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Lidocaine and benzalkonium analysis and titration in drugs using new ISFET devices[1]

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Abstract

Two new ISFETs recently developed by us have now been applied to some pharmaceutical determinations in real matrices; the first device, responsive to cationic surfactants, was employed in the determination of benzalkonium chloride contained in two different disinfectant solutions and in three types of commercial collyrium; the second device, responsive to cocaine hydrochloride, showed an appreciable response also to lidocaine hydrochloride and was used in the determination of lidocaine hydrochloride contained in some injectable antibiotics. The repeatability and accuracy of measurements performed in the analysis of these pharmaceutical matrices using new solid state sensors were evaluated. A further aspect of the research involved the use of two sensors to record complete titration curves for the determination of benzalkonium chloride, cocaine hydrochloride and lidocaine hydrochloride, respectively. Applications to real matrices were also performed by analysing by titration pharmaceutical formulations containing benzalkonium chloride, or lidocaine hydrochloride and an illicit powder containing cocaine hydrochloride and sugars.

Keywords: ISFETs; Analysis; Titrations; Benzalkonium; Lidocaine; Cocaine

1. Introduction

In the last few years many ISEs sensitive to organic cations that play an important role in pharmaceutical applications have been reported in the literature [1–3].

We recently carried out basic research to develop new ISEs and ISFETs sensitive to cationic surfactants [4,5]. The best results were obtained using a polymeric membrane (PVC and sebacate) ISFET with dodecyltrimethyl-ammonium reineckate (DDTMAR) as exchanger [5].

Further basic research performed by us in recent months has led to the development of polymeric membrane ISFETs for cocaine analysis [6]; the best results were obtained using cocaine reineckate as exchanger in the PVC-sebacate membrane [7].

These new ISFETs were fully characterised from the electrochemical point of view [5,6], and were found to be more efficient than the corre-

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sponding classical ISEs, and also cheap and simple to assemble; in addition, they displayed good selectivity and short response time (≤ 30 and ≤ 25 s, respectively).

During this research it was also observed that the ISFET sensitive to cationic surfactants showed a near Nernstian response to benzalkonium chloride, a general antiseptic, frequently used in pharmaceutical solutions, while the ISFET responsive to cocaine hydrochloride showed an under Nernstian, but still significant, response to lidocaine hydrochloride, a local anaesthetic employed in several pharmaceutical preparations.

The two new ISFETs were thus employed in several determinations in real matrices of pharmaceutical interest, the first to determine benzalkonium chloride in two different disinfectant solutions and in three types of commercial collyrium; the second, also responsive to lidocaine, was used to determine lidocaine hydrochloride in some injectable pharmaceutical preparations in which an antibiotic sodium salt was used as 'active agent'. Repeatability and accuracy of the measurements performed in the analysis of these pharmaceutical matrices using new solid state sensors were also evaluated. An interesting new application of the two new sensors was to record complete titration curves for the determination of benzalkonium chloride, cocaine hydrochloride, and lidocaine hydrochloride, both in standard solution and in pharmaceutical matrices, using as titrant, sodium dodecylsulfate and sodium tetraphenylborate, respectively.

2. Experimental

2.1. Materials and apparatus

High molecular weight poly(vinylchloride) (PVC), bis(2-ethylhexyl)sebacate and Jeffamine D-230 were from Fluka, AG, Buchs, Switzerland; benzalkonium chloride, lidocaine hydrochloride, sodium tetraphenylborate and ammonium reineckate were from Sigma, St. Louis, MO; dodecyltrimethyl-ammonium bromide (DDTMABr) from Aldrich-Chemie, EPON 825 from Shell (USA), tetrahydrofuran and all other solvents or reagents were of analytical reagent grade and obtained from Carlo Erba, Milan, Italy. The cocaine hydrochloride standard was obtained from S.A.L.A.R.S., Como (Italy), with the authorization of the Italian Ministry of Health.

