Preparation of nalbuphine hydrochloride potentiometric sensors and their applications in pharmaceutical formulations and biological fluids

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ABSTRACT

The construction and performance characteristics of nalbuphine hydrochloride selective electrodes were developed. Three types of electrodes: plastic membrane I, coated wire II, and coated graphite rod III were constructed based on the incorporation of nalbuphine hydrochloride with phosphotungestic acid. The influences of membrane composition, kind of plasticizer, pH of the test solution, soaking time, and foreign ions on the electrodes were investigated. The electrodes showed a Nernstain response with a mean calibration graph slope of 57.29 ± 0.7, 56.16 ± 0.2 and 55.82 ± 0.9 mV decade^{-1} at 25ºC for electrode I, II and III respectively, over nalbuphine hydrochloride concentration range from 1×10^{-2}-1×10^{-6}M, 1×10^{-2}-5×10^{-6}M and 5×10^{-3}-5×10^{-6}M, and with detection limit 4.9×10^{-7} M, 2.4×10^{-6}M and 2.5×10^{-6}M for electrode I, II and III respectively. The constructed electrodes gave average selective precise and usable within the pH range 3.5-7.4. Interferences from common cations, alkaloids, sugars, amino acids and drug excipients were reported. The results obtained by the proposed electrodes were also applied successfully to the determination of the drug in pharmaceutical preparations and biological fluids.   © 2010 Trade Science Inc. - INDIA

INTRODUCTION

Nalbuphine hydrochloride, 17-Cyclobutylmethyl-7, 8-dihydro-14-hydroxy-17 nor-morphine hydrochloride, (Figure 1). Nalbuphine is a synthetic opioid used commercially as an analgesic under a variety of trade names, including Nubain. It is noteworthy in part for the fact that at low dosages, it is found much more effective by women than by men, and may even increase pain in men. Nalbuphine is a synthetic narcotic agonist-antagonist analgesic of the phenanthrene series. It is chemically related to the widely used narcotic antago-

nist, naloxone, and the potent narcotic analgesic, oxymorphone. Nalbuphine is indicated for the relief of moderate to severe pain. It can also be used as a supplement to balanced anesthesia, for preoperative and
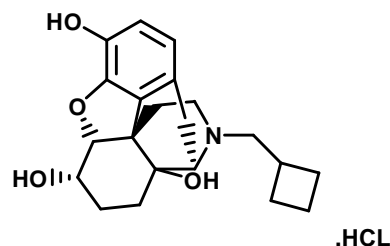

Figure 1 : Chemical structure of nalbuphine hydrochloride

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postoperative analgesia, and for obstetrical analgesia during labor and delivery[1].

Several methods have been reported for determination of nalbuphine hydrochloride, including high performance liquid chromatography[2-7], potentiometry[8]. This work describes new selective membrane sensors, of three types: plastic membrane, coated wire and coated graphite electrodes for the determination of doxepin hydrochloride in pure solutions, pharmaceutical preparations and biological fluids.

EXPERIMENTAL

Standard drug solution

Stock nalbuphine hydrochloride solution ($1x10^{-1}$M) was prepared daily by dissolving an appropriate amount of the drug in double distilled water. More dilute solutions were prepared by appropriate dilution.

Recommended procedures

Preparation of nalbuphine-phosphotungstate ion-pair

The ion-pair was prepared by mixing stoichiometric amounts of $1x10^{-2}$M phosphotungestic acid with an equimolar solution of nalbuphine hydrochloride, stirred for 10 min. The resulting white precipitate was filtered through G_4 sintered glass crucible and washed thoroughly with deionized water then dried at room temperature for 24 hours. The ion-pair should be stored in a desiccator.

