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Cholate Liquid Membrane Ion-selective Electrode for Drug Analysis*

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A cholate liquid membrane electrode employing benzyldimethylcetylammonium cholate as sensor was prepared, characterised and applied to the analysis of commercially available drugs containing cholanic acids. The results are comparable to those obtained using a benzoate electrode.

Keywords: *Cholate*; *liquid membrane*; *ion-selective electrode*; *benzyldimethylcetylammonium cholate*; *drug analysis*

The determination of cholic acids is becoming increasingly important for drug control and their quantitative determination is currently performed by high-performance liquid chromatography (HPLC),[1] thin-layer chromatography (TLC)[2] and gas chromatography (GC)[3] and by enzymatic,[4] spectrophotometric,[5] radiochemical[6] and calorimetric[7] methods. Each of these methods presents some problems, such as toxicity of the reagents, high cost and complexity of the apparatus or the procedure. In a previous study[8] we proposed a potentiometric method based on the use of a liquid membrane electrode indicator containing tributylcetylphosphonium benzoate dissolved in nitrobenzene as sensor. The results obtained were of a

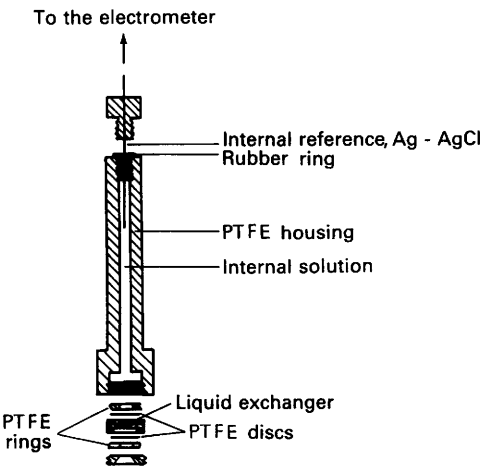

Fig. 1. Liquid membrane electrode assembly.

lower precision (but almost the same accuracy) compared with those obtained by traditional methods, but the method is both simpler and cheaper. In this paper we present a new liquid membrane electrode indicator, containing a quaternary ammonium cholate salt, benzyldimethylcetylammonium cholate, as sensor. This new sensor has been characterised and employed for the determination of the cholic acids in some commercial drugs. Results are compared with those obtained by a benzoate liquid membrane electrode and by the enzymatic - spectrophotometric method of Talalay.[4]

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Experimental

Reagents

All reagents were of analytical-reagent grade. Cholic acid, sodium cholate, deoxycholic acid and chenodeoxycholic acid were supplied by Merck, ursodeoxycholic acid by Giuliani and lithocholic acid and benzyldimethylcetylammonium chloride (BDMCACl) by Fluka. All reagents for the enzymatic - spectrophotometric (ultraviolet) tests for bile salts, using a previously reported procedure,[9] were provided by Nyegaard, Oslo.

Apparatus

Potentiometric measurements were carried out using an electrometer (Radiometer PHM64), a recorder (Varian G-14 A2) and an automatic burette (Radiometer ABU-11). A saturated calomel electrode was employed as reference electrode. Spectrophotometric measurements were performed with a Perkin-Elmer 320 spectrophotometer.

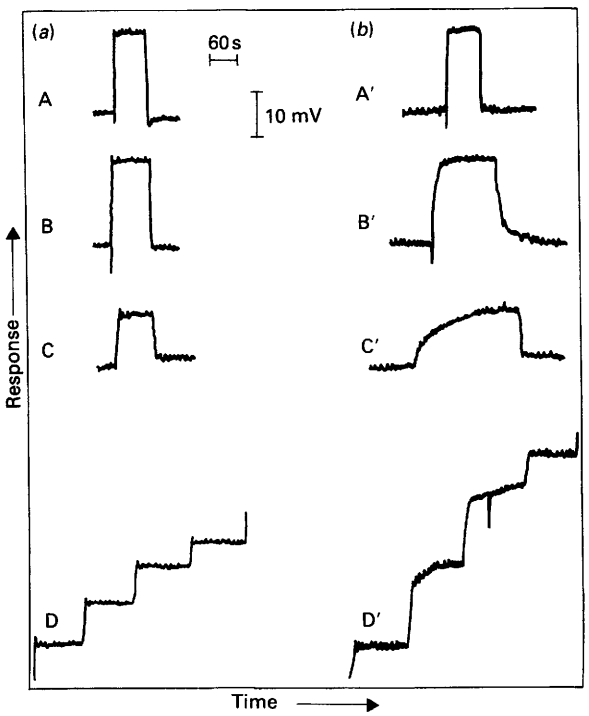

Fig. 2. Comparison between the response of (*a*) the cholate electrode with that of (*b*) the benzoate electrode in standard solutions of sodium cholate with changing cholate concentration (*C*). A and A′: *C* (initial) = 2.0×10^{-3} M; *C* (after addition) = 4.5×10^{-3} M; *C* (after dilution) = 1.9×10^{-3} M. B and B′: *C* (initial) = 3.8×10^{-4} M; *C* (after addition) = 9.0×10^{-4} M; *C* (after dilution) = 3.8×10^{-4} M. C and C′: *C* (initial) = 4.0×10^{-5} M; *C* (after addition) = 6.8×10^{-5} M; *C* (after dilution) = 4.4×10^{-5} M. D and D′: responses of the electrodes for successive increases of cholate concentration, D, *C* = $6.7 \times 10^{-4} - 2.2 \times 10^{-3}$ M; D′, *C* = $8.0 \times 10^{-4} - 2.5 \times 10^{-3}$ M.

