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Flow injection analysis of cholic acids in pharmaceutical preparations using a polymeric membrane ISE as detector

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Abstract

The results reported in this paper regard the setting up of a polymeric membrane ISE that is selective for cholic acids (CA) and able to work in a flow system, especially in flow injection analysis (FIA), based on the exchanger (tetrakisdecylammoniumcholate, TDACh), which has proved effective, is of very simple but suitable structure and is above all easy to synthetise starting from commercially available chemicals. A complete analytical characterisation of the sensor was performed working both in batch conditions and in FIA, using in the latter case a 'wall jet' type of flow cell. The response toward different bile acid sodium salts such as the CA, deoxycholic (DCA), chenodeoxycholic (CDCA), ursodeoxycholic (UDCA), taurocholic (TCA) sodium salts was checked. The application to the analysis of different commercial drugs by FIA was also performed to determine the UDCA or CDCA acid content of several pharmaceutical formulations. Lastly, a preliminary study is presented concerning the use of the investigated electrochemical sensor as high performance liquid chromatography (HPLC) detector.

Keywords: FIA; ISE; Bile acids; Drug; Analysis

1. Introduction

The importance of bile acids is well known in gastroenterology [1]. Moreover, some bile acids are also employed in the treatment of gallstone diseases and are thus contained in several commercial pharmaceutical formulations [2,3]. For a number of years the research group has been engaged, albeit not continuously, in developing electrochemical devices suitable for the analysis of bile acids [2–5] as bile acids are known to be important in the pharmaceutical and biomedical fields.

Nevertheless, so far the most commonly used methods for bile acid analysis have been chromatographic methods [6–8].

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The high performance liquid chromatography (HPLC) determination of bile acids [6,9] in biological fluids or drugs can be performed using different detectors: although electrochemical (voltammetric) detectors have been proposed, generally fluorescence or UV detection is used [10,11]. However, some problems remain to be solved: bile acids absorb weakly at about 200 nm, where it is possible to work only after oxygen removal; otherwise a derivatisation must be performed.

In this paper a potentiometric detector for flow injection analysis (FIA) analysis of bile acid sodium salts based on a classic polymeric membrane electrode using tetrakisdecylammoniumcholate (TDACh) as exchanger is proposed.

The electrode response toward different bile acids such as cholic (CA), deoxycholic (DCA), chenodeoxycholic (CDCA), ursodeoxycholic (UDCA), taurocholic (TCA), taurodeoxycholic (TDCA) and glycocholic (GCA) acids was tested both in batch conditions and by FIA.

CDCA, or UDCA acid content of some commercial drugs was also determined by FIA, while preliminary data concerning the possibility of using the potentiometric probe as HPLC detector are presented.

2. Experimental

2.1. Chemicals

All bile acid sodium salts were supplied by Sigma (St. Louis, MO). Polyvinylchloride (PVC, high molecular mass), and bis(2-ethylhexyl)sebacate (BEHS) were purchased from Fluka, Buchs, Switzerland. The dibutylphthalate (DBP) was supplied by Merck, Darmstadt, Germany. The tetrakisdecylammonium bromide (TDABr), employed to prepare the new exchanger (TDACh) was supplied by Aldrich (Milwaukee, WI). All other reagents were of analytical reagent grade, supplied by Carlo Erba, Milan, Italy and used without further purification.

2.2. Apparatus and measurements

Batch potentiometric measurements were carried out using a digital pH meter (Orion mod. 720, Orion, MA), an Ag/AgCl reference electrode and a recorder, both supplied by Amel Instrument, Milan, Italy. The temperature of the test solution was kept constant at 25 ± 1°C during the experiments by using a thermostatted water jacket.

2.3. Preparation of the exchanger

The new exchanger, TDACh, was prepared following a procedure similar to that described in previous papers [2,3] for other exchangers: 0.5 g of TDABr was dissolved in 50 ml of chloroform; the organic phase was partitioned with 80 ml of an aqueous solution of sodium cholate (0.01 M, pH 8), and the two phases were then separated. This procedure was repeated several times, until no bromide ions were found in the aqueous phase. The organic phase was washed three times with distilled water. After the solvent of the organic phase was evaporated at room temperature, the residue consisted of TDACh.

