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Short Communication

Benzylpenicillin PVC membrane electrode for the determination of antibiotics in formulations

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Abstract: A PVC (polyvinychloride) ion selective electrode, employing benzyldimethylcetylammoniumbenzylpenicillin as sensor, was prepared, characterized and applied to the analysis of commercially available formulations containing benzylpenicillin and other antibiotics of the penicillin or cephalosporin group. The results are compared with those obtained by using the corresponding liquid membrane electrode.

Keywords: *Benzylpenicillin; sensors; PVC membrane electrodes.*

Introduction

In recent years, several new electrodes have been developed as electrochemical sensors suitable for the rapid assay of commercially available drugs [1–5]. One of these was electrode selective for benzylpenicillin based on a liquid membrane [5]. The membrane consisted of a solution in 1-decanol of the ion-exchanger benzyldimethylcetylammonium-benzylpenicillin (BDMCABP), which was prepared from commercially available materials, and of benzyldimethylcetylammonium chloride (BDMCA). In the absence of the BDMCA the life of the electrode is short (3–4 days compared to 7–8 days and when BDMCA is also present [5]), but the slope was closer to Nernstian values. Since polyvinylchloride (PVC) membrane electrodes, based on an ion-exchanger dispersed in a polymeric (PVC, silicon rubber) matrix [6–8] have much longer life times, a PVC membrane electrode based on BDMCABP in a matrix of PVC and sebacate was constructed for the assay of antibiotics. In this paper, a comparison is made between liquid and PVC membrane electrodes, for the assay of antibiotics in synthetic matrices and in injectable and pellet preparations containing penicillin or cephalosporin antibiotics.

Experimental

Reagents

All reagents were of analytical reagent grade. Benzylpenicillin potassium salt was supplied by Squibb S.p.A., Rome, Italy. Methicillin sodium salt and carbenicillin

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disodium salt were supplied by Farmitalia-Carlo Erba, Milano, Italy. Piperacillin sodium salt was supplied by Cyanamid S.p.A., Catania, Italy. Cephalothin sodium salt was supplied by Istituto Biochimico Italiano, Pomezia, Italy. Cephalexin sodium salt was supplied by Bonomelli S.p.A., Como, Italy. Benzyldimethylcetylammonium chloride, 1-decanol, high molecular polyvinylchloride (PVC) and bis(2-ethyl-hexyl)-sebacate by Fluka, Switzerland. Other reagents were supplied by Merck, Darmstadt, FRG.

Apparatus and measurements

Potentiometric measurements were carried out using an Orion research potentiometer microprocessor ionalyzer model 901 and a Radiometer REC 61 Servograph recorder. An automatic burette (Schott-Gerate TA 50) was coupled to the potentiometer–recorder system in which a saturated calomel electrode served as the reference electrode. The initial volume of the solutions for potentiometric measurements by the direct calibration and standard addition methods was 25 ml. The solutions were magnetically stirred and thermostated at 20°C during analysis. The benzylpenicillin solutions, and those of the other antibiotics and their formulations, were obtained by dissolving accurately weighed quantities of the potassium or sodium salt, or of the formulation, in distilled water and adjusting the pH to 6.0. If turbidity due to insoluble excipients was observed after dissolution of the formulations, filtration was carried out before the potentiometric measurements. The construction of the calibration curve and the calculation of the selectivity coefficients were carried out by means of a personal computer HP 86 and a suitable program [9].

Exchanger preparation

Benzyldimethylcetylammoniumbenzylpenicillin (BDMCABP) was prepared in a separating funnel as described before [5], by the reaction between commercially available benzyldimethylcetylammonium chloride in chloroform and an aqueous solution of potassium benzylpenicillin at pH 6.0. When the reaction was complete the chloroform was evaporated and the product obtained was purified by chromatography and characterized by elemental analysis, thermal analysis, TLC and spot test on silica gel, infrared spectroscopy and X-ray powder spectra [5].

Liquid membrane electrode. The sensor was prepared by placing 0.1 ml of a 0.01 M solution of BDMCABP in 1-decanol between two porous teflon discs (diameter 13 mm; thickness 0.1 mm; pore size 0.2 μm) and mounting these on the bottom of a sensor with Ag/AgCl as the reference, dipping in a 0.01 M solution of potassium benzylpenicillin and of KCl in water. The assembly of the sensor is shown in Fig. 1. The electrical resistance of the liquid membrane is of the order of k ohms.

PVC membrane electrode. To 165 mg of PVC dissolved in 3 ml of tetrahydrofuran and 330 mg of bis(2-ethyl-hexyl)sebacate as plasticizer, 5% w/w of BDMCABP was added. The solution was allowed to evaporate in a Petri plate (5 cm diameter). The residue which was a membrane (diameter 10 mm; thickness 0.1 mm), was glued to the bottom of a PVC tube by an adhesive obtained by dissolving PVC in cyclohexanone. The inner solution of the PVC tube was 0.01 M potassium benzylpenicillin and KCl into which a Ag/AgCl reference was dipped. The electrical resistance is of the order of M ohm. The assembly is shown in Fig. 1.

