributions

‖A.K.B.-V. and J.Z. contributed equally to this work.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the National Institutes of Health (Grant EB-000784) and the European Union in the framework of the Regional Development Fund through the Joint UW and WUT International PhD Program of Foundation for Polish Science (Grant MPD/2010/4) for funding this research.

REFERENCES

(1) Ma, S.; Meyerhoff, M. E.; Yang, V. C. *Anal. Chem.* **1992**, *64*, 694−697.
(2) Ma, S.; Yang, V. C.; Fu, B.; Meyerhoff, M. E. *Anal. Chem.* **1993**, *65*, 2078−2084.
(3) Fu, B.; Bakker, E.; Yun, J. H.; Yang, V. C.; Meyerhoff, M. E. *Anal. Chem.* **1994**, *66*, 2250−2259.
(4) Fu, B.; Bakker, E.; Yang, V. C.; Meyerhoff, M. E. *Macromolecules* **1995**, *28*, 5834−5840.
(5) Ye, Q.; Meyerhoff, M. E. *Anal. Chem.* **2001**, *73*, 332−336.
(6) Yun, J. H.; Meyerhoff, M. E.; Yang, V. C. *Anal. Biochem.* **1995**, *224*, 212−220.
(7) Hassan, S. S. M.; Meyerhoff, M. E.; Badr, I. H. A.; Abd-Rabboh, H. S. M. *Electroanalysis* **2002**, *14*, 439−444.
(8) Wang, L.; Buchanan, S.; Meyerhoff, M. E. *Anal. Chem.* **2008**, *80*, 9845−9847.
(9) Wang, L.; Meyerhoff, M. E. *Electroanalysis* **2010**, *22*, 26−30.
(10) Shvarev, A.; Bakker, E. *J. Am. Chem. Soc.* **2003**, *125*, 11192−11193.
(11) Shvarev, A.; Bakker, E. *Anal. Chem.* **2005**, *77*, 5221−5228.
(12) Gemene, K.; Meyerhoff, M. E. *Anal. Chem.* **2010**, *82*, 1612−1615.
(13) Du, W.; Fang, Q.; He, Q. H.; Fang, Z. L. *Anal. Chem.* **2005**, *77*, 1330−1337.
(14) VCCLAB, Virtual Computational Chemistry Laboratory, http://www.vcclab.org (accessed Aug 15, 2013).
(15) Despotis, G. J.; Joist, J. H.; Goodnough, L. T. *Clin. Chem.* **1997**, *43*, 1684−1696.
(16) FitzGerald, D. J.; Patel, A.; Body, S. C.; Garvin, S. *Perfusion* **2009**, *24*, 93−96.
(17) Gautam, N. K.; Schmitz, M. L.; Harrison, D.; Zabala, L. M.; Killebrew, P.; Belcher, R. H.; Prodhan, P.; Mckamie, W.; Norvell, D. C. *Pediatr. Anesth.* **2013**, *23*, 233−241.
(18) Koster, A.; Fischer, T.; Praus, M.; Haberzetti, H.; Kuebler, W. M.; Hetzer, R.; Kuppe, H. *Anesthesiology* **2002**, *97*, 837−841.
(19) Despotis, G. J.; Joist, J. H.; Hogue, C. W.; Alsoufiev, A.; Kater, K.; Goodnough, L. T.; Santoro, S. A.; Spitznagel, E.; Rosenblum, M.; Lappas, D. G. *J. Thorac. Cardiovasc. Surg.* **1995**, *110*, 46−54.
(20) Bhaskar, U.; Sterner, E.; Hickey, A. M.; Onishi, A.; Zhang, F. M.; Dordick, J. S.; Linhardt, R. J. *Appl. Microbiol. Biotechnol.* **2012**, *93*, 1−16.
(21) Ando, T.; Watanabe, S. *Int. J. Protein Res.* **1969**, *3*, 221−224.