Talanta 88 (2012) 66–72

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Microspheres aided introduction of ionophore and ion-exchanger to the ion-selective membrane

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ARTICLE INFO

Article history:
Received 27 July 2011
Received in revised form
26 September 2011
Accepted 12 October 2011
Available online 17 October 2011

Keywords:
Polyacrylates
Microspheres
Ion-selective electrodes
Diffusion

ABSTRACT

In this work a novel method for introduction of ionophore and ion-exchanger to the ion-selective polyacrylate based membrane is proposed. These compounds (and optionally primary ions) are introduced to polyacrylate microspheres, used to prepare ion-selective membrane. The approach proposed here can be used to prepare membranes containing primary ions equally distributed through the receptor phase, i.e. membranes that do not require conditioning in primary ions solution and are free from problems related to slow diffusion of primary ions. Thus obtained sensors were characterized with linear responses (also at relatively high activities) and high selectivities, despite considerable reduction of ionophore and ion-exchanger amount introduced to the membrane.

To be able to prepare ion-selective membranes using this approach, a method for quantification of ionophore and ion-exchanger introduced into microspheres is required. In this work a novel method utilizing high performance liquid chromatography (HPLC) with DAD or FLD detection is proposed.

Incorporation of ionophore and ion-exchanger into the microspheres was achieved either by absorption into ready spheres or in course of photopolymerization of polymeric beads. The obtained results have proven that both procedures led to incorporation of ionophore/ion-exchanger into polymeric spheres, however, the content of the compounds in the spheres post process is different from their ratio in solution from which they had been introduced. These effects need to be considered/compensated while preparing microspheres containing ion-selective membranes.

As a model system poly(n-butyl acrylate) spheres, silver selective ionophore and sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate were chosen, resulting ultimately in silver-selective electrodes.

1. Introduction

Fabrication of polyacrylate ion-sensing membranes, allows elimination of plasticizer from membrane composition, which is especially attractive, as often toxic plasticizer present in relatively high amounts in the membrane tends to leach, negatively affecting sensor lifetime [1]. Moreover, application of polyacrylates has also analytical advantages. One of the most important features of ion-selective electrodes (ISEs) with polyacrylate based membranes is two to three orders of magnitude slower transport (ions, ionophore) compared to poly(vinyl chloride) counter parts [2–4]. Benefits arising from low ions diffusion coefficients include lower detection limits and often more favorable selectivity coefficients [e.g. 5]. On the other hand, disadvantages include higher electrical resistance of membranes, moreover, to achieve full saturation much longer conditioning times are required (typically months instead of hours) [3,5]. As a result of relatively slow ions diffusion within the sensors membranes responses can be exclusively related to surface phenomena contributing to substantial differences, both in membrane contents and in recorded potentiometric responses, between polyacrylate and PVC based membranes [5–7]. It was demonstrated recently [3], on the example of silver selective electrode with polyacrylate membranes, that conditioning in primary ions solution ($AgNO_3$ concentration range from 10^{-1} to 10^{-4} M) results in pronounced accumulation of silver ions in the outer layer of the membrane [3] (which was not observed for plasticized PVC based membranes). In consequence, potentiometric responses of sensors with poly(n-butyl acrylate) membranes were much deviated from Nernstian pattern, especially for high activities of silver ions in solution, detection limits obtained were in the range of 10^{-6} M $AgNO_3$. On the other hand, more diluted $AgNO_3$ solutions applied for pretreatment did not result in improved slopes within higher activities; moreover, for low activities deviation from linearity was also observed (super-Nernstian slope region) [3], unless special and time consuming pretreatment protocol involving different and highly diluted primary ions solution was applied [6].

These results clearly suggest that to prepare ion-selective electrodes with polyacrylate membranes approaches other than

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doi:10.1016/j.talanta.2011.10.008

conditioning in primary ions have to be considered to avoid accumulation of those ions within the outer membrane layer and to prepare sensors characterized with responses equivalent with those of PVC based membranes.

A few years ago [8] we described a method based on introduction of a mixture of two ion-exchangers, lipophilic salts, to the membrane: one containing primary ions and the other one containing interfering ions. Thus obtained membranes were showing responses ranging from classical (μM detection limit for membranes saturated with primary ions) through extended linear responses range to calibration lines with super-Nernstian slope regions, dependent on the ratio of applied ion-exchangers [8].

Another approach that can be used, which to our best knowledge has not been considered before, is taking advantage of miniature polymeric objects, i.e. minimizing the distance [9] without changing the value of diffusion coefficient. Miniature portions of membrane, for example microspheres, can be prepared, then equilibrated with primary ions and combined to form a membrane, assuring equal distribution of primary ions in the membrane, without accumulation on the surface. This approach to our best knowledge has not been tested earlier.