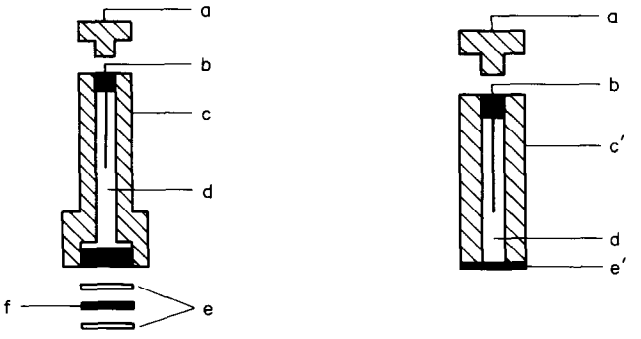

Figure 1
(a) to potentiometer; (b) inner reference electrode Ag/AgCl; (c) teflon body; (c') PVC body; (d) inner solution; (e) teflon porous discs; (e') PVC membrane; (f) liquid membrane.

Results

The principal analytical characteristics of the PVC membrane electrode (slope, precision, pooled standard deviation, response time, accuracy of data obtained by operating with different procedures, such as direct potentiometry or standard addition) in benzylpenicillin solutions, are reported in Table 1. The reproducibility of the slope and the correlation coefficient of the calibration curve are shown in Table 2. In these two tables data are compared with those found by using the corresponding liquid membrane sensor. To evaluate the extent of interference from other anions present in the solution, when measurements were made by using the PVC membrane sensor, the selectivity coefficients, obtained by the mixed solutions method, for ten of the most common anions, were measured and are shown in Table 3. The response of the electrode

Table 1
Characterization of PVC membrane ion selective benzylpenicillin electrode in standard solutions of potassium benzylpenicillin and comparison with the data obtained with the corresponding liquid membrane electrode

	PVC membrane sensor	Liquid membrane sensor
response time:	<15 s	<15 s
slope $\Delta V/\Delta \log a$: (at 20°C and pH 6.0)	−0.0582 (±0.0001)	−0.0569 (±0.0002)
linearity range: ($mol\ l^{-1}$)	5.80×10^{-4}–7.06×10^{-3}	1.96×10^{-4}–3.05×10^{-3}
repeatability of measurements (as pooled standard deviation %) in the linearity range	1.9%	1.2%
inaccuracy of measurement:		
(1) direct method		
1.23×10^{-3} mol l^{-1}	+0.8% (RSD% = 0.8)	−0.4% (RSD% = 3.2)
3.05×10^{-3} mol l^{-1}	−1.0% (RSD% = 1.5)	+2.2% (RSD% = 3.9)
(2) standard addition method		
1.23×10^{-3} mol l^{-1}	+3.0% (RSD% = 1.0)	+0.7% (RSD% = 3.3)
3.05×10^{-3} mol l^{-1}	+1.3% (RSD% = 1.6)	+3.3% (RSD% = 2.2)

Table 2
Calibration curve reproducibility in standard solutions of benzylpenicillin (pH = 6.0, 20°C)

Sample	Linearity range (mol l^{-1})	Liquid membrane electrode Slope $\Delta V/\Delta \log a$	Correlation coefficient
1	1.96×10^{-4}–3.05×10^{-3}	−0.0567	−0.9990
2	1.96×10^{-4}–3.05×10^{-3}	−0.0569	−0.9996
3	1.96×10^{-4}–3.05×10^{-3}	−0.0571	−0.9999
Mean value	1.96×10^{-4}–3.05×10^{-3}	−0.0569 (SD = ±0.0002)	−0.9995
Sample	Linearity range (mol l^{-1})	PVC membrane electrode Slope $\Delta V/\Delta \log a$	Correlation coefficient
1	5.80×10^{-4}–7.06×10^{-3}	−0.0581	−0.9980
2	5.80×10^{-4}–7.06×10^{-3}	−0.0581	−0.9980
3	5.80×10^{-4}–7.06×10^{-3}	−0.0583	−0.9996
Mean value	5.80×10^{-4}–7.06×10^{-3}	−0.0582 (SD = ±0.0001)	−0.9985

Table 3
Selectivity coefficients by Moody–Thomas mixed solutions method at pH 6

j^{n-}	$K_{bp,j^{n-}}$	Interfering ion concentration (mol l^{-1})
Benzoate	0.45	5.00×10^{-3}
Acetate	6.02×10^{-2}	1.00×10^{-2}
Citrate	5.66×10^{-2}	1.00×10^{-2}
Oxalate	1.84×10^{-2}	1.00×10^{-2}
Nitrate	0.14	5.00×10^{-3}
Sulphate	2.01×10^{-2}	5.00×10^{-3}
Chloride	1.00	5.00×10^{-3}
Bicarbonate	9.61×10^{-2}	5.00×10^{-3}
Phosphate monobasic	7.35×10^{-2}	1.00×10^{-2}
Hydroxide	0.41	1.00×10^{-2}

to the sodium salts of penicillin and cephalosporin antibiotics other than benzylpenicillin was measured, with the aim of assessing the possibility of using the electrode for the determination of these compounds. The results in Table 4 summarize the main analytical characteristics of the electrode (precision, accuracy, slope, correlation coefficient, linearity range). Finally, analytical data showing the results obtained by the use of the benzylpenicillin PVC membrane electrode for the assay of the content of the antibiotics in commercial preparations by direct potentiometric analysis and the standard addition method are reported in Table 5.