Procedure

Benzyldimethylcetylammonium cholate (BDMCACh) is prepared by the reaction between commercially available BDMCACl in chloroform and an aqueous solution of cholic acid at pH 9. To the chloroform phase diethyl ether is added with stirring and the BDMCACh is

precipitated. The product obtained is purified by recrystallisation and characterised by melting-point determination (104–106 °C), elemental analysis, thermal analysis, TLC on silica gel, infrared spectroscopy, NMR spectroscopy and X-ray powder diffraction. The electrode assembly characteristics are as follows: electrode body, PTFE; sensors, BDMCACh ($C_{49}H_{85}O_5N.H_2O$); membrane solvent, decan-1-ol (dielectric constant 8.1 relative to vacuum; viscosity 12.5 cP); membrane solution concentration, 0.01 M in BDMCACh; internal solution, sodium cholate 0.01 M, potassium chloride 0.01 M; internal reference electrode, Ag - AgCl - Cl^-; PTFE discs; and Millipore supports, 1.3×10^{-2} m diameter, 1×10^{-4} m thickness and 2×10^{-7} m pore size.

The electrochemical cell is operated under the following conditions: Ag - AgCl - 0.01 M KCl, 0.01 M sodium cholate ‖ 0.01 M BDMCACh in decan-1-ol ‖ solution under test ‖ saturated calomel electrode. The arrangement details of the electrode are shown in Fig. 1.

TABLE I

SELECTIVITY CONSTANTS ACCORDING TO THE MOODY - THOMAS METHOD

j^{n-}	$K_{chol^-}(j^{-n})$	Background level of interference/M
Benzoate	0.11	1×10^{-3}
Acetate	8.30×10^{-3}	1×10^{-2}
Nicotinate	1.92×10^{-2}	1×10^{-1}
Citrate	3.80×10^{-4}	1×10^{-2}
Oxalate	4.00×10^{-4}	1×10^{-1}
Nitrate	1.37×10^{-2}	1×10^{-2}
Sulphate	1.77×10^{-4}	1×10^{-1}
Chloride	8.50×10^{-3}	1.2×10^{-2}
Phosphate	8.70×10^{-3}	1×10^{-5}
Hydroxyl	0.10	1.6×10^{-3}

Results

The electrode was checked for electrochemical and analytical characteristics using standard sodium cholate solutions. The response time was 10 s maximum. Fig. 2 shows the response of the cholate electrode compared with that of the benzoate electrode with varying cholate concentration.

The linearity range was 4.00×10^{-5}–0.01 M; the slope of the calibration graph was −0.0577 (± 0.0006) volts per decade of mean activity, at 16 °C; and the relative standard deviation[10] was 0.5% over the same range.

The accuracy, for sodium cholate solutions of concentrations ranging between 1×10^{-4} and 0.01 M, differed according to the technique employed (*i.e.* titration method, direct potentiometry, standard additions and Gran's plot[11,12]) and the best results were obtained by a Gran's plot. The error was not higher than 3% compared with about 5.5% for the standard additions method and 6% for the other two methods. The electrode was also checked for selectivity constants relative to several common anions; the values, obtained according to the method of Moody and Thomas,[13,14] are shown in Table I. Values of the selectivity constants, K_{ij}, were obtained by measuring the e.m.f. in a solution containing a fixed amount (see Table I) of the interferent j and a varied activity of the primary ion i for which the electrode is selective. The value of K_{ij} is calculated from $K_{ij} = a_i/a_j^{z/y}$ where z and y are the charges of i and j; a_i and a_j are the values that correspond to the intersection of the part of the calibration

TABLE II

LINEARITY RANGE AND SLOPES OF THE CALIBRATION GRAPHS FOR CHOLIC ACIDS DETERMINATION

Acid	Slope, volts per decade of concentration	Linearity range/M
Cholic acid	−0.0567	4.00×10^{-5}–1.00×10^{-2}
Deoxycholic acid	−0.0598	3.98×10^{-4}–3.98×10^{-3}
Chenodeoxycholic acid	−0.0593	2.00×10^{-4}–3.16×10^{-3}
Ursodeoxycholic acid	−0.0521	1.00×10^{-4}–5.01×10^{-3}
Lithocholic acid	−0.2000	2.51×10^{-5}–7.94×10^{-5}

TABLE III

COMPARISON BETWEEN POTENTIOMETRIC DETERMINATIONS WITH A BENZOATE ELECTRODE, A CHOLATE ELECTRODE AND BY ENZYMATIC DETERMINATION OF CHOLANIC ACIDS IN COMMERCIAL DRUGS

Each value is the mean of at least five determinations; values in parentheses are standard deviations (%).