Polymeric microspheres can be conveniently prepared by emulsion polymerization, e.g. polyacrylate ones [9–15] and have many potential applications [16–19], mostly as fluorescent probes [e.g. 9–15,20]. Different approaches can be used to load polymeric spheres with compounds responsible for sensitivity and selectivity. In principle, incorporation of those compounds can be achieved either during polymerization or afterwards in course of absorption [9,21,22]. The choice of method of introduction is dependent on properties of the compounds to be introduced. Some compounds cannot be introduced in course of polymerization and they need to be absorbed into the microspheres post polymerization. Most often it is not straightforward to quantify the amount of compounds introduced to the spheres especially as relatively small quantities of substances of different chemical nature are in question, although this information is essential for preparation of successful electrochemical sensors. Especially ion-selective electrodes require that ratio of ionophore and ion-exchanger and their amount is controlled. Therefore, for preparation of sensor using microspheres preloaded with ionophore or/and ion-exchanger a method allowing quantification of incorporation of ionophore and ion-exchanger during microspheres preparation is required and is proposed herein.

It should be also stressed that either emulsion polymerization or absorption procedures require large quantities of solvent (e.g. water poly(vinyl alcohol) solution). As ionophore and ion-exchanger are soluble in water to some extent, and are used in relatively low quantities, the solvent can further influence the amount of species incorporated. To authors best knowledge no quantitative estimation or appropriate procedure for quantification of different compounds incorporated to the spheres was published so far.

In this report we present, for the first time, ion-sensing membrane prepared using microspheres that have been (pre)loaded with ionophore, ion-exchanger and then, optionally, with primary ions advantageously resulting in sensors characterized with stable, Nernstian potentiometric responses.

As a model we have studied silver-selective electrodes based on silver selective ionophore IV (O,O″-bis[2-(methylthio)ethyl]-tert-butylcalix[4]arene) and sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB) ion-exchanger. To study incorporation of ionophore and ion-exchanger into the polymeric matrices we have applied HPLC coupled with DAD/FLD detection.

The obtained microspheres before introduction to the membrane were contacted with silver solution – resulting, after formulation of the membrane, in sensor of ready-made saturated membrane (without necessity of conditioning to introduce primary ions to the membrane).

2. Experimental

2.1. Reagents

Silver selective ionophore IV (O,O″-bis[2-(methylthio)ethyl]-tert-butylcalix[4]arene), sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), poly(vinyl chloride) (PVC), and bis(2-ethylhexyl sebacate) (DOS) were from Fluka AG (Buchs, Switzerland), 1,6-hexanedioldiacrylate (HDDA) and 2,2-dimethoxy-2-phenylacetophenone (DMPP), n-butyl acrylate (nBA), tetrahydrofuran (THF), poly(vinyl alcohol) (PVA) and poly(3-octylthiophene-2,5-diyl), regioregular (POT) were from Aldrich (Germany).

Doubly distilled and freshly deionised water (resistance 18.2 MΩ cm, Milli-Qplus, Millipore, Austria) was used throughout this work. All salts used were of analytical grade and tetrahydrofuran (THF) HPLC grade were obtained from POCh (Gliwice, Poland).

2.2. Apparatus

In the potentiometric experiments a multi-channel data acquisition setup and software, Lawson Labs. Inc. (3217 Phoenixville Pike, Malvern, PA 19355, USA) was used. Pumps systems 700 Dosino and 711 Liquino (Metrohm, Herisau, Switzerland) were used to obtain sequential dilutions of calibrating solutions.

In the potentiometric experiments membrane coated glassy carbon electrode (GC, surface area 0.07 cm^2) and a double junction Ag/AgCl reference electrode with 1 M lithium acetate in outer sleeve (Möller Glasbläserei, Zürich, Switzerland) were used. The recorded potential values were corrected for the liquid junction potential calculated according to Henderson approximation [23].

2.3. Synthesis of polyacrylic spheres

Synthesis of poly(n-butyl acrylate) spheres has been carried out according to Ruedas-Rama and Hall recipe [9]. For *blank spheres* (type A) (made in the absence of ionophore and ion-exchanger) the first mixture of n-butyl acrylate (nBA) (480 μl), HDDA (220 μl) and DMPP (7.5 mg) as initiator was prepared and then it was dispersed in 5 ml of aqueous 1% (w/v) poly(vinyl alcohol) solution, using ultrasound bath (5 min).

For absorption experiment a portion of the blank spheres (type A) suspension (20, 200 or 400 μl) was added to 1 ml of THF/water (1:9 volume ratio) solution containing 0.2 mg/ml of ionophore and 0.1 mg/ml of ion-exchanger. Following 12 h time contact the mixture was centrifuged and the supernatant was analyzed. Resulting polymeric microspheres containing ionophore and ion-exchanger are denoted microspheres type AB.