The instrumentation used for the ISFET measurements was supplied by 'CPL Elettronica s.r.l.', Rome, Italy. A saturated calomel electrode was used as external reference electrode.

2.2. Samples

Samples of pharmaceutical interest, determined using the two ISFET devices, were all commercial formulations. Lidocaine was determined in formulations of injectable antibiotics and was sealed in phials, while benzalkonium chloride was contained in sealed plastic or small glass bottles of disinfectant solutions for external use or was one of constituents of collyrium solutions for ophthalmic use.

The illicit cocaine powder (samples from the illegal market analysed for legal purpose) was obtained from the 'Istituto di Medicina Legale', University 'La Sapienza', Rome (Italy) which also supplied the composition and the nominal cocaine content of the powder.

2.3. Methods

2.3.1. Exchanger preparation and characterization

The cocaine reineckate (COCR) and dodecyltrimethylammonium reineckate (DDTMAR) exchangers were prepared, in the first case [6], by mixing 10 ml of 10^{-1} M cocaine hydrochloride solution and 10 ml of 10^{-2} M of ammonium reineckate and, in the second case [4], by mixing 100 ml of 5×10^{-3} M dodecyltrimethylammonium bromide and 100 ml of 5×10^{-3} M of ammonium reineckate in aqueous solution.

In both cases, the precipitate was filtered off, washed with distilled water and dried at room temperature. Characterization and elemental analysis data [4,6] of synthesized compounds resulted in good agreement with the stoichiometry of the described compounds when used as exchangers.

These salts were used as exchangers in PVC membranes stratified over the area of the ISFET gate.

Table 1
(A) Composition of the examined drugs containing lidocaine hydrochloride and (B) composition of the examined drugs containing benzalkonium chloride

Drug	Pharmaceutical forms	Components	Content[a] (as % w/w)
(A)			
1	Phials	Lidocaine hydrochloride	2.0
		Distilled water	98.0
2	Phials	Lidocaine hydrochloride	3.7
		Cefotaxime sodium salt	96.3
3	Phials	Lidocaine hydrochloride	1.8
		Imipenem monohydrate	48.4
		Cilastatine sodium salt	48.5
		Sodium chloride	1.3
4	Phials	Lidocaine hydrochloride	1.0
		Sulbactam sodium salt	33.6
		Ampicillin sodium salt	65.4
(B)			
1	Solution (disinfectant)	Benzalkonium chloride	3.5
		Essential oils (lemon, bergamot, thyme)	0.5
		Glycerol	10.0
		Nonylphenol oxyethylate	5.0
		Ethylene glycol	0.5
		depurated water	80.5
2	Solution (disinfectant)	Benzalkonium chloride	0.175
		Lemon essence	0.05
		Thyme essence	0.025
		Ethyl alcohol	4.0
		Nonoxinol 30	1.5
		Tetrasodium EDTA	0.03
		E102 (tartrazine),	0.00075
		E131(blue patent)	0.00004
		Depurated water	94.22
3	Solution (collyrium)	Benzalkonium chloride	0.01
		Thymolol maleate	0.68
		Sodium phosphate monobasic	0.54
		Sodium phosphate bibasic	1.21
		Water for injectable solution	97.56
4	Solution (collyrium)	Benzalkonium chloride	0.01
		Naphazoline nitrate	0.013
		Sodium chloride	0.6
		Potassium phosphate monobasic	0.3
		Hamamelis virginiana	0.2
		Ethyl alcohol	0.01
		Camphor	0.004
		Water for injectable solution	98.86
5	Solution (collyrium)	Benzalkonium chloride	0.01
		Tetrizoline chloridrate	0.05
		Boric acid	1.42
		Sodium tetraborate	0.05
		Sodium chloride	0.25
		Methylene bleu	0.003
		Water for injectable solution	98.22

[a] Nominal values given by the manufacturers.