Membrane composition

The membrane composition was studied by varying the percentages (w/w) of the ion pair, poly (vinyl chloride) PVC and plasticizer di-octyl phthalate (DOP), until the optimum composition that exhibits the best performance characteristics was obtained. The membranes were prepared by dissolving the required amount of the ion-pair, PVC and (DOP), in 5 mL tetrahydrofuran (THF). The solution mixture was poured into a petri dish (3 cm in diameter), covered with a filter paper and the solvent was allowed to evaporate slowly at room temperature. To obtain the uniform membrane thickness, the amount of (THF) was kept constant, and its evaporation was fixed for 24h.

Electrode construction

Plastic membrane electrode: A punched circular membrane was attached to a poly-ethylene tube (8 mm in diameter) in an electrode configuration by means of PVC-THF solution. A mixture containing equal volume of $1x10^{-3}$M nalbuphine hydrochloride and potassium chloride was used as internal reference solution in which the Ag/AgCl reference electrode was dipped. The constructed electrode was pre-conditioned after preparation by soaking for at least 8 h in $1x10^{-3}$M nalbuphine hydrochloride and stored in the same solution. All potentiometric measurements were performed using the following cell assembly: Ag/AgCl/Internal solution /membrane/test solution//KCl salt bridge//SCE.

Coated wire electrode: Pure aluminum wire of 4.0 cm length was tightly insulated by polyethylene tube leaving 1.0 cm at one end for the coating and 0.5 cm at the other end for connection. The coating solution was (described previously under membrane composition). Prior to coating, the polished aluminum surface was washed with a detergent and water, thoroughly rinsed with water, and dried with acetone. Then the wire was rinsed with chloroform and allowed to dry. Afterwards, the aluminum wire was coated by quickly dipping it into the coating solution several times, and allowing the film left on the wire to dry for about 3 min. The process was repeated several times until a plastic membrane of approximately 1.0 mm thickness was formed. The prepared electrode was conditioned by soaking for 6 h in $1.0x10^{-3}$ M nalbuphine hydrochloride solution. All potentiometric measurements were performed using the following cell assembly: Al/membrane/test solution//KCl salt bridge//SCE.

Coated graphite electrode: A pure graphite rod of 4 mm diameter was insulated by tight polyethylene tube, leaving 2 cm at one end for coating and 1 cm at the other end for connection. The polished electrode surface was coated with the active membrane by dipping the exposed end into the coating solution was (described previously under membrane composition) ten times and allowing the film left on graphite rod to dry in air for 1 min each time. The process was repeated until 1.0 mm thickness formed. The prepared electrode was preconditioned by soaking for 8 h in $1.0x10^{-3}$ M nalbuphine hydrochloride solution.

Electrode calibration

Ten mL aliquots of 1×10^{-1}-1×10^{-6}M standard nalbuphine hydrochloride solutions were transferred into 50 mL beaker and the sensor(s) in conjunction with double junction Ag/AgCl reference electrode were immersed in the solution. The measured potential was plotted against the logarithm of drug concentration. The electrode(s) was washed with deionized water and dried with tissue paper between measurements.

Determination of nalbuphine hydrochloride in dosage forms

Nalbuphine hydrochloride ampoules

The prepared solution was diluted with deionized water to give serial dilutions ranging from 5×10^{-3}-1×10^{-5}M for nalbuphine hydrochloride injections. These solutions were transferred into 50 mL beaker, adjusted to pH 5 using 0.1N hydrochloric acid. The nalbuphine-electrode(s) was immersed in the solution. The electrode(s) system was allowed to equilibrate with stirring and the e.m.f. recorded and compared the calibration graph.

Content uniformity assay of nalbuphine ampoules

Ten individual ampoules of 20 mg/mL ampoule nalbuphine hydrochloride were placed in separate 100 mL beakers and dissolved in 90-100 mL of distilled water. The electrode(s) was directly immersed into 100 mL of each sample for five times and should be washed with deionized water to reach steady potential between the individual measurements. The mean potential was used to evaluate the content uniformity from the calibration graph.

