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Cholanic acids determined in commercial drugs by means of a new ISFET device*

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Abstract: An ISFET device selective for cholanic acids, based on a PVC–sebacate membrane, containing benzyldimethylcetylammoniumcholate as exchanger, has been prepared, characterized and applied to the determination of cheno or ursodeoxycholic acid content of commercial pharmaceutical drugs and critical micellar concentration (CMC) values for cholate, deoxycholate and chenodeoxycholate. The results are compared with those obtained using previously described polymeric membrane sensors based on the same exchanger.

Keywords: *ISFET; sensor; cholanic acids; analysis.*

Introduction

In recent years, work has been done to investigate the possibility of determining the cholanic acid content of pharmaceutical preparations of great importance in the therapy of diseases caused by gallstone affections [1]. Effective and rapid analysis has successfully been performed using a liquid membrane electrode [2] developed by the authors containing a new anionic exchanger (benzyldimethylcetylammoniumcholate) synthesized by the authors [2, 3].

This exchanger was subsequently used by the authors' research group dispersed in a polymeric membrane based on PVC and sebacate [4].

More recently considerable work has been done to develop solid state sensor ISEs or ISFETs selective for different chemical species [5–7]. In this way, using the same polymeric membrane [4] and the above-mentioned exchanger [2, 3] the authors assembled a new sensor using, instead of the classical potentiometric electrode, a more recently developed apparatus based on a field effect transistor transducer. Thus, an ISFET selective for cholanic acids was obtained.

The present paper shows the results of the analysis of commercial pharmaceutical preparations containing cheno or ursodeoxycholic acid, as well as the determination of critical micellar concentration (CMC) values for three different cholanic acids using the new ISFET device.

Experimental

Materials

High molecular weight poly(vinylchloride) (PVC), bis(2-ethyl-hexyl)sebacate, benzyldimethylcetylammonium chloride and Jeffamine D-230 were obtained from Fluka, AG (Buchs, Switzerland); cholic acid sodium salt, deoxycholic acid, chenodeoxycholic acid, ursodeoxycholic acid, taurocholic acid, glycocholic acid, taurodeoxycholic acid were supplied by Sigma (St Louis, MO, USA); Epon 825 was from Shell Italia (Milan, Italy); tetrahydrofuran and all other reagents and solvents were of analytical grade and obtained from Carlo Erba (Milan, Italy).

Samples

Two commercially available formulations containing chenodeoxycholic acid and three containing ursodeoxycholic acid, used in the therapy of cholesterol gallstones, were analysed. Each sample consisted of a finely crushed and homogenized powder obtained

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from five capsules; a weighed amount of the powder was then dissolved in distilled water adjusted to pH 9 with sodium hydroxide.

Electrochemical measurements for drug analysis were carried out after filtration, if any turbidity was observed, and after appropriate dilution of the solution obtained. A summary of the percentage composition of the products examined according to the indications of the manufacturers is given in Table 1.

Methods

FET preparation. The integrated chips (UUO3 type) were supplied by the HEDCO Laboratory of Utah University (USA); each chip (overall dimension 1.28 mm × 2.16 mm) contains two 400 μm × 20 μm gates and two metal gate control devices. The device was washed with isopropyl alcohol and then mounted on a plastic stick subsequently connected to the electrical measurement system. After making the electrical connections with an ultrasonic wire-bonder (model 4123, Kulicke and Soffa, Zurich, Switzerland), the device was encapsulated in epoxy resin (EPON 825 + Jeffamine D-230) body, leaving only a two gates area free, as shown in Fig. 1.

Exchanger preparation. The preparation, purification and characterization of the benzyldimethylcetylammoniumcholate (BDMCACh) exchanger were performed as previously described [2, 3]; the preparation involved shaking equal volumes of a 5×10^{-3} M benzyldimentylcetylammonium chloride chloroform solution and a 1×10^{-2} M sodium cholate aqueous solution in a separatory funnel. After separation the organic solvent was evaporated at room temperature.