Discussion

From the results it is concluded that the benzylpenicillin PVC membrane electrode has satisfactory analytical characteristics when evaluated in terms of its response time, precision, accuracy (Table 1), linearity range, quasi-Nernstian slope values, reproducibility (Table 2) and selectivity. The selectivity coefficient values for several anions

Table 4
Response of PVC membrane electrode in standard solutions of different sodium penicillins or cephalosporins, at 20°C and pH 6.0

Antibiotic	Linearity range (mol l^{-1})	Slope $\Delta V/\Delta \log c$	r	Pooled RSD in the linearity range	Inaccuracy by standard addition method
Potassium benzylpenicillin	5.9×10^{-4}–7.7×10^{-3}	−0.0582 (±0.0001)	−0.9985	1.9%	−1.4%
Sodium carbenicillin	4.0×10^{-4}–6.5×10^{-3}	−0.0268 (±0.0002)	−0.9950	1.8%	−2.0%
Sodium methicillin	9.9×10^{-4}–5.8×10^{-3}	−0.0503 (±0.0002)	−0.9994	1.7%	−1.8%
Sodium piperacillin	4.0×10^{-4}–8.7×10^{-3}	−0.0444 (±0.0007)	−0.9996	1.6%	−1.0%
Sodium cephalotine	7.9×10^{-4}–1.0×10^{-3}	−0.0524 (±0.0012)	−0.9984	2.3%	−0.5%
Sodium cephalexine	3.6×10^{-4}–1.2×10^{-3}	−0.0510 (±0.0005)	−0.9991	2.8%	−2.7%

Table 5
Inaccuracy (as % differences between experimental and nominal value) of antibiotic determinations in formulations. Each value is the mean of at least three determinations

Antibiotics contained in the drug	Direct method	RSD %	Standard addition method	RSD %
(1) Sodium carbenicillin (injectable)				
(a) Liquid membrane electrode	+1.4%	5.5	−1.9%	2.4
(b) PVC membrane electrode	+1.3%	3.1	−2.0%	3.2
(2) Sodium methicillin (injectable)				
(a) Liquid membrane electrode	+1.4%	6.3	−2.2%	6.9
(b) PVC membrane electrode	+1.4%	1.3	−1.8%	6.7
(3) Sodium piperacillin (injectable)				
(a) Liquid membrane electrode	+1.7%	6.7	+1.2%	5.9
(b) PVC membrane electrode	+1.0%	8.7	−1.0%	6.0
(4) Sodium cephalotine (injectable)				
(a) Liquid membrane electrode	−1.2%	2.1	+0.5%	3.4
(b) PVC membrane electrode	+1.0%	4.0	−0.5%	4.0
(5) Sodium cephalexine* (pellets)				
(a) Liquid membrane electrode	−2.7%	2.8	−3.0%	7.5
(b) PVC membrane electrode	−1.8%	3.9	+0.5%	4.0
(6) Sodium cephalexine† (pellets)				
(a) Liquid membrane electrode	−6.2%	6.5	−6.7%	2.9
(b) PVC membrane electrode	−3.5%	3.8	+2.4%	4.0

*Excipients: polyvinylpyrrolidone (2.1%), corn starch (1.8%), magnesium stearate (0.6%), hydroxypropyl cellulose (1.6%), acetyl monoglycerides (0.4%), E-110 (dye) (0.1%), titanium dioxide (0.3%).
†Excipients: granular cellulose (6.0%), sucrose (0.9%), natural flavour (1.8%), amylopectin (2.2%), magnesium stearate (0.9%), hydroxypropylmethylcellulose (1.7%), titanium dioxide (0.03%).

were generally sufficiently low that no significant interference is likely to occur (Table 3). These analytical data are of the same order as those for the corresponding liquid membrane electrode (Tables 1 and 2), but the life-time of the PVC membrane electrode is much longer (at least two months) and its assembly is much easier. The slope of each antibiotic differs (Table 4). Benzylpenicillin displays the highest slope which is almost Nernstian in behaviour, but the other antibiotics have a slope that is close to the Nernstian value, when the number of the charges involved is taken into account. The slope values obtained with the PVC membrane electrode are generally a little higher than those obtained with the liquid membrane sensor [5].

The results obtained in the assay of the antibiotics in the commercial preparations (Table 5), show that the precision and accuracy are generally higher, with the PVC membrane sensor, than with the liquid membrane, especially when the drugs are formulated as pellets. Comparable results were obtained with direct potentiometric analysis and with the direct standard addition method.

When the behaviour of the PVC membrane electrode is compared with that of other sensors selective for antibiotics, particularly the enzyme electrode prepared by Papariello [10], or the enzyme-coupled field effect transistor prepared by Caras and Janata [11], the present method is simpler, cheaper and more direct.

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