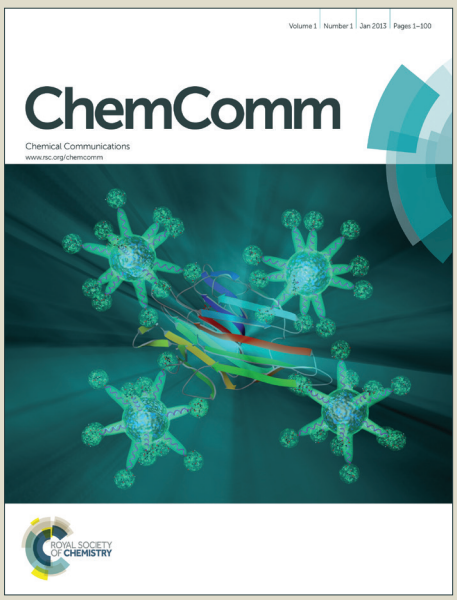
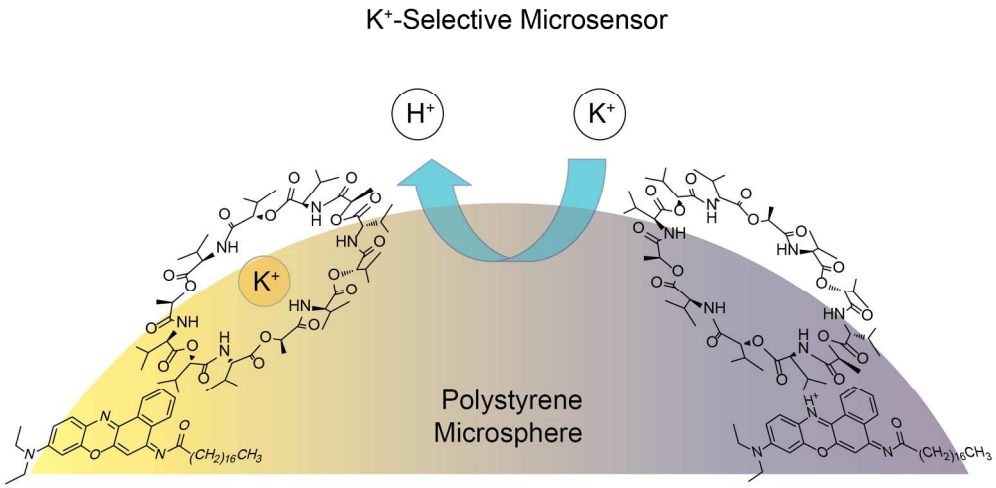
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm



781x384mm (72 x 72 DPI)

Journal Name

RSCPublishing

COMMUNICATION

Potassium-Selective Optical Microsensors Based On Surface Modified Polystyrene Microspheres

Cite this: DOI: 10.1039/x0xx00000x

Xiaojiang Xie*, Gastón A. Crespo, Jingying Zhai, Istvan Szilágyi, and Eric Bakker*

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Ion-selective microspheres based on surface modification of polystyrene particles (0.8 and 2.4 µm, diameter) are presented here for the first time. The traditional lipophilic sensing components of K^+ selective optodes (chromoionophore, ion-exchanger and ionophore) are adsorbed on the surface of the polystyrene particles using a simple mixed solvent method. The resulting microparticles respond to K^+ in an exhaustive sensing mode with excellent selectivity and rapid response time (t95% = 5 s).

Ion selective optodes are one of the most recognized optical ion sensors and have been intensively explored in the past few decades.[1-6] Classical optodes are composed of a polymeric solvent cast film containing a lipophilic pH indicator (also called chromoionophore), an ion exchanger and an optically silent ionophore selective to the analyte. Cation selective optodes function on the basis of extraction equilibria between the aqueous phase and the bulk of the sensing film.[5] The extraction competition between the analyte ion and H^+ defines the protonation degree of the chromoionophore in the optode film, where the signal readout comes from.