Table 2
ISFET characterisation in standard solution of (a) lidocaine hydrochloride and (b) in standard solution of benzalkonium chloride

	(a)	(b)
Response time	$\leq$25 s	$\leq$30 s
Equation of the calibration graph	$y = -25.1\ (\pm 0.8)\ \log C + 250.0$ (± 4.9) (y = mV; C = M)	$y = -52.2\ (\pm 0.4)\ \log C + 944.9$ (± 8.7) (y = mV;C = M)
Correlation coefficient	-0.9974	-0.9981
Linearity range	$(2.5 \times 10^{-5} \div 9.6 \times 10^{-3})$ M	$(2.5 \times 10^{-6} \div 2.6 \times 10^{-3})$ M
Minimum detection limit	8.0×10^{-6} M	8.3×10^{-7} M
Inaccuracy	$(-5.1 \div +4.0)$%	$(-2.8 \div +5.4)$%
Repeatability of measurements in the linearity range (as 'pooled' standard deviation %)	6.0%	2.0%

(a) COCR used as exchanger (pH = 5; T = 25°C).
(b) DDTMAR used as exchanger (pH = 6; T = 25°C; KCl 10^{-2} M).

2.3.2. FET device assembly

The integrated chips (UUO3 type) were supplied by HEDCO Laboratory of Utah University; each chip (overall dimension 1.28×2.16 mm) contained two 400×20 μm gates and two metal gate control devices. The chips were accurately washed with isopropyl alcohol and then mounted on plastic sticks subsequently connected to the electrical measurement system [5]. After making the electrical connections with an ultrasonic wire-bonder (Kulicke and Soffa, model 4123; Switzerland), the devices were encapsulated in an epoxy resin (EPON 825 + Jeffamine D-230) body, leaving only a two gate area free.

2.3.3. ISFET preparation

The polymeric selective membrane of the ISFET for lidocaine and cocaine analysis was prepared by stirring a suspension of 113 mg of polyvinylchloride (PVC) as base polymer, 279 mg of bis(2-ethylhexyl)-sebacate as plasticizer, and 8 mg (2% by weight) of the cocaine reineckate exchanger (COCR) in 3 ml of tetrahydrofuran for $\sim$5 h.

The polymeric ion selective membrane of the ISFET for benzalkonium analysis was prepared by stirring a suspension consisting of 30 mg of polyvinylchloride (PVC) as base polymer, 66 mg of bis(2-ethylhexyl)sebacate as plasticizer, and 4 mg (4% by weight) of the dodecyltrimethylammonium reineckate (DDTMAR) exchanger in 0.5 ml of tetrahydrofuran for 5 h.

In both the cases the solvent was allowed to evaporate partially in order to obtain a sufficiently viscous suspension. A drop of this suspension ($\sim$40 μl) was deposited on the ISFET gate area, taking care to avoid air bubble formation, and left to dry at room temperature for 24 h.

2.3.4. ISFET measurement procedure

The ISFET measurements were carried out using a saturated calomel electrode as external reference electrode and equipment operating 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 $\sim$100 μA and 1.5 V, respectively, using an operational amplifier in a feedback loop, as described in reference [8] and the gate output voltage (V_g) was then directly displayed in mV by the measurement apparatus.

For lidocaine hydrochloride or benzalkonium chloride measurements the ISFET device, together with the reference electrode (saturated calomel), was immersed in 30 ml of 0.05 M acetate buffer solutions at pH 5, or in 10^{-2} M KCl, pH 6, respectively, contained in a thermostated cell, kept at 25°C, under magnetic stirring. The signal was allowed to stabilise for $\sim$10 min, after which fixed volumes of standard solutions of benzalkonium chloride or lidocaine hydrochloride were successively added to 30 ml of the initial solution and, after $\sim$60 s, the gate output voltage variation of the ISFET was recorded.