2.4. Electrode assembly

The sensitive PVC based polymeric membrane was obtained as follows: 50 mg of the TDACh exchanger, 0.81 g of plasticiser (DBP) and 0.38 g of PVC were solubilised under stirring in 15 ml of tetrahydrofuran (THF). The solution obtained was then poured into a Petri dish (i.d. 48 mm); after solvent evaporation, a membrane (0.3 mm thick) was obtained. A disk (10 mm diameter) was then cut from the membrane and glued on one end of a PVC tube using a PVC solution in THF (6% w/w). An Ag/AgCl wire was used as inner reference electrode and a solution 0.01 M of sodium cholate and 0.01 M of potassium chloride as inner solution (Fig. 1).

2.5. FIA apparatus

The apparatus for FIA measurements is also shown in Fig. 1. The indicating electrode was

forced (0.3 cm deep) into a perspex cylindrical flow cell (2.0 cm high, 1.5 cm i.d.); one teflon capillary (0.6 in. o.d.; 0.007 in. i.d.) was inserted from the opposite side of the cell until it approached the surface of the electrode membrane; the other end was joined (stainless junction 1/16 in.) to a stainless steel tube (0.66 in. o.d.; 0.02 in. i.d.) ending in a valve (Rheodine, Cotati, CA) equipped with a 20 μl loop through which the mobile phase (sodium cholate 10^{-6} M, pH 8.0) was forced to circulate by a peristaltic pump (Bio-Rad, Hercules, CA). The mobile phase left the cell through three holes in the cell wall. The cell and the external reference electrode (Ag/AgCl) were dipped into a 500 ml beaker containing the same phase maintained at a constant level by a second peristaltic pump (Minipuls 3, Gilson, WI).

For HPLC measurements the first pump and the steel tube were respectively substituted by a pump for HPLC (Jasco PU-980, Tokio, Japan) and a LC-18 column (Supelcosil, Supelco, Bellefonte, PA).

Batch and flow measurements were performed using a mod. EA 940 Expandable Ion Analyser (Orion, MA) and a mod. 868 Amel recorder.

2.6. Pharmaceutical formulations tested

All the samples were common commercial drugs in the form of capsules or tablets. The content of the capsules or the fine ground tablets was homogenised, accurately weighed and dissolved in aqueous solution at pH 8.0. After filtration through a G4 Gooch and suitable dilution, the samples were ready for the potentiometric FIA analysis.

The sample compositions, as declared by the manufacturers, are reported in Table 1.

3. Results

As in previous research, it was necessary to select the plasticiser to be used in the polymeric membrane preparation. To this end DBP and BEHS were tested, as in previous work they had proved to be the most suitable for the construction of ISEs for CAs [3,5].

Table 2 compares the main data referring to the calibration graphs obtained for the CA by ISEs constructed with membranes containing respectively one of the two different plasticisers. Best

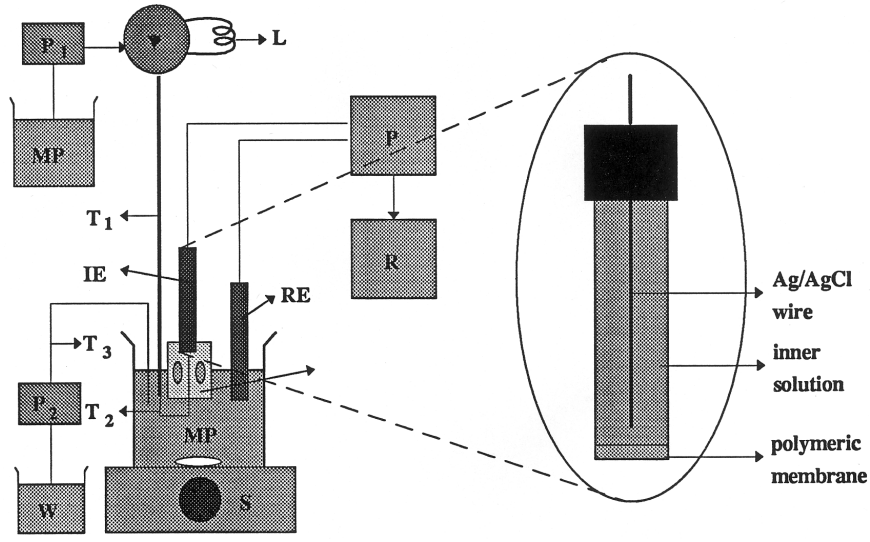

Fig. 1. Experimental apparatus for flow injection analysis (FIA) and ISE assembly. (P1) feed pump, (P2) escape pump, (M) mobile phase, (V) injection valve, (L) loop, (W) waste, (T1) stainless tube, (T2) teflon tube, (T3) waste tube, (C) flow cell, (RE) reference electrode, (IE) indicating electrode, (P) potentiometer, (R) recorder.