While film based optodes are rather robust and effective, they are bulky and therefore cannot be used in small spaces such as cells. The response time can be also quite long, making it difficult to monitor dynamic processes.[7] Recently, miniaturized forms of optodes have emerged to overcome the disadvantages. Nanometer-sized sensing spheres based on acrylamide and poly(vinyl chloride) (PVC) have been shown to be successful in interrogating intracellular environments.[8-12] Polymeric microspheres and nanospheres that behave on the same basis of bulk ion-selective optodes have been developed in our group.[13, 14] Microtiter plate based optodes, microbeads and nanospheres have also been prepared by surface coating, polymerization and sonication.[15]

However, in general, the number of matrix materials for these micro/nanosensors are very limited and the procedures to prepare the sensors are sophisticated. In this work, we report on the preparation and characterization of K^+-selective microspheres through simple surface modification on polystyrene (PS) particles without any added plasticizer.

PS exhibits a slow self-diffusion coefficient of below 10^{-13} cm^2 s^{-1} at room temperature[16], making optode based equilibria rather unrealistic. Indeed, ion-selective optical microsensors using polystyrene as matrix have not yet been reported, and cast optode films based on PS did not give functional optode response in this work (see below). Instead of the typical bulk phase equilibria encountered with ion optodes, the mass extraction equilibrium is here established between the surface region and the aqueous surrounding, which unusual and quite surprising. To our knowledge, it is the first example of ion selective optodes functioning on the basis of surface modification.

The surface of PS microspheres (non-modified) is very hydrophobic in character and provides for strong physical adsorption of molecular species with hydrophobic regions, including drugs and proteins.[17-19] The ion-selective optodes components are always highly hydrophobic and therefore should be easily adsorbed on the surface of the PS microsphere surface. Here, PS microspheres with average diameter of 0.8 µm and 2.4 µm (characterized by dynamic light scattering (DSL)) in the form of aqueous suspensions are used as templates. To produce the K^+-selective PS microspheres, a tetrahydrofuran (THF) solution containing chromoionophore I (Ind), sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (Na^+R^-) and potassium ionophore I (valinomycin, L) is prepared (see supporting information for the detailed composition). As shown in Fig. 1(a), the THF cocktail is then injected into the aqueous PS microsphere suspension on a vortex with a spinning speed of 1000 rpm. A clear blue suspension is immediately obtained, indicating that Ind is in its protonated state. THF is subsequently removed by purging compressed air on the surface of the suspension for 20 min. The preparation process, compared with others such as polymerization, is much more convenient and less time consuming. In addition, it is

University of Geneva, 30 Quai Ernest Ansermet, 1211 Geneva 4, Switzerland. E-mail: xiaojiang.xie@unige.ch, eric.backker@unige.ch
Tel: +41 22 379 6429

† Electronic Supplementary Information (ESI) available: Experimental details, confocal image of Lumogen Red modified PS particle, K^+ response from 2 µm PS particle, selectivity, calibration in pH 5.6 Mes-NaOH solution. See DOI: 10.1039/c000000x/