ISFET device assembly. The polymeric membrane was prepared by stirring for about 5 h a suspension consisting of 165 mg of polyvinylchloride (PVC) as base polymer, 330 mg of bis(2-ethylhexyl)sebacate as plasticizer and 10% by weight of benzyldimethyl-

Table 1
Composition of the pharmaceutical products examined (all in capsule form)

Drug no.	Components	Content* (as % by weight)
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	Urosodeoxycholic acid	69.4
	Talc	26.7
	Carboxymethyl–starch	3.3
	Colloidal silica	0.6
4	Ursodeoxycholic 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

*Nominal values given by the manufacturers.

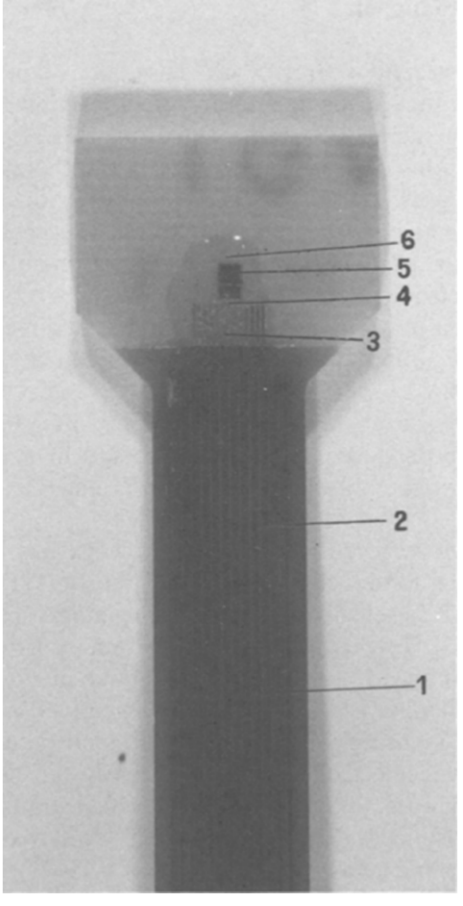

Figure 1
The ISFET device assembly and its electrical connections on the plastic stick: (1) plastic stick; (2) connection wires; (3) covering made of epoxy resin; (4) electrical connections obtained by an ultrasonic wire-bounder; (5) polymeric ion-selective membrane.

cetylammoniumcholate as exchanger in 3 ml of tetrahydrofuran. The solvent was allowed to partially evaporate in order to obtain a sufficiently viscous suspension. A drop of this suspension (about 40 μl) was deposited on the ISFET gates area, taking care to avoid air bubble formation, and left to dry at room temperature for 24 h.

Measurements procedures. The measurements using ISFET devices were carried out in steady-state conditions; the ISFET-containing plastic stick and a saturated calomel reference electrode, both connected to a electrical circuit and recorder, were immersed in standard aqueous solutions of cholanic acid sodium salts, or in authentic matrices (i.e. aqueous solution containing the solubilized drug, appropriately diluted). The various solutions to be analysed were stirred magnetically and kept at 25°C. Standard solutions of cholanic acid sodium salts were obtained by dissolving weighed amounts of each cholanic acid sodium salt in distilled water adjusted to pH 9 with sodium hydroxide; alternatively the drug analysis was also performed using the standard addition method.

The calibration graphs of the cholates in aqueous solution were obtained by means of subsequent additions of small volumes of standard solutions of the analyte to increase the concentration, starting from an initial volume of 50 ml of distilled water at pH 9 and recording the signal variation on the measurement apparatus.

The ISFET measurement equipment, supplied by CPC Elettronica s.r.l. (Rome, Italy), operated at constant applied drain voltage conditions in feedback mode: the source-drain current (I_D) and the drain potential (V_D) were maintained constant at about 100 μA and 1.5 V, respectively, using an operational amplifier in a feedback loop, as described in ref. 8. The operational amplifier adjusts the voltage in order to keep a constant current between the drain and the source. The readout of the gate output voltage (V_g) was then obtained directly in mV on a suitable display of the measurement apparatus.

The calibration graphs and the selectivity coefficients were calculated using a Hewlett–Packard HP 86 personal computer and software developed by the authors [9], based on the classical algorithm relative to the 'mixed solution' method [10].

The determination of the critical micellar concentration (CMC) of the various cholanic acids was performed by graphical analysis of the voltage vs log*c* curves as illustrated in Fig. 2 and described in a previous paper [11].