Table 1
Composition of the analysed drugs, containing ursodeoxycholic acid (UDCA) or chenodeoxycholic acid (CDCA) as active principle

Drug no.	Drug composition	(mg)
1	Ursodeoxycholic acid	50
	Starch	7.25
	Gel	0.6
	Mg stearate	0.5
2	Ursodeoxycholic acid	150
	Lactose	99.9
	PVP	10.1
	Mg stearate	2.9
	Colloidal silica	2.1
3	Ursodeoxycholic acid	125
	Talc	47.0
	Carboxymetilic starch	5.9
	Gelatine	1.0
4	Chenodeoxycholic acid	100
	Starch	37.3
	Aerosill	2.0
5	Mg stearate	0.6
	Chenodeoxycholic acid	50
	Lactose	38.4
	Starch	2.0
	Talc	2.0
	Sodiumglycolate starch	6.0
	Gelatine	0.8
	Mg stearate	0.8
6	Chenodeoxycholic acid	150
	PVP	3.8
	Gelatine	1.1
	Mg stearate	0.8

results were obtained using DBP with respect to BEHS: a quasi Nernstian slope, linear range about one and half decades wider, better correlation coefficient and overall the minimum of the linear range is about one decade lower; DBP was thus used in all the following tests aimed at studying the ISE's response to the main bile acids.

3.1. Results in batch conditions

Table 3 shows results concerning main analytical data obtained working in batch conditions for the bile acid sodium salts considered. As can be seen, a very quick response (< 8 s) was obtained together with good precision and quasi Nernstian slope values in the case of CA, CDCA and UDCA. In the case of DCA and TCA, the slope values were about ten percent lower. The slope values are actually quite similar, proving that the sensitivity of the electrode is almost the same for all the bile acids. The linear range was about 3 decades for CA and at least 2 decades for the other cholanic acids; for all the bile acids, the limit of quantitation (LOQ) was 10^{-5} M, except for CA (10^{-4} M).

Selectivity coefficients [12,13] were calculated for the most common organic and inorganic anions and listed in Table 4. The only real interferent was found to be the phthalate ion, while OH^- can interfere only at relatively high concentrations. No interference was found from citrate, phosphate and borate, which means that no interference is due to the buffers commonly used in HPLC analysis.

3.2. Results in FIA

After the characterisation of the ISE in batch conditions, the possibility of using it as a detector for FIA was evaluated.

The response of the ISE as a function of the pH was tested using the CA sodium salt (Fig. 2); quasi Nernstian values were obtained working at pH 7.0–9.2 while at pH 6.3 a sudden decrease (more than 56%) was observed; this is probably due to the fact that this pH value approaches the

Table 2
Characterisation of the response of the potentiometric ISE with the membrane containing one of the two different plasticisers (dibutylphthalate, DBP, or bis(2-ethyl-hexyl)sebacate, BEHS), to sodium cholate standard solutions (pH 7.8, $T = 25$°C)[a]

Plasticiser	Linear range (M)	Slope ($\Delta mV/\Delta \log C$) $\pm$ S.D.	Correlation coefficient
DBP	2.0×10^{-5}–1.8×10^{-2}	-58.9 ± 0.5	-0.9994
BEHS	1.0×10^{-4}–1.5×10^{-2}	-54.2 ± 1.8	-0.9920

[a] Exchanger: tetrakisdecyilammoniumcholate (TDACh).

Table 3
Response of the proposed ISE to different bile acids in batch conditions[a]

Acid	Response (s)	Linear range (M)	Slope ($\Delta mV/\Delta \log C$) ± S.D.	Correlation coefficient
Cholic	≤8	2.0×10^{-5}–1.8×10^{-2}	-57.5 ± 0.9	−0.9991
Deoxycholic	≤8	5.2×10^{-5}–5.6×10^{-3}	-52.1 ± 0.7	−0.9982
Chenodeoxycholic	≤8	1.6×10^{-5}–8.5×10^{-3}	-59.2 ± 1.5	−0.9984
Ursodeoxycholic	≤8	1.6×10^{-5}–3.1×10^{-3}	-57.5 ± 1.5	−0.9978
Taurocholic	≤8	1.0×10^{-4}–2.0×10^{-2}	-51.5 ± 1.9	−0.9854

[a] Experimental conditions: 10^{-2} M sodium cholate and 10^{-2} M potassium chloride as inner solution of ISE; $T=25$°C.

pK_a value (about 6) [14]. This problem does not occur in the case of the conjugate acids as their pK_a values are lower, and so their complete dissociation is ensured also at pH 6.