Application to serum and urine

Adjust urine pH to 5 (using 0.1N hydrochloric acid) and pH of serum to 6 (use phosphate buffer). Add hydrochloric acid to urine and phosphate buffer to serum drop wise until the suitable pH obtained. Transfer 5 mL previously adjusted urine or serum into small separatory funnels, and then separately add 5 mL, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}M standard drug solution, followed by the addition of 20 mL toluene for urine or 20 mL diethyl ether for serum. Shake each funnel for 5 min, and transfer aqueous layer to centrifuge tube. Centrifuge for 2 min at 1500 rpm, transfer each solution to a 50 mL volumetric flask, and dilute to volume with deionized water. Apply above procedure as described under electrode calibration[9].

RESULTS AND DISCUSSION

Optimization of membrane composition

It is known that sensitivity, selectivity and the lifetime of the ion-selective electrodes depend on the nature of ion-pair used as well as on the membrane composition and the properties of the plasticizer employed. In this study three membrane compositions were investigated, the results were summarized in TABLE 1. The results showed that the electrode(s) made by membrane of type (c) with 0.7 wt% nalbuphine-phosphotungstate ion pair, 33.0 wt% PVC and 66.0 wt% plasticizer DOP exhibits the best performance characteristics (slope 57.29 ± 0.7, 56.17 ± 0.2 and 55.82 ± 0.9 mV decade^{-1} at 25ºC for electrode I, II and III respectively, over nalbuphine concentration range from 1×10^{-2}-1×10^{-6}M,

TABLE 1 : Optimization of membrane composition (wt/wt %)

Type of Sensor	M	PVC wt%	DOP wt%	Ion-Pair wt%	Slope	RSD%	r	Linear Conc. Range
Plastic Membrane Electrode	(a)	38.0	60.0	2.0	54.73	1.1	0.9987	1.0×10^{-3}-1.0×10^{-5}
	(b)	35.5	64.0	0.5	56.48	1.2	0.9997	5.0×10^{-3}-1.0×10^{-6}
	(c)	33.0	66.0	1.0	57.29	0.7	0.9999	1.0×10^{-2}-1.0×10^{-6}
Coated Wire Electrode	(a)	38.0	60.0	2.0	52.94	0.8	0.9994	1.0×10^{-3}-1.0×10^{-5}
	(b)	35.5	64.0	0.5	55.68	0.7	0.9996	1.0×10^{-3}-9.0×10^{-5}
	(c)	33.0	66.0	1.0	56.17	0.2	0.9999	1.0×10^{-2}-5.0×10^{-6}
Coated Graphite Electrode	(a)	38.0	60.0	2.0	49.98	1.1	0.9989	1.0×10^{-4}-5.0×10^{-5}
	(b)	35.5	64.0	0.5	53.57	1.3	0.9995	1.0×10^{-3}-9.0×10^{-5}
	(c)	33.0	66.0	1.0	55.82	0.9	0.9999	5.0×10^{-3}-5.0×10^{-6}

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1×10^{-2}-5×10^{-6}M and 5×10^{-3}-5×10^{-6}M, for electrode I, II and III respectively.

Nature and response characteristics of the electrode(s)

Nalbuphine reacts with phosphotungestic acid to form a stable nalbuphine-phosphotungstate ion-pair complex which is water insoluble but readily soluble in an organic solvent such as tetrahydrofuran. The complex was prepared and tested as active material with di-octylphthalate DOP as a solvent mediator in a poly (vinyl chloride) membrane response for nalbuphine. The critical response characteristics of plastic membrane, coated wire, and coated graphite-electrodes were determined and results are summarized in TABLE 2. The electrode(s) exhibits a Nernstain response over the concentration range from 1×10^{-2}-1×10^{-6}M, 1×10^{-2}-5×10^{-6}M and 5×10^{-3}-5×10^{-6}M nalbuphine for electrode I, II and III respectively, with a cationic slope of 57.29 ± 0.7, 56.17 ± 0.2 and 55.82 ± 0.9 mV decade^{-1} change in concentration for electrode I, II and III respectively as in Figure 2. The choice of membrane sol-