Results

The first observation is that the response of the ISFET device developed by us, stored without any special precautions, remained almost stable over a period of more than 2 months of discontinuous (roughly daily) usage.

The complete characterization of the device response in aqueous solutions for four different cholanic acids is shown in Table 2 and that for a further two conjugated cholanic acids in Table 3.

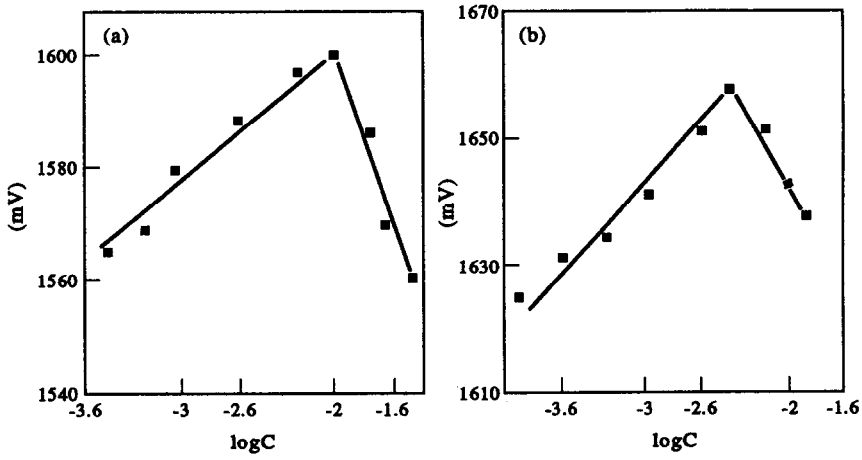

Figure 2
Graphical determination of critical micellar concentration (CMC) values: (a) for sodium cholate; (b) for sodium deoxycholate, by the ISFET device.

Table 2
Sensor characterization in standard solutions of cholanic acids sodium salts

Cholanic acid sodium salt	Response time (s)	Linearity range (M)	Regression line* (y in V; x in M)	Correlation coefficient (r)	Minimum detection limit (M)	Repeatability of the measurements (as pooled SD %)
Cholic	20–30	$(2.5 \times 10^{-5}$–$9.2 \times 10^{-3})$	$y = 0.038(\pm0.001)\log x + 1.76(\pm0.01)$	0.995	1.0×10^{-5}	7.3
Deoxycholic	20–30	$(1.2 \times 10^{-5}$–$4.3 \times 10^{-3})$	$y = 0.027(\pm0.001)\log x + 1.61(\pm0.01)$	0.983	1.0×10^{-5}	5.2
Chenodeoxycholic	20–30	$(1.0 \times 10^{-5}$–$2.0 \times 10^{-3})$	$y = 0.034(\pm0.003)\log x + 1.33(\pm0.04)$	0.989	1.0×10^{-5}	15.9
Ursodeoxycholic	20–30	$(7.5 \times 10^{-6}$–$1.0 \times 10^{-3})$	$y = 0.021(\pm0.001)\log x + 1.29(\pm0.02)$	0.994	7.5×10^{-6}	12.4

*Nine samples were analysed in order to determine each regression line and linearity range. SD values in parentheses; $n = 3$.

Values of slopes reported in the table are those found after sensor stabilization, while, immediately after sensor preparation, slope values are: for cholic acid, for deoxycholic acid, for chenodeoxycholic acid and for ursodeoxycholic acid sodium salt 0.062; 0.059; 0.050 and 0.023 ($\Delta V/\Delta\log c$), respectively.

Table 3
Sensor characterization in standard solutions of conjugated cholanic acids sodium salts

Cholanic acid sodium salt	Response time (s)	Linearity range (M)	Regression line* (y in V; x in M)	Correlation coefficient (r)	Minimum detection limit (M)	Repeatability of the measurements (as pooled SD %)
Glycocholic	20–30	$(1.25 \times 10^{-5}$–$9.1 \times 10^{-3})$	$y = 0.020(\pm0.003)\log x + 1.59(\pm0.00)$	0.992	1.25×10^{-5}	14.4
Taurodeoxycholic	20–30	$(1.25 \times 10^{-5}$–$2.0 \times 10^{-3})$	$y = 0.029(\pm0.002)\log x + 1.61(\pm0.02)$	0.993	1.25×10^{-5}	8.1

*Nine samples were analysed in order to determine each regression line and linearity range. SD values in parentheses; $n = 3$.