For silver ionophore and sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate *polymerization loaded spheres* 6 mg of silver ionophore IV (O,O″-bis[2-(methylthio)ethyl]-tert-butylcalix[4]arene) and 3 mg of NaTFPB were dissolved in prepared as described above mixture of nBA, HDDA and DMPP. Then the mixture was dispersed in 5 ml aqueous 1% (w/v) poly(vinyl alcohol) solution, yielding spheres type AP(I + E).

The effect of polymerization conditions on incorporation of *ionophore only* was studied for polymer spheres prepared using mixture of nBA (480 μl), HDDA (220 μl) and DMPP (7.5 mg) containing either 6 mg or 12 mg of silver ionophore (no ion-exchanger added, denoted spheres type AP-I) and dispersed (if not stated otherwise using above given conditions) in 5 ml or 10 ml of 1% PVA

Table 1
Incorporation of ionophore into the poly(n-butyl acrylate) spheres for different polymerization conditions, spheres type AP-I. All spheres were prepared from mixture of nBA (480 μl), HDDA (220 μl) and DMPP (7.5 mg) as initiator containing different amount of silver ionophore and dispersed in different amount of 1% (w/v) PVA solution.

Spheres	Amount of ionophore added to polymerization solution	Volume of 1% (w/v) PVA solution used	Amount of ionophore incorporated during polymerization (relative to amount present before polymerization, %)
AP-I 1	6 mg	10 ml	76.5 ± 0.2 (FLD)
AP-I 2	12 mg	5 ml	71.4 ± 0.1 (FLD)

solution; the composition of polymerization solution is given in Table 1.

Polymerization step was conducted under nitrogen atmosphere for 5 min using vigorous stirring of the emulsion (UV lamp of peak output 360 nm).

Following polymerization, spheres were separated using centrifugation at 5800 rpm for 5 min. The supernatant was analyzed and spheres were resuspended in a new 5 ml portion of PVA of the same volume as used for polymerization.

2.4. *HPLC analysis*

Agilent 1200 HPLC system (Agilent, USA) was used. The entire HPLC system was composed of a Binary Pump, an Autosampler, a Column Thermostat, a Diode Array Detector (DAD) and a Fluorescence Detector (FLD). A 5 μm Eclipse XDB-C18 column, 150 mm × 4.6 mm i.d. (Agilent, USA) was used.

The 0.01 M acetic buffer was prepared by dissolving an appropriate amount of sodium acetate in Milli-Q water to get the required concentrations at pH = 4.8. The mobile phase flow rate was 1 ml/min. Filtered solutions were degassed before use. The gradient elution consisted of four stages as described in Table 2. After separation, the column was rinsed for 4 min by 55:45 THF in 0.01 M acetic buffer (v/v). Then 15 μl of sample solution was injected into the column, at 25 °C. Diode Array Detector wavelength was 270 nm. Fluorescence Detector parameters were: excitation wavelength: 260 nm; emission wavelength: 290 nm.

2.5. *Preparation of silver-selective membrane*

Glassy carbon electrodes were polished with Al_2O_3, 0.3 μm and rinsed well with water. Polyoctylthiophene (POT) layers (used as solid contact – ion-to-electron transducers) were prepared by applying 5 μl of polymer solution in chloroform (2.75 mg/ml) on the GC electrode, prepared as described above, placed in upside down position. After drying of conducting polymer (CP) layer solvent the membrane was applied.

Ag-selective, microspheres based, membranes were prepared by mixing 23 mg of microspheres loaded with ionophore and ion-exchanger in course of absorption of these compounds, spheres type AB-(I + E), prepared using mixture of 200 μl microspheres for 1 ml of THF/water mixture, 18 mg of microspheres type AB-(I + E) prepared as above but additionally, after completion of preparation, contacted with 10^{-3} M $AgNO_3$ solution and 22 mg of spheres containing ionophore only introduced to the microspheres during photopolymerization, spheres type AP-I. Microspheres were dispersed in 300 μl of THF and to this mixture 400 μl THF solution containing 40 mg of dissolved poly(n-butyl acrylate) – prepared as described earlier [24] was added. The composition of the membrane calculated taking into account results of HPLC aided determination of ionophore and/or ion-exchanger incorporation to microspheres was as follows (in wt%): 0.2% of ionophore and 0.07% of ion-exchanger.

Table 2
Gradient elution program for chromatographic separation of sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate and O,O″-bis[2-(methylthio)ethyl]-tert-butylcalix[4]arene.

Time (min)	Tetrahydrofurane (%)	0.01 mol/l acetic buffer, pH 4.8 (%)
0.0	55	45
2.5	55	45
3.0	80	20
7.5	80	20

The membranes were obtained by application of 60 μl (4 × 15 μl) of above described cocktail per electrode.

Thus obtained electrodes were conditioned prior measurements in 0.05 M EDTA solution of pH 6.75 (due to addition of NaOH), pNa = 0.76 (free from silver ions).

Membrane samples intended for LA-ICP-MS studies were prepared from above described cocktail applying 2 × 10 μl on acetate foil intended to use for laser-printers.