TABLE 2 : Critical response characteristics of nalbuphine-PT sensors

Parameter[a]	Nalbuphine-PT plastic membrane electrode	Nalbuphine-PT coated wire electrode	Nalbuphine-PT coated graphite electrode
Slope (mV per decade)	57.29 ± 0.7	56.17 ± 0.2	55.82 ± 0.9
Intercept	587.46	530.52	510.46
Correlation coefficient r.	0.9999	0.9999	0.9999
Linear range (M)	1.0×10^{-2}-1.0×10^{-6}	1.0×10^{-2}-5.0×10^{-6}	5.0×10^{-3}-5.0×10^{-6}
Detection limit (M)	4.9×10^{-7}	2.4×10^{-6}	2.5×10^{-6}
Response time for 10^{-3}M (s)	20	?15	?40
Working pH range	3.5-7.4	3.5-7.4	3.5-7.4
Lifetime /day	30	35	27
Accuracy (%)	99.51	99.43	99.48
Standard deviation (%)	0.4	0.4	0.5
Repeatability (CV$_w$ %)	0.5	0.5	0.6
Between day variability (CV$_b$ %)	0.7	0.7	0.8
Robustness[b]	100.0 ± 0.1	99.88 ± 0.5	99.47 ± 0.5
Ruggedness[c]	99.98 ± 0.2	99.68 ± 0.2	99.21 ± 0.9

[a] **Mean of three measurements.**
[b] **A small variation in method parameters were studied as pH of buffer.**
[c]**Comparing the results by those obtained by different sensors assemblies using - Orion 420 A.**
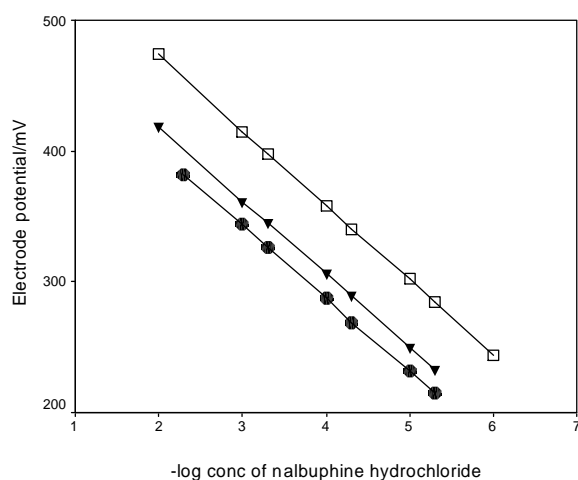

Figure 2 : Typical calibration graph of nalbuphine sensors: (□) nalbuphine- phosphotungstate-plastic membrane electrode, (▲) coated wire- nalbuphine-phosphotungstate electrode, (●) coated graphite nalbuphine- phosphotungstate electrode

vent to achieve the required selectivity is based on its electric permittivity and its immiscibility with aqueous phase, high viscosity, low solubility of the matrix in the membrane and ability to dissolve ion-pair complex.

Life time

The response time of the electrode(s) was tested for 1×10^{-1}-1×10^{-6}M nalbuphine solutions. The sequence of measurements was from low to high concentrations. The electrode(s) exhibits a fast dynamic response of 20, =15, and =40 for electrode I, II and II respectively. The electrode(s) used for a period of 30, 35 and 27 days for electrode I, II and III respectively, without significant change in the electrode(s) parameters.