The response of the ISE to CA was also evaluated as a function of the mobile phase flow rate; results are illustrated in Fig. 3; no strong variation of the response was found at different flow rates; in any case, the higher sensitivity (expressed as slope of the calibration curve) and the nearest Nernstian value were obtained at a flow rate of 4 ml min^{-1}; this value was then chosen for all the subsequent tests.

Fig. 4 shows typical FIA peaks for increasing concentrations of CA. The good reproducibility of the peaks throughout all the linear range must be emphasised.

Table 5 shows main data for different bile acids analysis obtained by FIA. The linear ranges are generally found to be comparable to those obtained in batch conditions. However, a small shift at higher concentrations is evidenced for CA and CDCA, while a broader interval (3 decades) is found for UDCA. With a view to using the sensor as HPLC detector, its response to different CA was also tested using FIA, although in the presence of a mobile phase commonly used in HPLC, i.e. phosphate buffer containing 15% by volume of methanol; a comparison of the results obtained for CA and CDCA, both with and without the methanol in the mobile phase, shows no significant differences (Table 5).

Six different pharmaceutical formulations containing urso- or CDCA were analysed using FIA. For this purpose, the content of three capsules or tablets was accurately weighed and solubilised in aqueous solution at pH 8.0; then, after centrifugation and filtration, FIA was performed. Results are shown in Table 6.

There is good agreement between experimental and nominal values as well as satisfactory precision (S.D. generally lower than ± 2% by weight).

3.3. Preliminary results as detector for HPLC

In order to evaluate the possibility of using the ISE as detector for HPLC, the effect of the most common solvents (i.e. CH_3CN, CH_3OH) on the FIA analysis was tested.

First of all, no real damage was observed when the polymeric membrane of the ISE was left in contact with CH_3CN for about 3 h; in any case, the slow detachment of the membrane from the electrode body was evidenced when CH_3CN was present in the mobile phase; this problem must be solved by using a different glue (new experiments

Table 4
Selectivity coefficients (K_{ij}) of the polymeric membrane electrode for different cations and anions[a]

Interferent ion	Background level	K_{ij}
SO_4^{2-}	1.0×10^{-2}	1.0×10^{-4}
$H_2PO_4^-$	2.5×10^{-2}	1.0×10^{-4}
HPO_4^{2-}	1.5×10^{-2}	1.0×10^{-4}
NO_3^-	1.0×10^{-2}	2.0×10^{-2}
Cl^-	1.0×10^{-2}	1.5×10^{-2}
OH^-	1.0×10^{-2}	1.0×10^{-1}
Benzoate	1.0×10^{-3}	3.0×10^{-3}
Acetate	1.0×10^{-2}	1.0×10^{-3}
Citrate	1.0×10^{-2}	1.4×10^{-3}
Borate	1.0×10^{-2}	1.0×10^{-3}
Oxalate	$1.0\times\cdot10^{-2}$	1.0×10^{-3}
Phthalate	1.0×10^{-2}	11

[a] *i*, cholate; *j*, interferent ion.