For a control experiment silver selective membranes were also prepared in traditional way, i.e. by direct introduction of ionophore and ion-exchanger to membrane matrix [e.g. 3]. Poly(vinyl chloride) based membranes contained (in wt%) 2% of silver ionophore, 0.5% of NaTFPB, 67.5% of DOS, 30% of PVC, total 200 mg of membrane components were dissolved in 2 ml of THF. Poly(n-butyl acrylate) based membranes contained (in wt%): 2.4% of silver ionophore, 0.6% of NaTFPB, 0.2% HDDA and 1.4% DMPP of initiator in n-butyl acrylate. To obtain poly(n-butyl acrylate) membranes 10 μl of n-butyl acrylate based cocktail were applied per electrode (on the top of POT layer) and photopolymerization was performed using UV lamp (360 nm) for 5 min under argon atmosphere. For poly(vinyl chloride) based membranes 20 μl of PVC cocktail in THF were applied per electrode covered with POT layer and left to dry in laboratory atmosphere for 20 h. Thus prepared sensors were conditioned priori to measurement in the same way as microspheres containing sensors, in above described EDTA solutions.

2.6. *LA-ICP-MS measurements*

An inductively coupled plasma mass spectrometer ELAN 9000 (Perkin Elmer, Germany) equipped with the laser ablation system LSX-200+ (CETAC, USA) was used. The LA-ICP-MS setup used was as described earlier [25], using 3 mJ/pulse energy, repetition rate 5 Hz, spot size 50 μm. As target for laser ablation centrifuged spheres or membranes samples were used. The spheres used for this experiment were previously in contact with 10^{-3} M solution of $AgNO_3$ for 12 h (spheres to $AgNO_3$ solution volume ratio 1:10), then the spheres were centrifuged, washed with water and resuspended in excess of PVA, again centrifuged and then analyzed. LA-ICP-MS studies were conducted for blank spheres and spheres with ionophore and ion-exchanger accumulated in course of absorption. Alternatively, the same LA-ICP-MS experiment parameters were used to study samples of microspheres based membranes, prepared as described above.

3. Results and discussion

Preparation of analytically useful ion-selective membranes requires precisely balancing amount of ionophore and ion-exchanger present in the membrane. To use microspheres to

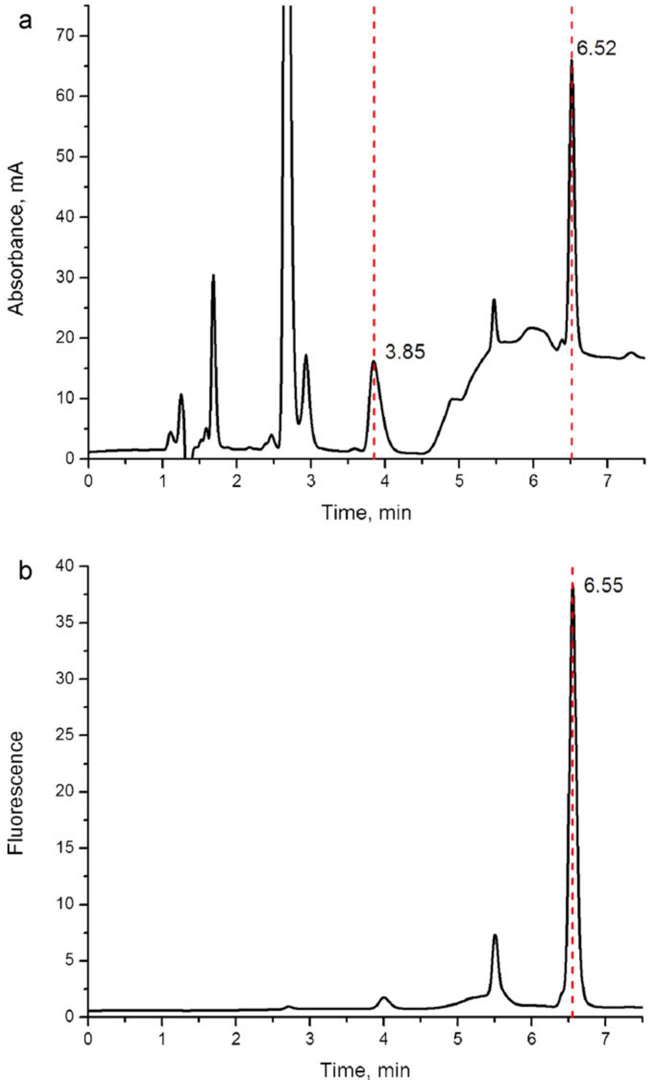

Fig. 1. (A) Chromatogram of sodium tetrakis[3.5-bis(trifluoromethyl)phenyl]borate (ion-exchanger) – retention time 3.85 min and O,O″-bis[2-(methylthio)ethyl]-tert-butylcalix[4]arene (silver ionophore) – retention time 6.52 min. DAD at wavelength 270 nm. (B) Chromatogram of O,O″-bis[2-(methylthio)ethyl]-tert-butylcalix[4]arene (silver ionophore) – retention time 6.55 min. FID: excitation wavelength 260 nm; emission wavelength 290 nm.

introduce these compounds to the membrane, effectiveness of incorporation of compounds mentioned above to the membrane needs to be tested.