			Cholanic acid found, %				Differences between found and nominal values, %			
Drug	Cholanic acid	Nominal value, %	Benzoate electrode (standard addition method) (1)	Cholate electrode (standard addition method) (2)	Cholate electrode (Gran's plot method) (3)	Enzymatic - spectro-photometric method (4)	Method 1	Method 2	Method 3	Method 4
1	Chenodeoxy-cholic acid	71.4	62.1 (9.1)	75.0 (0.0)	76.4 (2.1)	76.4 (2.1)	−13.0	+5.0	+7.0	+7.0
2		50.0	55.0 (8.1)	51.0 (7.1)	51.0 (8.2)	53.2 (3.0)	+10.0	+2.0	+2.0	+6.4
3		94.3	108.4 (9.8)	96.8 (9.7)	96.4 (9.9)	97.1 (2.9)	+15.0	+2.7	+2.2	+3.0
4	Ursodeoxy-cholic acid	83.3	82.1 (6.2)	85.8 (0.0)	85.0 (1.5)	82.0 (2.1)	−1.4	+3.0	+2.0	−1.6
5		56.6	53.8 (6.5)	57.4 (7.0)	58.3 (0.7)	56.6 (1.0)	−4.9	+1.4	+3.0	0.0

graph that has an approximately zero slope. This part corresponds to the complete interference by ion j with the Nernstian (or approximately Nernstian) part and corresponds to the electrode function for the primary ion i. For $K_{ij} < 1$ the electrode responds preferentially to ion i and for $K_{ij} > 1$ the electrode responds preferentially to ion j.

In order to evaluate the possible uses of the electrode in the analysis of aqueous solutions containing the other cholanic acids (deoxy-, chenodeoxy-, ursodeoxy- and lithocholic acid), all of biological and pharmaceutical interest, linearity concentration ranges and slopes were determined for these unconjugated acids (Table II).

Finally, the electrode was applied to the determination of two cholanic acids (chenodeoxycholic and ursodeoxycholic acids), both of which are contained in a commercial drug used for dissolving biliar gallstones, using the following procedure. A weighed amount of each of five examined drugs was dissolved in water at pH 11 and after sedimentation of a little insoluble matter the solution was filtered and appropriately diluted. Each was then analysed

TABLE IV

COMPOSITIONS OF THE EXAMINED DRUGS

Drug	Component	Content, % *m/m*
1	Chenodeoxycholic acid	71.4
	Corn starch	26.6
	Aerosil	1.4
	Magnesium stearate	0.6
2	Chenodeoxycholic acid	50.0
	Lactose	38.4
	Starch	2.0
	Talc	2.0
	Starch - sodium glycolate	6.0
	Precipitated silica	0.8
	Magnesium stearate	0.8
3	Chenodeoxycholic acid	94.3
	Polyvinylpyrrolidone	3.8
	Colloidal silica	1.1
	Magnesium stearate	0.8
4	Ursodeoxycnolic acid	83.3
	Starch	10.0
	Precipitated silica	3.3
	Magnesium stearate	3.3
5	Ursodeoxycholic acid	56.6
	Lactose	37.7
	Polyvinylpyrrolidone	3.8
	Magnesium stearate	1.1
	Colloidal silica	0.8

by an enzymatic - spectrophotometric and by a potentiometric method with a cholate electrode and a benzoate electrode. The better results for precision and accuracy were obtained by standard additions and Gran's plot methods, using the cholate electrode. In Table III experimental data, obtained by this sensor, are reported and compared with those obtained by both a benzoate electrode[8] and enzymatic method.[9] In Table IV the nominal percentage compositions of all the examined drugs are reported.

Conclusions

From the results several conclusions were drawn, evidencing the superiority of cholate electrodes over benzoate electrodes.

In general, faster response times are obtained using a cholate electrode—10 s was the maximum time obtained. The accuracy in cholate standard solutions is almost the same as for a benzoate electrode and the best results are obtained by a Gran's plot. The precision is higher for a cholate electrode than for a benzoate electrode and the linearity range is wider and the minimum detection limits are lower; also the values of the selectivity constants for anions are low enough to prevent any common interference when using the cholate electrode. Consideration of the slopes of the calibration graph for the five cholanic acids examined (Table II) leads to the conclusion that the value of the slope increases as the number of hydroxyl groups in the steroid ring decreases or if one of these hydroxyl groups is in the β-position towards the plane of the ring.

The results of drugs analysis are, however, less precise using a cholate electrode compared with an enzymatic - spectrophotometric method but comparably accurate, and the analysis with the cholate electrode is faster and cheaper than that by the enzymatic method; moreover it has no problems of reagent availability and storage.

Finally, in comparison with other possible methods such as calorimetry[7] and chromatography,[3] potentiometry is perhaps less precise but does not require expensive and complicated apparatus or need any pre-treatment of the sample.

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