Effect of plasticizer

In this study, three plasticizers, di-butyl sebacate

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(DBS), di-butylphthalate (DBP) and di-octylphthalate (DOP), were used to examine the optimization of the membrane with plasticizer entailed the use of plasticizer content ratio, 60.0, 64.0 and 66.0 wt%, and the use of PVC contents of 38.0, 35.5 and 33.0 wt%. The electroactive compound (nalbuphine-phosphotungstate) contents of 2.0, 0.5, 5.0 and 1.0 wt%. The results obtained showed that the response performances of the prepared membranes were rather different depending on the use of plasticizer, the proportion of the plasticizer toward PVC and of the electroactive compound. The typical potential responses of the electrodes constructed with three plasticizers were given in Figure 3. As shown in Figure 3, the DOP-PVC electrodes were superior to DBS- and DBP-PVC electrodes in both the response slope and linear concentration range. So DOP was selected as the plasticizer of the membranes. The best membrane composition of the DOP-PVC electrode(s) was 66.0 wt % PVC, 33.0 wt % DOP and 1.0 wt % ion-pair.
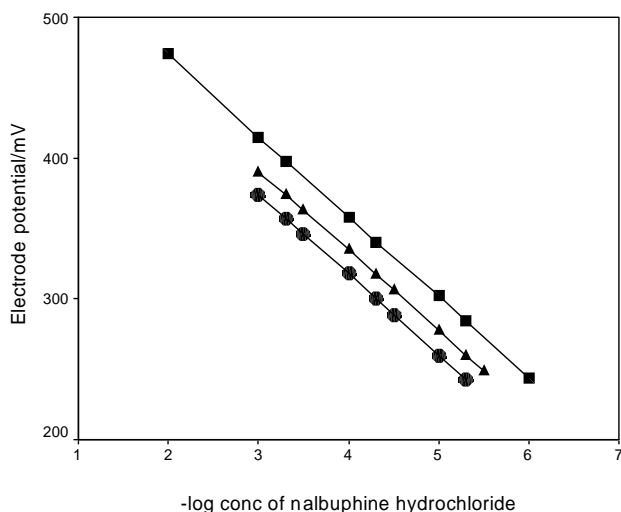

Figure 3 : Optimization of plasticizers. DBS (●) (PVC membrane composition: DBS 60.0 wt%, PVC 38.0 wt%, ion-pair, 2.0 wt %) DBP (▲) (PVC membrane composition: DBP 64.0 wt%, PVC 35.5 wt%, ion pair, 0.5 wt %) DOP (■) (PVC membrane Composition: (DOP 66.0 wt %, PVC 33.0wt%, ion-pair, 1.0 wt %)

Effect of soaking

The performance characteristics of nalbuphine-phosphotungstate electrode(s) was studied as a function of soaking time. For this purpose the electrode(s) was soaked in 1×10^{-3}M solution of nalbuphine hydrochloride and the calibration graphs were plotted after

8, 6, and 8 h. the optimum soaking time was found to be 8, 6 and 8 h at which the slope of the calibration curve was 57.29 ± 0.7, 56.17 ± 0.2 and 55.82 ± 0.9 mV decade^{-1}, at 25 ºC for electrode I, II and III respectively. The influence of prolonged soaking on the lifetime nalbuphine-phosphotungstate electrode(s) was followed by constructing calibration plots. The electrode(s) was soaked continuously on 1×10^{-3}M solution of nalbuphine hydrochloride for 24 h, 7, 10, 21, 27, 30 and 35 days. The calibration plot slopes decreased to 54.51, 53.57 and 50.42 mV decade^{-1} after 30, 35 and
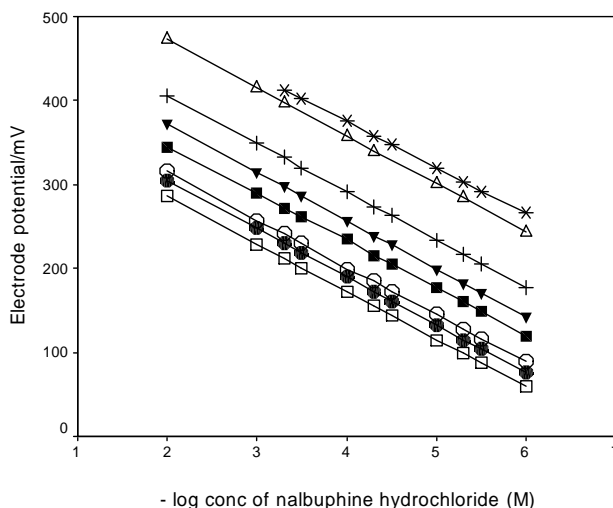