Taking into account relatively small concentrations of colorless ionophore and ion-exchanger that were of interest for these studies, the analytical technique of choice was HPLC coupled with DAD/FLD detection. In order to monitor the possible variation in the concentration of compounds of interest by HPLC, the optimization of the elution as well as the detection mode used was performed. The optimized elution program is presented in Table 2. The typical chromatograms obtained with UV ($\lambda = 270$ nm) and fluorescence detector ($\lambda_{excitation} = 260$ nm; $\lambda_{emission} = 290$ nm) are shown in Fig. 1. The use of UV detector allows to monitor both signals for sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate at retention time 3.85 min and O,O″-bis[2-(methylthio)ethyl]-tert-butylcalix[4]arene at retention time 6.52 min. When UV detector was used (Fig. 1A), the signal at 3.85 min is well shaped and situated on the baseline, however, the signal at 6.52 min is overlapped with the relatively high background, thus the fluorescence detection was also used. Fig. 1B represents a typical chromatogram obtained in course of separation of sample containing both silver ionophore and ion-exchanger NaTFPB. It is clear that the signal for silver ionophore is narrow and well situated on the baseline which enables the calculation of the peak area. Taking into account the retention times of both compounds tested it is clear that ion-exchanger is less lipophilic compared to ionophore, as for the latter one retention time is 6.52 min compared to 3.85 min retention time of ion-exchanger. It is worth to mention that similar chromatograms were obtained regardless nature of sample analyzed: (i) solution used for polymerization; (ii) post polymerization solution; (iii) solution used for absorption of ionophore and NaTFPB in blank microspheres.

Table 3
Analytical parameters for the HPLC with different detection mode for the determination of ionophore and ion-exchanger.

	Detection mode used		
	UV–Vis		Fluorescence
	Ion-exchanger	Ionophore	Ionophore
Retention time (min)	3.85	6.52	6.55
Linear dependence	$y = 4566.6x + 0.1$	$y = 4027x - 8$	$y = 3510x + 35$
Correlation coefficient	0.999	0.995	0.998
Detection limit (g/l)	0.009	0.05	0.03
Concentration range (g/l)	0.036–0.221	0.11–0.41	0.06–0.41

The analytical parameters of the determination of both compounds by HPLC with UV absorption and fluorescence detection are summarized in Table 3. The quantification was performed with the use of the peak area for ionophore and/or ion-exchanger in the particular solution, respectively.

3.1. Absorption of ionophore and ion-exchanger in blank microspheres

Blank microspheres – prepared in the absence of ionophore and ion-exchanger were left in contact with solution (THF/water mixture) containing ionophore and ion-exchanger for 12 h. This procedure has been applied earlier to incorporate chromoionophore and ion-exchanger that cannot be easily introduced to spheres by polymerization [9].

Although studying the size of the beads formed was beyond scope of this work, the results (not presented) of electron dispersive analysis of X-rays (EDAX) clearly showed that diameter of predominant majority of studied spheres was close to 100 nm.

A very simple approximation, disregarding swelling/change of diffusion coefficient of ionophore and ion-exchanger due to the presence of THF in water during absorption process and assuming that spheres diameter is close to 100 nm, can be done for the diffusion time of the ionophore/ion-exchanger from the sphere solution interface to the sphere center. Taking the equation: $t = l^2/D$, where l is distance – here sphere radius ca. 50 nm, and D is the ionophore diffusion coefficient within polyacrylate membrane – ca. 10^{-11} cm^2/s [3] gives the diffusion time close to a few seconds. Taking into account that swelling of the polyacrylate membrane in the presence of THF is likely to speed up the process, 12 h time applied is well sufficient for tested compounds to reach whole volume of the spheres.

The weight ratio of ionophore to ion-exchanger was equal 2:1 similarly as it is usually used for preparation of ion-selective electrodes [3]. The concentration of ionophore and ion-exchanger in examined solution was monitored before adding microspheres (considered as 100%) and after 12 h contact (expressed as the ratio of the concentration with respect to initial 100%). Fig. 2 shows the changes of ion-exchanger and ionophore contents in the solution from which these compounds were incorporated to the blank spheres. Results presented in Fig. 2 demonstrate that ionophore