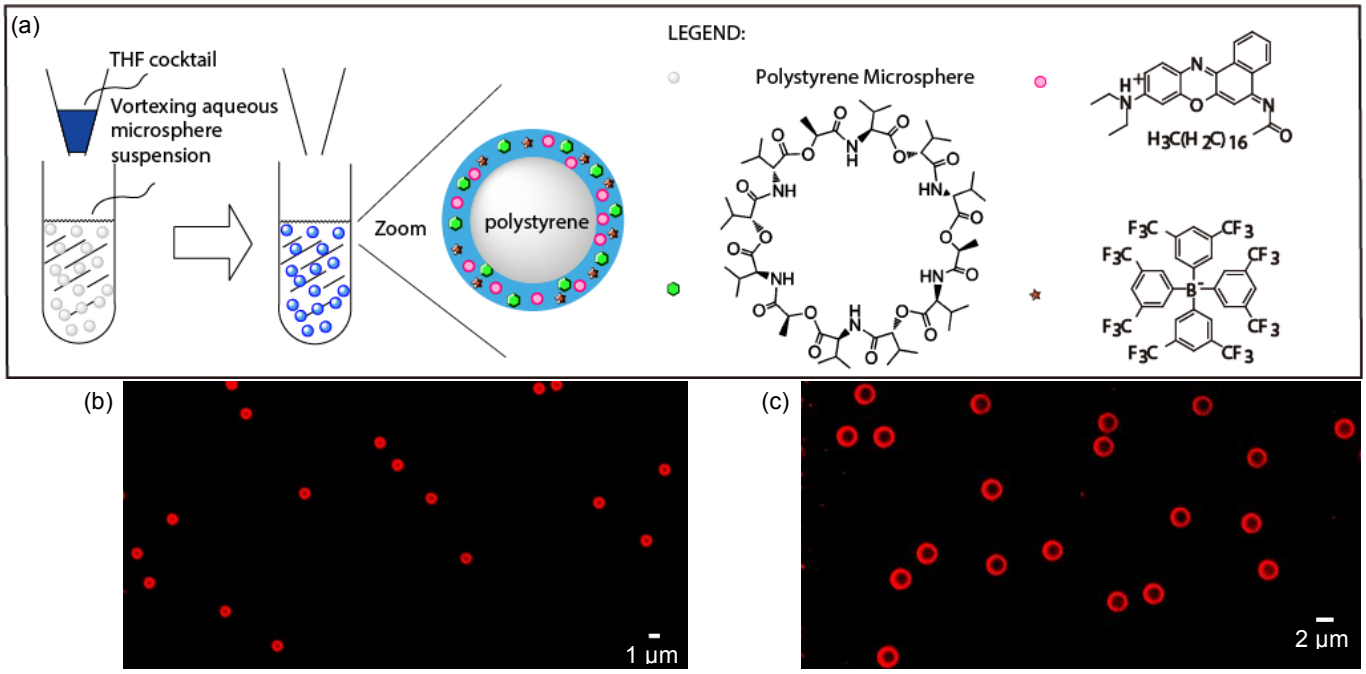


Fig. 1 Schematic illustration of modification process on PS microspheres with K^+-selective sensing components (a). Fluorescence confocal microscopic images for K^+-selective PS microspheres with 0.8 μm diameter (b) and 2.0 μm diameter (c) in H_2O.

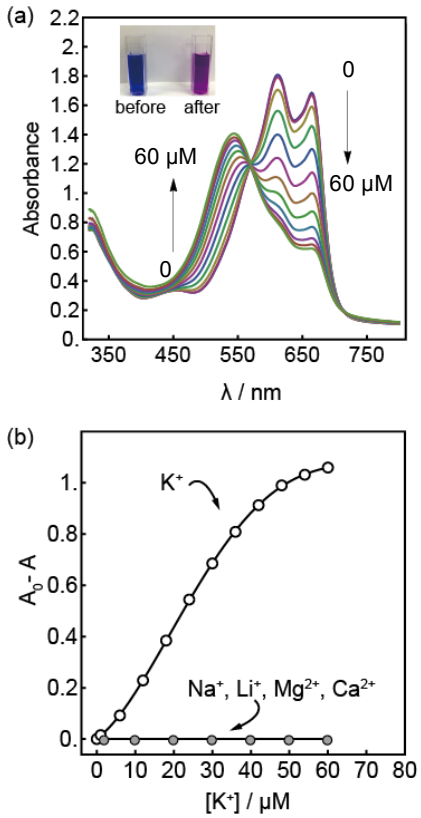


Fig. 2 (a) Absorbance change for the K^+-selective PS microspheres (0.8 μm) in 10 mM pH 7.4 Tris-HCl with different levels of KCl. Inset: pictures of the microsphere suspension before and after addition of K^+ (60 μM). (b) Calibration curves using absorbance difference at 663 nm (A_0-A) for K^+ and other interfering ions as indicated, where A_0 is the initial absorbance in buffer.

easier to produce in large quantity compared with previous methods using a particle caster.[13]

The average diameter of the resulting K^+-selective PS microspheres was determined by dynamic light scattering as 0.8 μm with a polydispersity index of 0.11 (2.4 μm for the carboxylate modified latex (CML) with polydispersity index (PDI) of 0.12). This means that the size of the particles was not drastically altered during doping. Confocal fluorescence microscopy was used to identify the localization of the sensing components with the microspheres, i.e., to assess whether the sensing components are distributed evenly in the microsphere or limited to a certain region. Fig 1 (b) and (c) show the confocal images of the modified PS microspheres, with the red color representing the fluorescence emission from the chromoionophore. The modification appears to have only taken in the surface region of the PS particles. Confocal images of microspheres modified with a reference fluorescent dye (Lumogen Red) using the same procedure also showed emission limited to the surface of the microspheres (Fig. S1, ESI†). The modified PS particles remained suspended over a number of weeks and the sensing components remained in the surface region as confirmed by confocal microscopy.