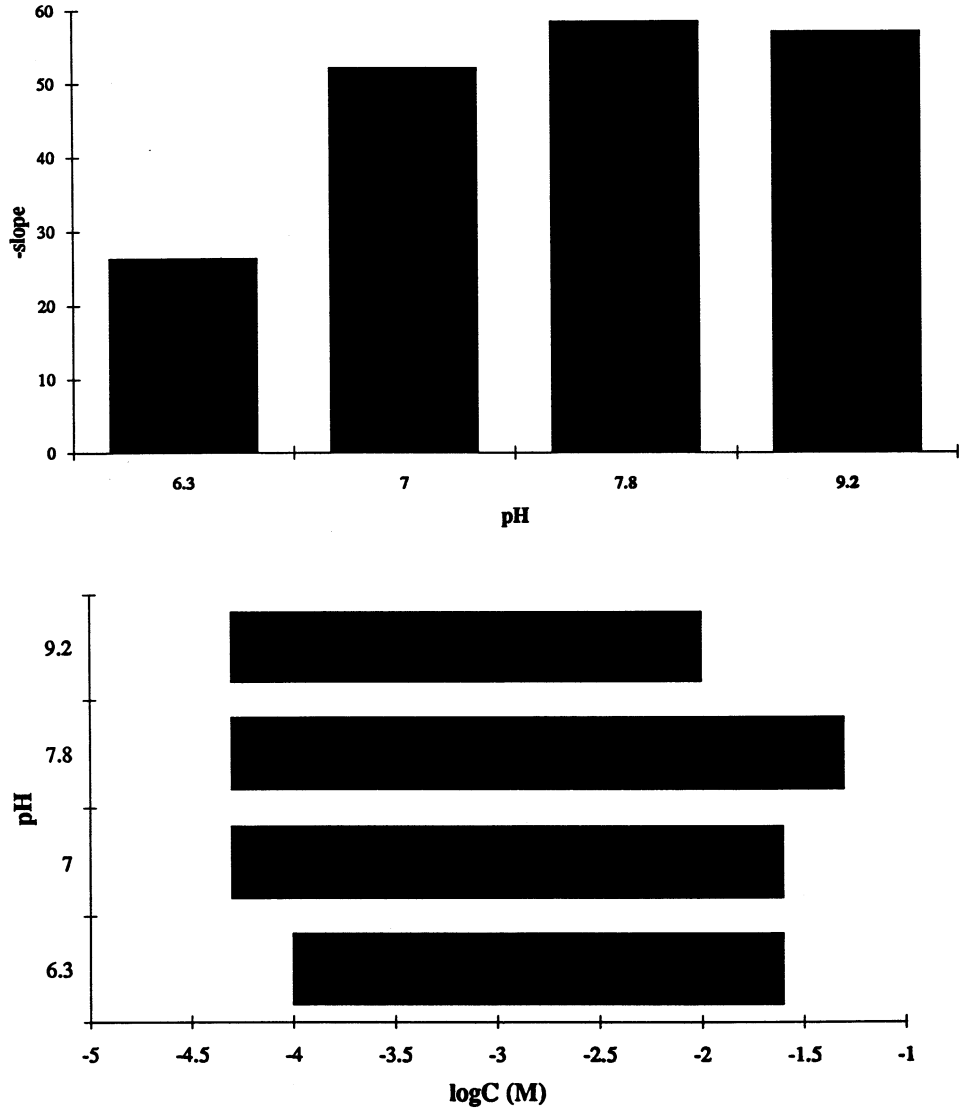

Fig. 2. Trend of the slope and of the linear range, for the cholic acid (CA) calibration curve, as a function of the pH.

are still in progress). No similar problem arises from the addition of methanol to the mobile phase for at least 3 months. Fig. 5 shows the response of the ISE in FIA at different percentages (0–50%) by volume of methanol in the mobile phase; the maximum decrease of the slope of the calibration curve was no higher than 10% with respect to the aqueous phase, while the linear ranges were not significantly different. The main results obtained by FIA analysis for the different bile acids in the presence of 15% methanol in the mobile phase are also reported in Table 5.

Lastly, Fig. 6 shows the repeatability of the peaks obtained for CA, CDCA, TDCA and GDCA eluted one by one through a Supelcosil LC-18 chromatographic column.

In the experimental conditions used (reverse phase chromatography with isocratic elution), all the bile acids were eluted with the same retention time (about 4 min); a good symmetry the same

half height width of the peak is observed for all the peaks; of course their separation requires an elution using a pH and a mobile phase gradient. At present, considerable but not insurmountable problems have to be solved in order to use the ISE as detector for HPLC.

In Table 7 the low detection limits (LOD) obtained using the ISE proposed herein as detector are reported; a comparison with values reported in literature for non derivatised bile acids, using UV detection in HPLC [10,11,15] is also made. LOD values obtained by ISE detection are of about the same order of magnitude or a decade higher than those reported in literature using UV detection. Of course, the results are only preliminary and so leave much room for improvement.

4. Discussion

On the basis of the results contained in the present note, the FIA system for CA determination under investigation for some time now in the laboratory may be said to be sufficiently mature