Values of slopes reported in the table are those found after sensor stabilization, while, immediately after sensor preparation, slope values were: for glycocholic acid and for taurodeoxycholic acid sodium salts: 0.042 and 0.038 ($\Delta V/\Delta\log c$), respectively.

The response time, linearity range, minimum detection limit, repeatability and accuracy of the measurements in standard aqueous solutions of different cholates, the slope and correlation coefficient values of the calibration graphs in the linearity range are reported in each table.

The selectivity coefficient K_{ij} values, (i.e. those which appear in the Nikol'skii equation [10]) of some common inorganic or organic anions, obtained by the 'mixed solutions' method [10, 12] with respect to cholate as primary ion, are shown in Table 4.

Tables 5 and 6 give the results obtained for the analysis of authentic matrices (antilithogenic commercial drugs) together with a comparison of the nominal values supplied by manufacturing firms and with those obtained, under the same experimental conditions, by liquid or polymeric ISE for cholanic acid analysis, as described in a previous paper [4]; in addition, repeatability of measurement and accuracy data (by standard addition method) for drug analysis are also illustrated.

In Table 7, the critical micellar concentration (CMC) values of three of the most common non-conjugated cholanic acids found by the ISFET developed in the research reported herein, are compared with data previously obtained by classical polymeric ISE [11] as well as with data obtained from surface tension measurements [13].

Lastly, in Table 8 the main analytical data obtained in the analysis of cholic acid by ISFET and by classical polymeric ISE [4], or solid state polymeric membrane ISEs, experimentally developed by us using the same assemblies as described in previous papers [5, 7], are compared.

Discussion

The experimental results displayed in Tables 2 and 3 show that, immediately after sensor preparation, ISFET shows quasi-Nernstian slope values for cholic, deoxycholic, chenodeoxycholic acids and under-Nernstian values for ursodeoxycholic, glycocholic, taurodeoxycholic, while after the ISFET response was stabilized (generally after being used to obtain the first two or three calibration curves), the slope values for all these cholanic acids in any case became under-Nernstian. Response time was fast, linear concentration range was about 2–3 decades or wider, and lifetime, precision and accuracy were satisfactory. If the results are compared with those obtained with the classical polymeric ISE investigated in a previous paper [4] overall marked differences are found for the slopes and linearity ranges and selectivity coefficient values, i.e. slopes and selectivity are better for the ISE, while the linearity range is better and the minimum detection limit lower for the ISFET. On the other hand, data given in Table 8 show that, for instance, in the case of cholic acid, comparing data obtained by ISFET with those from other solid state polymeric membrane ISEs, ISFET performance is seen to be better; in fact linearity range is wider by at least one decade, sensitivity (as slope value) higher and minimum detection limit narrower. On the other hand, when comparing the same data with those from classical polymeric ISE (with inner reference solution) only the sensitivity value is clearly better for the latter sensor.

A wider linearity range, also at higher concentrations, allows the use of the ISFET for the determination of the CMC of cholanic acids, which requires measurements of these compounds up to concentrations of 0.01 M.

CMC values determined by ISFET, listed in Table 7, are in good agreement with those obtained by polymeric ISE or by the surface tension method. Finally, the results of analysis of antilithogenic commercial drugs, shown in Tables 5 and 6, confirm that this sensor can be used for the quality control of matrices of commercial interest such as pharmaceutical preparations, showing precision and accuracy of the same order or better than using classical liquid or polymeric ISEs.