As shown in Fig 2, the K^+-selective PS microspheres (0.8 μm) suspension in 10 mM Tris-HCl (pH 7.4) exhibited a strong absorption around 630 nm which originated from the protonated chromoionophore ($HInd^+$), and the suspension exhibited a deep blue color. Compared to our previous work with nanospheres where the micro-environment is quite hydrophilic,[14] the basicity of the chromoionophore in the hydrophobic region of the PS particle surface is much higher. Valinomycin is able to bind with K^+ and form a stable complex (LK^+). As K^+ is added, the peak around 630 nm gradually decreases while the absorbance around 540 nm and a reddish color gradually arise, indicating the formation of deprotonated chromoionophore (Ind).

For traditional film based ion-selective optodes, electroneutrality must hold for the bulk of the film.[5] When a given amount of K^+ is extracted into the sensing phase, the same amount of H^+ will be released into the aqueous phase, so that the bulk of the sensor is electrically neutral. If this is not the case, a charge excess will

accumulate on the surface. Zeta-potentials were measured for the K^+-selective microspheres in 10 mM pH 7.4 Tris-HCl buffer. As the K^+ concentration was increased (up to 70 µM), the zeta-potential remained at ±0.2 mV (which is within the instrumental error range), confirming that the surface is neutral, and suggesting that electroneutrality still holds for these microsensors. Therefore, the sensing process can be expressed using Equation 1, with (s) and (aq) designating the microsphere surface and the surrounding aqueous phase.

$$HInd^+(s) + R^-(s) + L(s) + K^+(aq) \rightleftharpoons Ind(s) + R^-(s) + LK^+(s) + H^+(aq) \quad (1)$$

Under the conditions used here, the K^+-selective PS microspheres exhibited an exhaustive sensing mode, as recently established by our group using emulsion based (plasticizer and surfactant) nanospheres.[20] In an exhaustive mode, the added analyte will be consumed by the sensor and result in a response range that also depends on the quantity of sensor material. In this case, the K^+ response indeed depended on the amount of the microspheres. When smaller amounts of microspheres were used (A_0 =0.36, microsphere ion-exchange capacity of ca. 13 µM, Fig. S2, ESI†), a relative narrow detection range from 0 to 10 µM was observed. Higher microsphere concentration (A_0 =1.68, microsphere ion-exchange capacity of ca. 60 µ M) resulted K^+ response from 0 to 60 µM with a linear range from 10 to 40 µ M, as shown in Fig 2. The detection limits are adjustable through tuning the microsphere concentration and able to cover most environmental and biological samples. At lower pH, the equilibrium based sensing of K^+ without sample depletion should take place instead of the exhaustive mode. As shown in Fig. S3, a linear relationship between the sensor response and the logarithms of K^+ concentration from 10^{-6} to 10^{-1} M was observed in 10 mM Mes-NaOH buffer (pH 5.6).

The K^+ response from the modified PS microspheres of 2 µm diameter was similar (Fig. S4, ESI†). However, the initial absorbance at pH 7.4 without addition of K^+ showed both maxima from Ind and $HInd^+$, meaning that chromoionophore I was only partially protonated. Such difference is perhaps due to the presence of carboxylates on the surface of the PS microspheres that render the micro-environment of PS microsphere surface more hydrophilic. A decreased basicity was also observed in previous work reported for ion-selective nanospheres where the surface was covered by other surfactants such as Pluronic F-127.[14]

The selectivity of the K^+-selective PS microspheres was found to be excellent. As shown in Fig. 2(b), other commonly seen ions such as Na^+, Ca^{2+}, Mg^{2+} and Li^+ did not interfere in the active range for K^+. Interference became only visible when these ions reached high concentrations (> 10 mM) (Fig. S5, ESI†). The microspheres appear to exhibit the required selectivity for intracellular experiments.

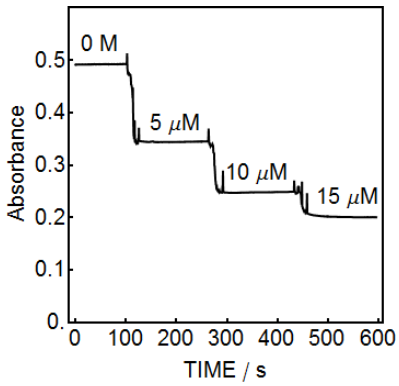


Fig. 3 Response time of the K+-selective PS microspheres in 10 mM pH 7.4 Tris-HCl. Addition of different concentrations of KCl and mixing by pipetting the suspension up and down with a glass pipette are indicated by the spikes.