Figure 4 : Calibration graphs obtained at 25±1ºC after soaking the nalbuphine-phosphotungstate-PVC membrane electrode for (□) 24h, (●) 7 days, (○) 10 days, (■) 15 days, (▼) 21 days, (+) 27 days, (Δ) 30 days, (*) 35days
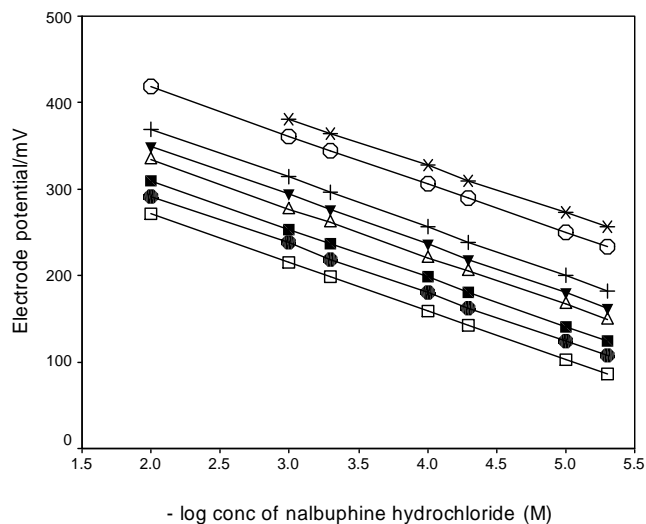

Figure 5 : Calibration graphs obtained at 25±1ºC after soaking the nalbuphine- phosphotungstate- coated wire electrode for (□) 24h, (●) 7 days (■)15 days, (Δ) 21 days, (▼) 27 days, (+) 30 days, (○) 30 days, (*) 40 days
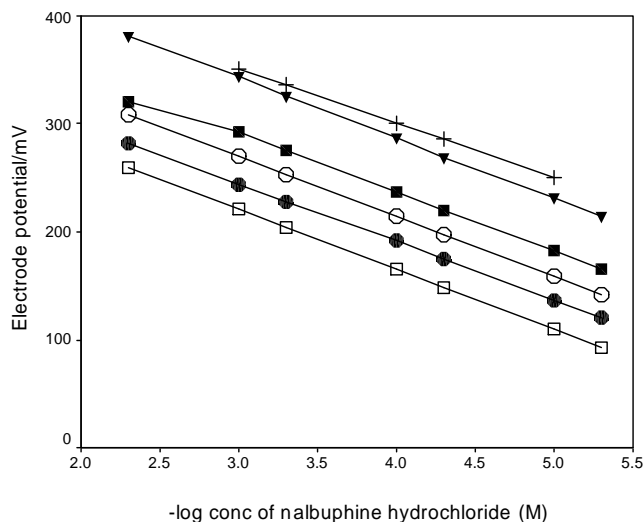
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Figure 6 : Calibration graphs obtained at 25±1°C after soaking the nalbuphine-phosphotungstate- coated graphite electrode for (□) 24h, (●) 7 days, (○)15 days, (■) 20 days, (▼) 27 days, (+) 30 days.

27 days for electrode I, II and III respectively. Figure 4-6, shows the effect of prolonged soaking time and the life span of the electrodes.