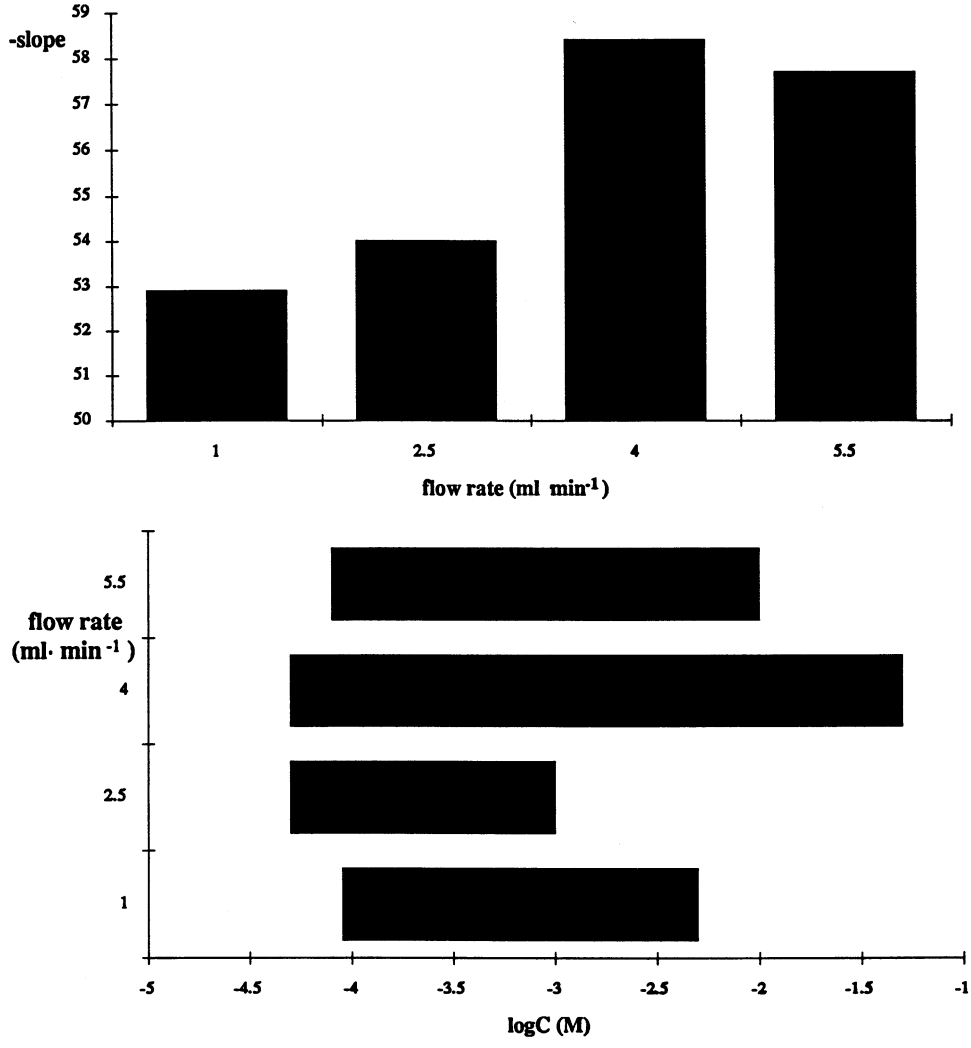

Fig. 3. Trend of the slope and of the linear range, for the cholic acid (CA) calibration curve, as a function of the flow rate.

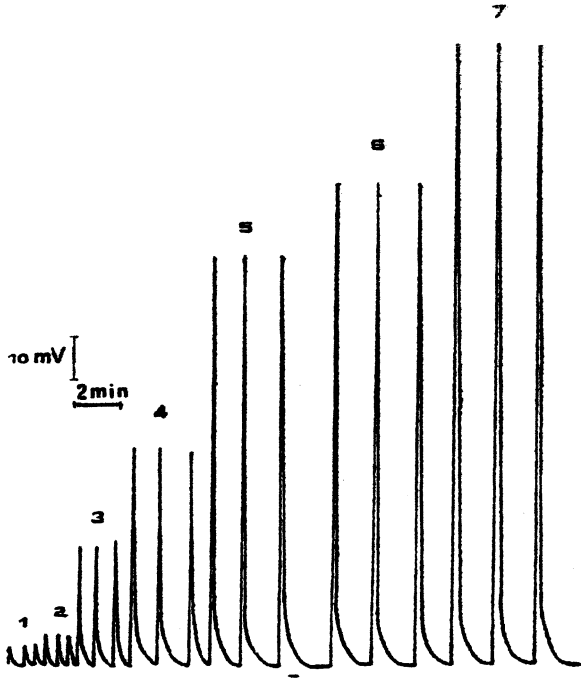

Fig. 4. Reproducibility of peaks obtained by flow injection analysis (FIA), increasing the concentrations of cholic acid (CA): (1) 5.0×10^{-5} M, (2) 1.0×10^{-4} M, (3) 5.0×10^{-4} M, (4) 1.0×10^{-3} M, (5) 5.0×10^{-3} M, (6) 1.0×10^{-2} M, (7) 2.5×10^{-2} M.