Table 3
(A) Lidocaine hydrochloride determination

Drug n. and pharmaceutical form	Nominal value contained (as percent by weight) (a)	Value found by ISFET* (as percent by weight) (b)	$\frac{b-a}{a}\%$
1 (phials)	2.00	1.98 (3.5)	−1.0
2 (phials)	3.70	3.80 (5.6)	+2.7
3 (phials)	1.80	1.86 (3.7)	+3.3
4 (phials)	1.00	1.02 (5.6)	+2.0

* RSD% in brackets. Each found value is the mean of at least five determinations.
COCR used as exchanger.
(B) Recovery (by the standard addition method) of lidocaine hydrochloride, in commercial pharmaceutical preparations by ISFET device

Drug n.	Lidocaine hydrochloride found* (Values as μg ml^{-1}). (RSD% $\leq$4.0). ($n>5$)	Lidocaine hydrochloride added (Values as μg ml^{-1}).	Total lidocaine hydrochloride found* (Values as μg ml^{-1}). (RSD% $\leq$4.0). ($n>5$)	Recovery%
1	49.5	49.8	98.7	99.4
	49.5	115.7	167.0	101.1
	49.5	213.8	273.5	103.9
2	53.3	49.6	105.2	102.2
	53.3	115.4	179.0	106.1
	53.3	213.3	283.1	103.1
3	53.3	49.6	107.1	104.1
	53.3	115.4	176.2	104.4
	53.3	213.3	274.5	103.0
4	53.3	49.4	105.2	102.4
	53.3	114.8	173.7	103.3
	53.3	212.2	270.5	101.9

* Final found values of the sample, after proper dilution, before each measurement.

All calibration graphs for benzalkonium chloride and lidocaine hydrochloride were obtained by plotting the gate output voltage variation (as Δ mV) versus the benzalkonium chloride or lidocaine hydrochloride final concentration values, respectively.

As far as titration of standard solutions of cocaine, lidocaine or benzalkonium was concerned, the ISFET device using COCR or DDT-MAR as exchanger, together with the saturated calomel electrode, was immersed in 20 ml of cocaine hydrochloride standard solution, or in 20 ml of lidocaine hydrochloride standard solution, buffered with acetate 10^{-3} M, pH 5, or in 20 ml of a benzalkonium chloride standard solution, 10^{-2} M in KCl, pH 6, respectively, using sodium tetraphenylborate as titrant in the first two cases and sodium dodecylsulfate in the latter case. The output voltage variation was recorded after each addition.

Titration of cocaine contained in an illicit powder and lidocaine and benzalkonium in pharmaceutical formulations was carried out in practically the same way (see also data in Table 5).

Table 4
(A) Benzalkonium chloride determination

Drug n. and pharmaceutical use	Nominal value contained (as percent by weight). (a)	Value found by ISFET (as percent by weight). (RSD% in brackets[a]) (b)	$\frac{b-a}{a}\%$
1 (Disinfectant)	3.50	4.02 (10.0)	+14.8
2 (Disinfectant)	$1.75\times10-^{1}$	1.76×10^{-1}(5.8)	+0.6
3 (Collyrium)	1.00×10^{-2}	1.16×10^{-2} (6.1)	+16.0
4 (Collyrium)	1.00×10^{-2}	1.04×10^{-2} (3.8)	+4.0
5 (Collyrium)	1.00×10^{-2}	0.95×10^{-2} (6.9)	−5.0

[a] Each found value is the mean of at least five determinations.
DDTMAR used as exchanger.
(B) Recovery (by the standard addition method) in commercial pharmaceutical preparations by the ISFET device

Drug n.	Benzalkonium chloride found*; (Values as μg ml^{-1}). (RSD%<8.0) ($n>5$)	Benzalkonium chloride added (Values as μg ml^{-1})	Total benzalkonium chloride found*; (Values as μg ml^{-1}). (RSD%<9.0) ($n>5$)	Recovery %
1	98.6	54.4	156.4	102.2
	98.6	163.2	268.6	102.6
	98.6	380.8	482.8	100.7
2	105.4	27.2	136.0	102.6
	105.4	81.6	183.6	98.2
	105.4	187.0	275.4	94.2
4	27.2	23.8	47.6	93.4
	27.2	71.4	98.6	100.0
	27.2	170.0	207.4	105.6

* Final found values of the sample, after proper dilution, before each measurement.