Regeneration of the electrode

The above discussion reveals that soaking of the electrode(s) in the drug solution for a long time has a negative effect on the response of the membrane. The same effect appears after working with the electrode(s) for a long time. The regeneration of the electrode(s) was tried simply by reformation of the ion-exchange on the external gel layer of membrane[10]. The regeneration of the nalbuphine membrane was successfully achieved by soaking the exhausted electrode(s) for 24 h in a solution that was $1x10^{-2}$M phosphotungestic acid, followed by soaking for 3 h in $1x10^{-2}$M nalbuphine hydrochloride solution. Figure 7-9, show the calibration graphs for an exhausted electrode(s) (slopes 54.51, 53.57 and 50.42 mV decade^{-1}) for electrode I, II and III respectively, and for the same electrode(s) after regeneration (slopes 56.15, 53.34 and 52.93 mV decade^{-1}) for electrode I, II and III respectively. It was found that the lifespan of the regenerated electrode(s) is limited to $= 6$ h due to the ease of leaching of the lipophilic salts from the gel layer at the electrode(s) surface compared with those that are attached homogeneously to the PVC network through the solvent mediator.

Figure 7 : Regeneration of nalbuphine-phosphotungstate-PVC membrane electrode (●) calibration graph of the exhausted electrode, (□) calibration graph of a regenerated electrode

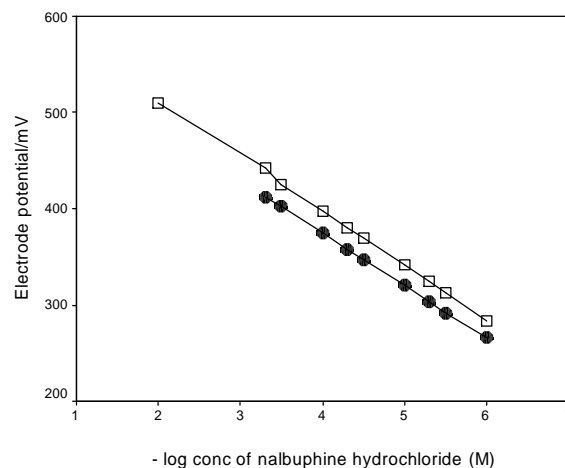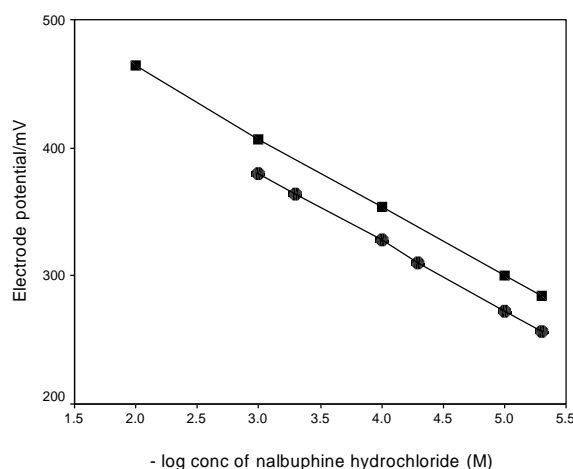**Figure 8 : Regeneration of nalbuphine-phosphotungstate-coated wire electrode (●) calibration graph of the exhausted electrode, (■) calibration graph of a regenerated electrode**

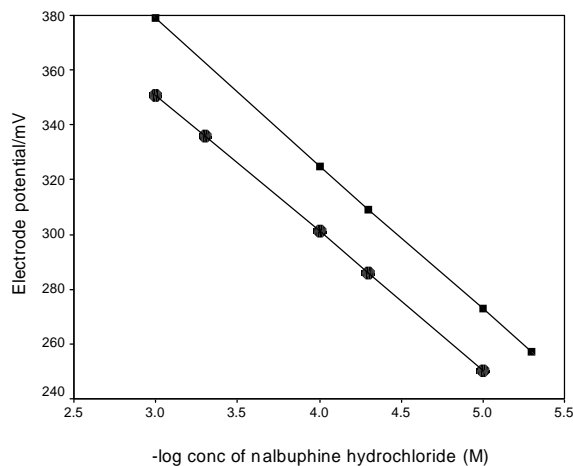**Figure 9 : Regeneration of nalbuphine-phosphotungstate-coated graphite electrode (●) calibration graph of the exhausted electrode, (■) calibration graph of a regenerated electrode**