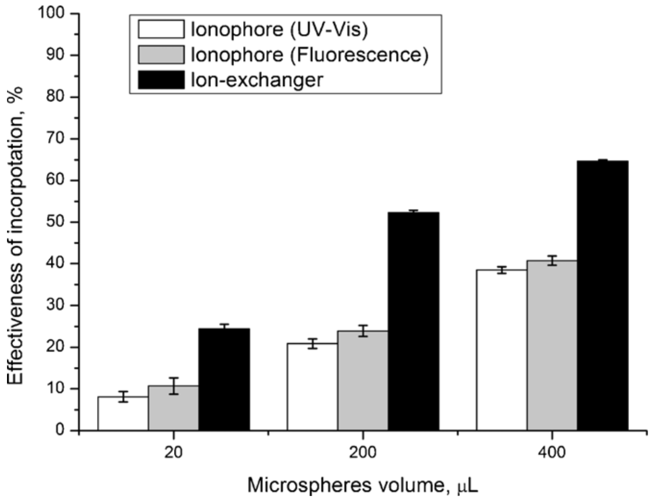

Fig. 2. Effectiveness of ionophore and ion-exchanger incorporation into the blank spheres for absorption during contact with water–THF mixture (absorption).

is incorporated to the spheres and as expected for excess of compound present in the solution the amount absorbed is proportional to the volume of the spheres added, i.e. it is proportional to the amount of spheres introduced to the solution. Interestingly, the incorporation of ion-exchanger is more effective, meaning that higher percentage of compound is absorbed into spheres domain. This observation is extremely important for preparation of microspheres based ion-selective electrodes. This effect was observed, nevertheless ion-exchanger concentration in the absorption solution was smaller than concentration of ionophore and according to chromatograms presented in Fig. 1, this compound is of lower lipophilicity compared to ionophore. Hence, it can be expected that the amount/ratio of the two species in the spheres is different from that expected taking into account their contents in solution from which absorption is performed and different from typically used in ion-selective membranes or optodes, i.e. molar excess of ionophore with respect to the ion-exchanger. In our opinion this is important finding which can affect selectivity and sensitivity of spheres while used as optical sensors [e.g. 9]; however, these issues are far beyond the scope of this report.

It is interesting to speculate on the origin of higher ratio of ion-exchanger absorption. It seems probable that due to local charge separation (e.g. the presence of H^+ ions trapped in the polymer resulting from polymerization process) or the presence of surfactant – poly(vinyl alcohol) – enhances incorporation of dissociated ions resulting from potassium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate present in absorption solution. Bearing in mind that the negative charge of ion-exchanger anion is well sheltered in the structure, this can lead to elevated ratio of ion-exchanger incorporation, especially at the outermost layer of the polymer. Similar effect of salt incorporation to extremely high levels was observed earlier for ion-selective membranes [3].

3.2. LA-ICP-MS studies of spheres following contact with Ag^+ ions solution

The effectiveness of absorption incorporation of ionophores and ion-exchanger could be proven indirectly, following contact of the spheres loaded with ionophore and ion-exchanger during absorption of these compounds from solution with Ag^+ ions in solution. The amount of silver ions is expected to increase in ionophore/ion-exchanger loaded spheres in comparison to blanks spheres. Therefore the LA-ICP-MS studies of blank and absorption loaded spheres were performed. In this case spheres where left for 12 h in the solution containing 10^{-3} M $AgNO_3$. As the quantitative measurements were not aimed, only the comparison of the intensity of the signal was considered as a measure of the relative content of silver [3,25]. For blank spheres (spheres that have been loaded with neither silver ionophore nor ion-exchanger) intensity of ^{107}Ag isotope was only close to 4×10^3 counts, whereas for loaded spheres intensity of the signal was close to 120×10^3 counts, pointing to 30-fold increase of the amount of silver present in loaded spheres. The significantly higher number of ^{107}Ag counts obtained for ionophore and ion-exchanger loaded spheres clearly shows that absorption of those compounds is leading to accumulation of silver within the spheres, which is a good stating point for further modification leading to preparation of silver sensitive light absorption or fluorescent spheres.

3.3. Incorporation of ionophore and ion-exchanger into the spheres in course of polymerization

As above described results clearly show that simple absorption of compounds to the spheres results in predominant loading of microspheres with ion-exchanger, we have tested alternative method of introduction, i.e. incorporation in course of polymerization.

For the spheres type AP(I + E), where both ion-exchanger and ionophore (in the same weight ratio as used for preparation of ion-selective electrodes' membranes), the ion-exchanger incorporation for the spheres prepared according to the above given protocol was equal to 16.9 ± 0.2% (DAD), whereas ionophore incorporation was equal to 12.3 ± 0.3% (FLD). Thus, similarly as in the case of absorption incorporation, ion-exchanger was incorporated into the spheres in higher amount compared to ionophore.

As incorporation in course of polymerization was not leading to proper, from the point of view of ion-selective membrane preparation, proportions of ionophore and ion-exchanger, to achieve desired composition of ion-selective membrane supplementation in ionophore containing spheres is required. Thus the process of polymerization incorporation of ionophore, spheres type AP-I was studied. The results are summarized in Table 1. It can be seen that regardless different composition of polymerization solution, both spheres AP-I 1 and AP-I 2 exhibit similar effectiveness in incorporation of silver ionophore into the polymeric spheres. However, in both cases in post polymerization solution remained about 25% of ionophore used.