also for routine use. This represents significant progress in the research which has been carried out in recent years in this sector [2–5], in which analysis was essentially possible in batch or flow conditions but not in flow injection. To this end, the use of the wall jet cell seems to have solved many of the problems encountered since one began developing a FIA system. Also the new exchanger proposed, although significantly more efficient than that proposed in previous papers [2–5], is very simple to synthetise starting from commercially available chemicals and does not need any particular further purification before being used. Also the selectivity of the ISE proposed here is undoubtedly effective versus the majority of inorganic and organic anions (except phthalate), including those deriving from the first terms of the fatty acid series. Lastly, the low detection limits shown in Table 7 are certainly effective, although, as it was already stated, it is believed they can be further improved.

As regards the robustness of the FIA method, the data in Figs. 2 and 3 indicate for example that even significant variations in important working conditions, such as pH or flow rate, while certainly affect system response, do not however compromise the system functionment rendering sensor response critical.

The possible use of the system as detector in HPLC apparatus is of course a different matter. The preliminary results indicate that only by operating in the specific conditions described (reverse phase with isocratic elution) is it possible to record sufficiently symmetrical and repeatable chromatographic peaks for all the CA. However, an essential part of the research clearly remains to be developed before the system can actually be proposed as HPLC detector. In other words, a suitable mobile phase must be found which, when coupled to a suitable stationary phase, will allow proper separation of the various CA and, at the

Table 5
Response of the proposed ISE to some bile acids in flow injection analysis (FIA)[a]

Bile acid	Linear range (M)	Slope ($\Delta mV/\Delta \log C$) ± S.D.	Correlation coefficient
Cholic	5.0×10^{-5}–5.0×10^{-2}	-58.4 ± 1.5	-0.9985
Chenodeoxycholic	8.0×10^{-5}–1.0×10^{-3}	-60.6 ± 2.1	-0.9976
Ursodeoxycholic	5.0×10^{-5}–2.5×10^{-2}	-59.2 ± 1.7	-0.9991
Cholic[b]	5.0×10^{-5}–5.0×10^{-2}	-58.1 ± 1.7	-0.9943
Chenodeoxycholic[b]	5.0×10^{-5}–5.0×10^{-2}	-58.2 ± 1.2	-0.9928
Glicodeoxycholic[b]	5.0×10^{-5}–5.0×10^{-2}	-59.6 ± 1.9	-0.9998
Taurodeoxycholic[b]	5.0×10^{-5}–5.0×10^{-2}	-59.1 ± 1.2	-0.9991

[a] Mobile phase: phosphate buffer (5.0×10^{-3} M), pH 8.0.
[b] Mobile phase: phosphate buffer (5.0×10^{-3} M) + 15% methanol, pH 7.5).

Table 6
Flow injection analysis (FIA) determination of the urso- (UDCA) or chenodeoxycholic acid (CDCA) content of some antilitogenic drugs

Drug no. and cholanic acid contained	Nominal value (% w/w) (a)	Found value ± S.D. (% w/w) (b)	$(b-a)/a$ (%)
1 UDCA	85.7	85.1 ± 0.9	−0.7
2 UDCA	56.6	54.4 ± 1.5	−3.9
3 UDCA	69.9	67.1 ± 2.9	−4.0
4 CDCA	71.5	70.8 ± 1.7	−1.0
5 CDCA	50.0	49.3 ± 1.1	−0.7
6 CDCA	96.3	95.7 ± 0.6	−1.4