One important reason that accounts for the vacancy of PS-based ion-selective optodes is the very slow diffusion of the sensing components in polystyrene. In this early study, sensing films of ca. 5 µm thickness and containing the same components as the PS microsheres were prepared by drop casting, but the films showed no response to K^+ even after days. This suggest that the micron-sized sensing beads exhibit much shorter response time owing to their reduced dimensions and the localization of the surface doping. Indeed, the $t_{95\%}$ response time was found as ca. 5 s (Fig. 3), noting that the convective mixing was not optimized for speed.

In summary, a novel method of preparing ion-selective optical microsensors by modification on the surface of polystyrene microparticles was introduced here. Physical entrapment of the sensing components on the surface of the PS particle iwass achieved by precipitation of the sensing components in aqueous PS particle suspension. The resulting microspheres showed a narrow size distribution, long shelf life, high sensitivity and selectivity to K^+ and a fast response time. The present study suggests that surface modification of PS particles may comprise a route to mass production of a new family of ion-selective optical sensors that work on the basis of ion-selective optodes. These microsensors may become valuable tools for the analysis of small ion quantities of environmental and biological samples.

Acknowledgements

The authors thank the Swiss National Science Foundation (SNF) for financial support. Jingying Zhai acknowledges the support by the China Scholarship Council.

Notes and references

1. M. Lerchi, E. Bakker, B. Rusterholz and W. Simon, *Anal. Chem.*, 1992, **64**, 1534-1540.
2. C. Krause, T. Werner, C. Huber and O. S. Wolfbeis, *Anal. Chem.*, 1999, **71**, 5304-5308.
3. X. Xie, G. Mistlberger and E. Bakker, *J. Am. Chem. Soc.*, 2012, **134**, 16929-16932.
4. I. H. A. Badr and M. E. Meyerhoff, *J. Am. Chem. Soc.*, 2005, **127**, 5318-5319.
5. E. Bakker, P. Bühlmann and E. Pretsch, *Chem. Rev.*, 1997, **97**, 3083-3132.
6. X. Xie, X. Li, Y. Ge, Y. Qin and H.-Y. Chen, *Sens. Actuators, B*, 2010, **151**, 71-76.
7. S. Dai, Q. Ye, E. Wang and M. E. Meyerhoff, *Anal. Chem.*, 2000, **72**, 3142-3149.
8. J. M. Dubach, D. I. Harjes and H. A. Clark, *J. Am. Chem. Soc.*, 2007, **129**, 8418-8419.
9. I. Tsagkatakis, S. Peper and E. Bakker, *Anal. Chem.*, 2001, **73**, 315-320.
10. E. J. Park, M. Brasuel, C. Behrend, M. A. Philbert and R. Kopelman, *Anal. Chem.*, 2003, **75**, 3784-3791.
11. A. Burns, P. Sengupta, T. Zedayko, B. Baird and U. Wiesner, *Small*, 2006, **2**, 723-726.
12. J. M. Dubach, S. Das, A. Rosenzweig and H. A. Clark, *PNAS*, 2009, **106**, 16145-16150.

13. M. Telting-Diaz and E. Bakker, *Anal. Chem.*, 2002, **74**, 5251-5256.
14. X. Xie, G. Mistlberger and E. Bakker, *Anal. Chem.*, 2013, **85**, 9932-9938.
15. I. H. A. Badr and M. E. Meyerhoff, *Anal. Chim. Acta*, 2005, **553**, 169-176.
16. M. Antonietti, J. Coutandin and H. Sillescu, *Macromol. Rapid Comm.*, 1984, **5**, 525-528.
17. M. Lücka, W. Schröderb, B.-R. Paulkec, T. Blunkd and R. H. Müller, *Biomaterials*, 1999, **20**, 2063-2068.
18. J. Lee, P. A. Martic and J. S. Tan, *J. Colloid. Interf. Sci.*, 1989, **131**, 252-266.
19. Z. Adamczyk, A. Bratek-Skicki, P. Dąbrowska and M. Nattich-Rak, *Langmuir*, 2012, **28**, 474-485.
20. X. Xie, J. Zhai and E. Bakker, *Anal. Chem.*, 2014, **ASAP**. DOI: 10.1021/ac403996s.