3.4. Microspheres based ion-selective membranes

The ultimate aim of this study was to prepare potentiometric sensors with polyacrylate based membrane that is characterized with conventional analytical parameters and is saturated with primary ions but offering Nernstian responses also in higher activity solutions, in contrary to previously described silver selective electrodes with polyacrylate based membranes [3] (lowering the detection limit was not aimed). Taking into account results of above studies of ionophore and ion-exchanger incorporation to the membrane, it is clear that due to more eager incorporation of ion-exchanger, composition of the membrane needs to be supplemented with microspheres containing ionophore only, to achieve excess of ionophore compared to ion-exchange in the membrane. Moreover, as we aimed preparation of ready "primary ions saturated membrane" a part of spheres was contacted before introduction to the membrane with $AgNO_3$ solution.

Sensors with microspheres containing membrane were conditioned in silver ions free EDTA solution.

This conditioning solution was chosen purposely to highlight effect of membrane saturation with primary ions beforehand. Typically (e.g. for calcium or potassium selective electrodes),

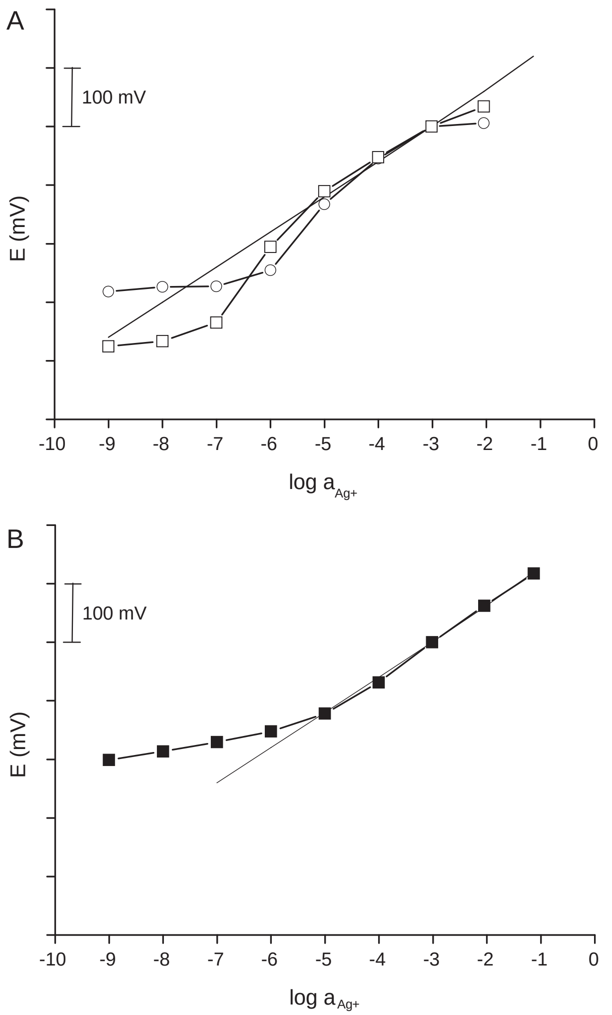

Fig. 3. Calibration lines recorded for Ag-selective sensors tested, following 20 h conditioning in EDTA solution. For easy comparison all curves were shifted to give equal potential at $\log a_{\mathrm{Ag}^+} = -3$. Line represents Nernstian slope. (A) Responses recorded for classical (□) plasticized poly(vinyl chloride) and (○) poly(n-butyl acrylate) based membranes. (B) Responses recorded for microspheres based Ag-selective sensor.

regardless if the ion-selective membranes is using plasticized PVC or polyacrylate in the absence of primary ions during membrane preparation/application step and pretreatment, linear Nernstian responses are observed for high activities followed by a super-Nernstian response range for lower ones [6,8,26]. Similar effects are observed also for conditioning in low primary ions activities solutions (e.g. 10^{-6} M) e.g. [3]. Fig. 3A presents responses obtained for silver selective classical membranes, with plasticized PVC or poly(n-butyl acrylate) following conditioning in EDTA solution, free from Ag^+ ions. As expected for membranes conditioned in the absence of primary ions, for $\mathrm{AgNO_3}$ activities lower than 10^{-5} M an abrupt potential decrease (super-Nernstian slope region) was observed. Moreover, similarly as reported earlier for silver selective membranes [3] and unlikely potassium or calcium sensors, change of Ag^+ concentration in solution from 10^{-2} to 10^{-3} M was accompanied with potential change significantly lower than theoretical, close to 6 mV and 35 mV, for poly(n-butyl acrylate) and plasticized PVC membranes, respectively. This effect results most probably from surface accumulation of silver ions in the either type membrane. Moreover, it is clearly indicating that in some cases preparation of good analytical parameters ion-selective electrode requires alternative approach.