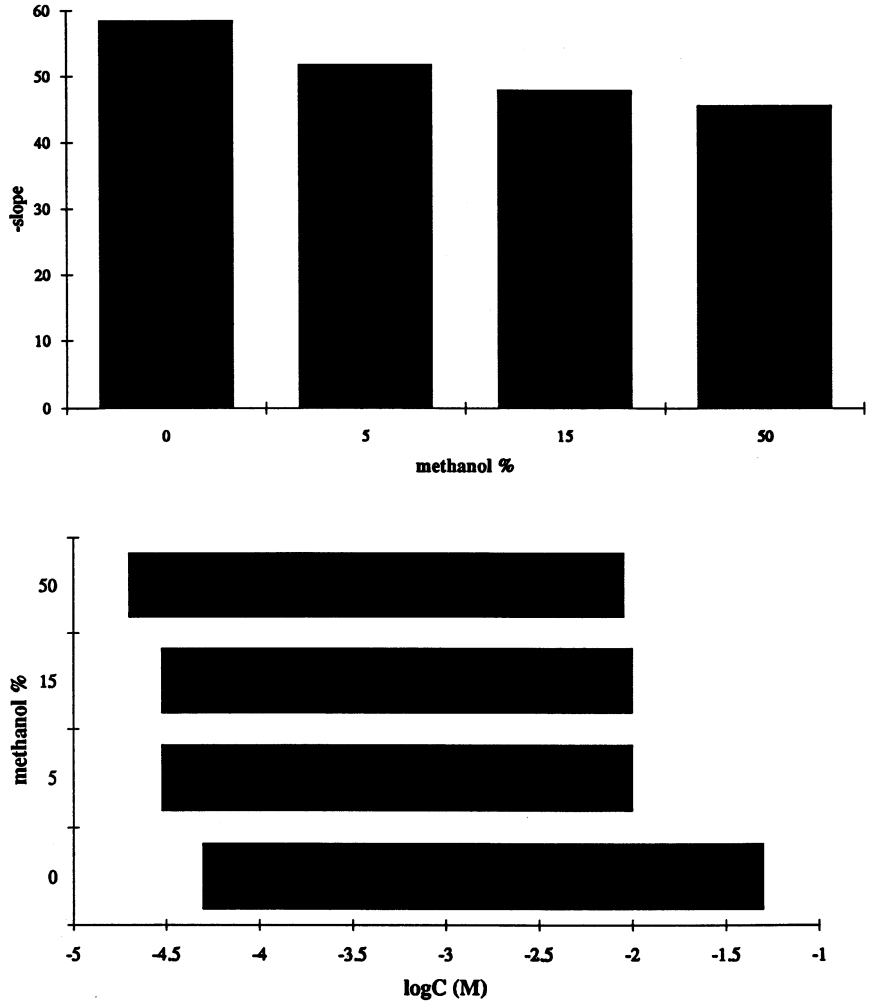

Fig. 5. Trend of the slope and of the linear range, for the cholic acid (CA) calibration curve, as a function of the methanol% in the mobile phase (sodium cholate 10^{-6} M + phosphate buffer 5 mM, pH 7.8).

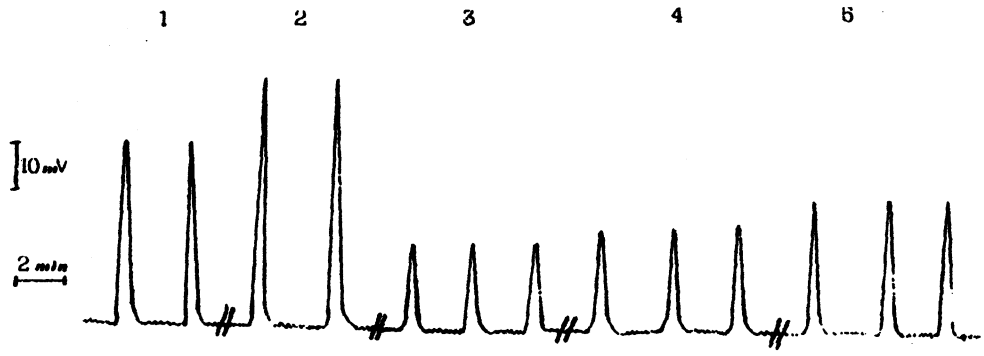

Fig. 6. Peaks obtained by high performance liquid chromatography (HPLC) for replicate injections of 20 μl standard solutions of: (1) deoxycholic acid (DCA), (2) cholic acid (CA), (3) taurodeoxycholic acid (TDCA), (4) taurocholic acid (TCA), (5) chenodeoxycholic acid (CDCA).

Table 7
Comparison of the low detection limit values (LOD), for different bile acids, obtained using the ISE herein proposed as detector, with those reported in literature, using UV detection

Acid	ISE as detector[a] HPLC (μg)	UV detector HPLC (μg)		
	[This paper]	[11]	[15]	[10]
CA	1.6	1.8	–	0.5
CDCA	2.9	1.3	–	0.5
UDCA	–	–	–	0.5
TDCA	3.3	–	0.2	0.15
GCA	3.1	–	0.2	0.10

[a] Using a 20 μl loop.

same time, will enable the sensor to produce a satisfactory response over a sufficiently long period of time without the polymeric membrane of the ISE being gradually deteriorated by the constant contact with the flow of solvent mixture used as eluent in the HPLC system.

Obviously these problems are purely technological in nature, although their solution does not appear to be neither easy nor rapid, as demonstrated by the work that has been carried out by the group for a considerable time now.

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