Table 4
Selectivity coefficients, determined by the 'mixed solutions' method [12]. Cholic acid sodium salt as primary ion (i)

Interferent ion (j)	Concentration of the interferent ion (i) (M)	Selectivity coefficient K_{ij}
Acetate	0.01	0.323
Benzoate	0.01	0.492
Nitrate	0.01	0.373
Chloride	0.01	0.313
OH^-	0.0001	5.78

Conclusions

The new sensor device can be used at lower or higher cholate concentrations than classical polymeric ISE, it is easy to handle and store

Table 5
Repeatability and recovery (by the standard addition method) of chenodeoxycholic and ursodeoxycholic acid in commercial pharmaceutical preparations by the ISFET sensor

Drug no.	Cholanic acid contained	Nominal value (%)	Found mean* % value by ISFET sensor	Cholanic acid found† (μM)	Cholanic acid added (μM)	Total cholanic acid found (μM)	Recovery (%)
2	Chenodeoxycholic	50.0	51.0 (1.7)	250.0	125.0	360.4	96.1
				250.0	250.0	484.0	96.8
				250.0	375.0	608.1	97.3
3	Ursodeoxycholic	69.4	67.9 (2.6)	45.7	22.9	70.7	103.1
				45.7	45.9	96.2	105.0
				45.7	68.6	108.8	95.2

*Samples were appropriately diluted before measurement. RSD % values in parentheses; $n = 4$.
†Final values found after appropriate dilution of the sample before each measurement.

Table 6
Comparison of found results, using the ISFET sensor, by nominal values and by values obtained using classical ISE for cheno and ursodeoxycholic acid analysis, in commercial pharmaceutical preparations. Found values are the mean of at least four determinations; values in parentheses are the percentage standard deviations

Drug no.	Cholanic acid	Nominal value contained (a)	Value found by ISFET (b)	Value found by ISE (c)	$\frac{b-a}{a}$ %	$\frac{c-a}{a}$ %
1	cheno-	71.4	70.8 (3.7)	74.8* (1.3)	−0.8	+4.8
2	cheno-	50.0	51.0 (1.7)	51.0† (7.1)	+2.0	+2.0
3	urso-	69.4	67.9 (2.6)	75.0† (6.6)	−2.2	+8.1
4	urso-	83.3	86.5 (1.2)	88.5* (0.8)	+3.8	+6.2
5	urso-	56.6	54.9 (4.8)	57.4† (7.0)	−3.0	+1.4

* Value found by polymeric membrane ISE [4].
† Value found by liquid membrane ISE [2, 3].

Table 7
Critical micellar concentration (CMC) for the most important cholanic acids sodium salts

Cholanic acid sodium salt	CMC for the ISFET sensor (mM)	CMC by the ISE sensor [11] (mM)	CMC by surface tension method [13] (mM)
Cholate	10.8	11.1	11.0
Deoxycholate	4.3	4.2	3.0
Chenodeoxycholate	4.5	4.7	4.0

Table 8
Analytical comparison of different solid state sensors (ISFET and ISEs) with polymeric classical ISE for cholic acid sodium salt analysis

Electrode assembly	Linearity range (M)	Slope ($\Delta V/\Delta \log c$) (c = M)	Minimum detection limit (M)	Response time (s)
ISFET with PVC membrane containing BDMCACh	2.5×10^{-5}–9.2×10^{-3}	0.0377	1.0×10^{-5}	20–30
ISE with graphite rod and PVC membrane [5] containing BDMCACh	2.5×10^{-4}–4.4×10^{-3}	−0.0240	1.0×10^{-4}	30
ISE with graphite rod and PVC–PVA–PVAc membrane* [7] containing BDMCACh	2.5×10^{-4}–4.4×10^{-3}	−0.0319	1.0×10^{-4}	30
ISE with inner reference solution and PVC membrane [4] containing BDMCACh	8.0×10^{-5}–5.3×10^{-3}	−0.0562	5.0×10^{-5}	10–15

*PVC = Poly(vinylchloride); PVA = Poly(vinylalcohol); PVAc = Poly(vinylacetate).

and has the typical advantage common to all sensors of this kind, i.e. it can be miniaturized and used as part of an array with other sensors; moreover, in practice, neither the measurement nor the experimental apparatus present any more difficulties than those encountered in common potentiometric measurements and even the cost of the equipment is comparable to that of a good potentiometer. In addition the authors believe that the ISFET obtained represents one of the few examples of anionic responsive ISFET developed so far, and is a clear advance in the development of a solid state sensor able to determine cholates and their CMC values.

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