Fig. 3B presents potentiometric responses of microspheres containing poly(n-butyl acrylate) membrane sensors. In contrary to above described membranes despite conditioning in silver ions free solution Nernstian (within the range of experimental error) responses were obtained, with slope equal to 57.5 ± 3.1 mV/dec within the activities range from 10^{-1} to 10^{-6} M, detection limit equal to $10^{-6.3}$ M. Thus (as aimed) obtained potentiometric dependence is typical for classical, primary ions saturated membranes [5,8]. It should be stressed that even for higher activities (10^{-1} to 10^{-3} M) Nernstian potential change was observed, in contrary to classical membranes tested in parallel, Fig. 3A. Thus obtained responses were also stable in time, no changes of responses pattern were observed.

Also LA-ICP-MS experiments performed on thus obtained and pretreated membranes (results not shown) have confirmed equal distribution of silver throughout the membrane thickness. The presence of silver in the membrane following conditioning in Ag^+ ions free EDTA solution, confirmed by LA-ICP-MS experiment is a clear evidence that silver was introduced to the membrane with microspheres. Obtained uniform, throughout the membrane thickness, distribution of silver cannot be relatively quickly (days at the most) obtained using typical method, i.e. during conditioning [3].

Selectivity of microsphere containing electrodes was tested using model interferents, separate solutions method within activities range form 10^{-2} to 10^{-4} M, selectivity coefficients were calculated using experimental electrodes slope. The mean $\log K^{\mathrm{pot}}_{\mathrm{AgJ}} \pm \mathrm{SD}$ were equal to -4.8 ± 1.3, -4.9 ± 1.2 and -5.0 ± 1.3 for Na^+, K^+ and H^+, respectively. Typically sensors of classical potentiometric responses are characterized by selectivity coefficients less favorable compared to those of the same membrane but with super-Nernstian slope region on the dependence [26]. Nevertheless, selectivity coefficients obtained for herein proposed membrane are comparable with values obtained for sensors characterized with super-Nernstian slopes and PVC based membranes. Moreover, $\log K^{\mathrm{pot}}_{\mathrm{AgJ}}$ values obtained in this work are significantly more favorable compared to plasticized PVC membrane based sensors of detection limit equivalent to herein proposed one [27].

Selectivity coefficients obtained for microspheres based membrane prove that herein proposed novel method of introduction of ionophore and ion-exchanger has resulted in analytical advantages, despite that as calculated in experimental section amount of ionophore and ion-exchanger present in the sensor was about 1/10 of that typically applied [27]. Thus proposed approach allows significant cost reduction without compromising performance, yet leading to more favorable analytical parameters of the sensor.

This effect is most probably resulting from surface area to volume ratio of microspheres used. Moreover further (fine) tuning of analytical performance of herein proposed sensors can be achieved using approach developed earlier in our group [8].

4. Conclusions

The results presented above clearly demonstrate that the novel method proposed here, i.e. application of microspheres, can be used to deliver ionophore, ion-exchanger and what even more challenging primary ions to the ion-selective membrane, resulting in sensor of good, classical performance even without contact of the sensor with primary ions (i.e. without conditioning in primary ions). However, this cannot be achieved without detail information about amount of ionophore/ion-exchanger present in the microspheres. Thus herein presented results give for the first time quantitative information about effectiveness of incorporation of colorless ionophore and ion-exchanger into highly lipophilic polyacrylic microspheres. The obtained results indicate that HPLC technique is effective tool giving access to this information. It was confirmed that both absorption and polymerization are similarly effective in loading microspheres with ionophore and ion-exchanger. The results obtained shown that ion-exchanger is incorporated at higher amounts (although its concentration in solution was nearly half that of ionophore) compared to ionophore. This result is even more surprising taking into account that ion-exchanger was less lipophilic compared to ionophore, as estimated from the chromatograms obtained.

Thus, it can be expected that regardless protocol applied, the contents of both compounds in spheres, and their ratio is different from that typical for ion-selective membranes.

Sensors with novel type of membranes were characterized with classical detection limits, however, were operating in Nernstian manner also in higher activities range compared to typical polyacrylate membranes. Selectivities obtained were much more favorable compared to plasticized PVC based membranes of equivalent linear response range.

Acknowledgements

A financial support from the research project 501/68-BW-179222 from University of Warsaw, Poland, is gratefully acknowledged.

This work was supported from scientific research funds, National Centre of Science (Poland) within the research project N N204 247640, in the years 2011–2014.

Dr Mikołaj Donten help in EDAX imagining is kindly acknowledged.

Professor Józef Mieczkowski help in synthesis of poly(n-butyl acrylate) is kindly acknowledged.

Authors are grateful to Professor Krzysztof Maksymiuk for valuable comments related to this work.

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