3. Results and discussion

All the examined drugs and their percent composition as declared by the manufacturers are shown in Table 1. The data required for a complete electroanalytical characterisation of the two kinds of ISFET devices using COCR or DDTMAR as exchangers in standard solution of lidocaine hydrochloride and benzalkonium chloride, respectively, are summarised in Table 2: the response time, slope, linearity range, minimum detection limit, repeatability, accuracy of the measurements, reproducibility of the slope and correlation coefficient values of the calibration graphs, in the linearity range, are shown in this table, while the characterization of the ISFET device using COCR as exchanger in cocaine hydrochloride standard solution was reported in a previous paper [6].

The selectivity coefficient values, K_{ij} (i.e. those that appear in the Nikol'skii equation [9]), of the most common organic interferents and inorganic cations obtained by the 'mixed solutions' method [9,10], were reported in previous papers for both the polymeric membrane ISFETs [4–7]. With reference to the long-term stability of the two sensors, the response of both kinds of ISFET device, which were developed by us and stored without any special precautions, may be said to have remained practically constant over a period of at least 2 months or more of discontinuous (roughly daily) usage. After characterization of the sensors, we employed the ISFET device using COCR as exchanger for the analysis of lidocaine in pharma-

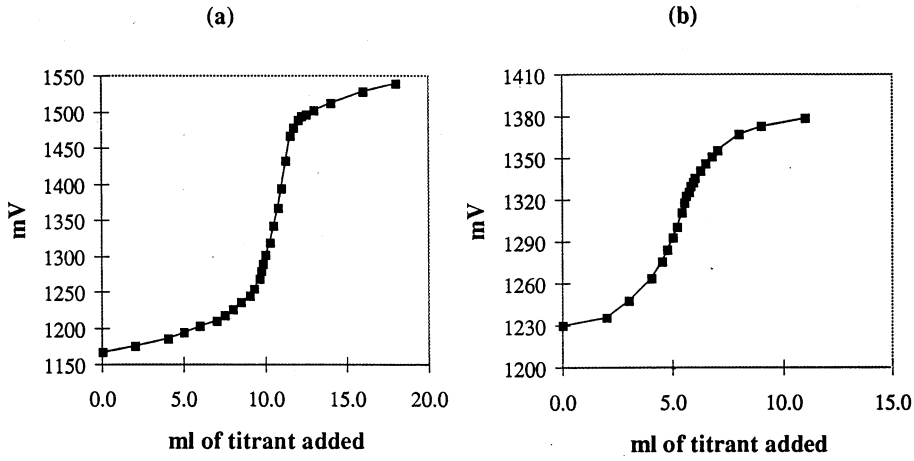

Fig. 1. Titration curves of benzalkonium chloride in standard solution (a) and in pharmaceutical formulation (b) by the ISFET using DDTMAR as exchanger.

ceutical real samples (phials) also in the presence of other active substances such as antibiotic sodium salts. The results obtained for the samples examined so far, by the classical method of analysis (i.e. using a calibration graph obtained as described in Section 2.3.4), are shown in Table 3(A). Besides, several pharmaceutical forms used as disinfectant solutions or collyria, were analysed in the same manner for their benzalkonium chloride content using the ISFET containing DDTMAR as exchanger; in this case the data obtained are summarised in Table 4(A). Also precision data (as RSD%) are reported in the last two tables, while data referring to the accuracy of measurements in pharmaceutical samples, evaluated using the standard addition method, are set out in Table 3(B), Table 4(B), respectively. The wide variance in the analytical data of recoveries sometimes observed, for instance in the case of drug n. 4 in Table 4(B), must be attributed to the very small percentage of the checked analyte contained in the drug (see Table 1).

Results were satisfactory from the analytical point of view and of considerable interest in the testing of pharmaceutical formulations. This may be considered strong evidence of the suitability of these sensors for rapidly and accurately checking industrial pharmaceutical products containing lidocaine or benzalkonium.

Using the ISFET device containing DDTMAR as exchanger, titrations were also carried out using standard solutions and real samples: standard solutions of benzalkonium chloride were titrated several times and this surfactant was determined also by titration in pharmaceutical formulations (see titration curves in Fig. 1 and some titration data in Table 5A). Employing the ISFET containing COCR as exchanger, several titrations of lidocaine hydrochloride standard solutions and pharmaceutical formulations containing lidocaine were carried out (see results in Table 5B). Lastly, using the same ISFET, titration curves were repeated several times for standard solutions of cocaine hydrochloride and a good repeatability was found for the titrations (see data reported in Table 5C). Titrations of real matrices using the latter ISFET were also performed, involving the analysis of an illicit powder containing cocaine hydrochloride and sugars (see analytical data in Table 5C).

In the applications using ISFETs for the titration of standard solution or pharmaceutical formulations, the precision of measurements (as RSD%) was ~ 0.5–4.0%; the response time of the sensors < 25 s, the drift was no higher than 1 $mV \cdot h^{-1}$ and the lifetime of ISFET devices exceeded 2–3 months.

Finally, comparing the data in Table 3(A), Table 4(A) and those for cocaine hydrochloride reported in previous papers [5,7] with those shown

Table 5
(A) Titration of benzalkonium chloride by the ISFET using DDTMAR as exchanger; titrant used was sodium dodecylsulfate; (B) titration of lidocaine hydrochloride by the ISFET using COCR as exchanger; titrant used was sodium tetraphenylborate; and (C) titration of cocaine hydrochloride by the ISFET using COCR as exchanger; titrant used was sodium tetraphenylborate

	Nominal concentration [M] (a)	Titrant concentration [M]	Found concentration [M] (b)	RSD (%)	$\frac{b-a}{a}\%$
(A)					
Standard	1.17×10^{-2}	2.26×10^{-2}	1.16×10^{-2}	1.0	−0.85
Pharmaceutical formulation	5.0×10^{-3}	2.26×10^{-2}	5.1×10^{-3}	4.0	+2.0
(B)					
Standard	1.00×10^{-2}	2.10×10^{-2}	0.95×10^{-2}	1.4	−5.0
Phials	1.20×10^{-2}	2.00×10^{-2}	1.25×10^{-2}	2.5	+4.1
(C)					
Standard	1.00×10^{-2}	4.00×10^{-2}	0.97×10^{-2}	1.5	−3.0
Illicit powder (glucose 25%, mannitol 25%)	1.00×10^{-2}	2.26×10^{-2}	0.98×10^{-2}	0.5	−2.0

in Table 5(A–C), it may be said that the ISFET sensors prepared by us generally give satisfactory results both in classical analysis involving direct potentiometry (i.e. using a previously constructed calibration curve) and when the analysis is based on potentiometric titration curves obtained by using the ISFET sensors proposed in the present work. The accuracy obtained in the titration analysis results is comparable with that found in classical analysis in the case of cocaine hydrochloride and benzalkonium chloride (the agreement with nominal values was found to be between +2.0 and −3.0%). As far as lidocaine hydrochloride is concerned, the results obtained via titration appear to be slightly less accurate (Table 5B) than those obtained using the classical method (see data in Table 3A), although equally precise.

4. Conclusions

In conclusion, if the ISFETs proposed herein are compared with the small number of ISEs described in the literature [11–15], there may be said to be small differences in some cases with regard to sensitivity or the selectivity, although the sensors proposed herein seem to offer numerous advantages in terms of response time, minimum detection limit, duration and above all the concrete possibility of being used to analyse real samples both by classical direct methods or using titration methods.

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