Effect of pH

The effect of pH of the nalbuphine hydrochloride solutions using 1×10^{-3} M nalbuphine on the electrode(s) potential was investigated. The solution was acidified by the addition of very small volumes of hydrochloric acid 0.1N then the pH value was increased gradually using sodium hydroxide 0.1N for each pH value, the potential was recorded and thus the potential-pH curves for nalbuphine concentrations were constructed as in Figure 10. As is obvious, within the pH range 3.5-7.4 for all electrodes. The electrode(s) potential is practically independent of pH, and in this range the electrode(s) can be safely used for nalbuphine hydrochloride determination. The potential decrease at higher pH values is most probably attributed to the formation of the ionization of the hydroxyl group, leading to a decrease in the concentration of the nalbuphine ion.
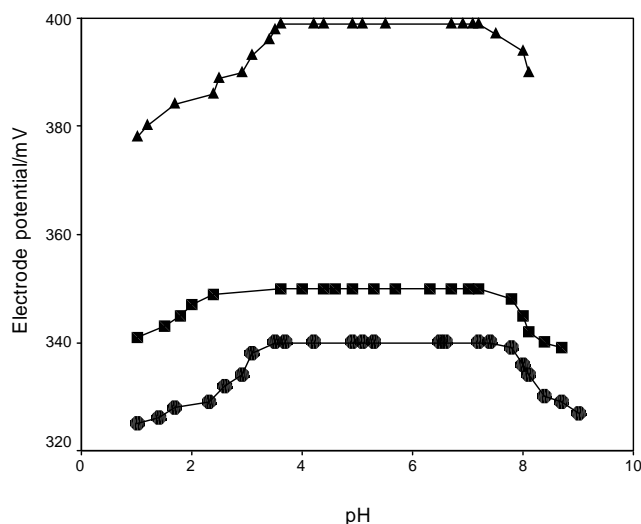

Figure 10 : Effect of pH on potential/mV of 1.0×10^{-3}M, (▲) plastic nalbuphine-phosphotungstate-PVC electrode, (■) coated wire-nalbuphine-phosphotungstate electrode, (●) coated graphite nalbuphine-phosphotungstate electrode

Selectivity of the electrode

Potentiometric selectivity coefficients were evaluated by the separate solution method. TABLE 3, showed that the proposed nalbuphine-phosphotungstate membrane electrode(s) is highly selective toward nalbuphine. The electrode(s) showed no response to a number of potentially interfering ionic excipients usually used in the manufacturing of the pharmaceutical preparations, such as starch and lactose. In the case of amino acids, the high selectivity is mainly attributed to the dif-

ference in polarity and lipophilic character of their molecules relative to nalbuphine.

TABLE 3 : Selectivity coefficients of the nalbuphine-PT sensors calculated by the separate solution method (1.0×10^{-3}M of both nalbuphine hydrochloride and the interferent) at 25°C.

Interferent	Nalbuphine-PT plastic membrane electrode $K^{pot}_{nal^+ Cl^-}$	Nalbuphine-PT coated wire electrode $K^{pot}_{nal^+ Cl^-}$	Nalbuphine-PT coated graphite electrode $K^{pot}_{nal^+ Cl^-}$
Na^+	6.4×10^{-4}	2.5×10^{-5}	4.2×10^{-4}
K^+	2.6×10^{-3}	1.3×10^{-4}	1.8×10^{-4}
NH^{4+}	8.2×10^{-4}	5.4×10^{-4}	3.0×10^{-4}
Mg^{2+}	4.5×10^{-4}	7.1×10^{-4}	9.2×10^{-3}
Ca^{2+}	7.7×10^{-3}	6.8×10^{-3}	5.4×10^{-3}
Ba^{2+}	6.6×10^{-3}	5.8×10^{-3}	4.8×10^{-3}
Cr^{3+}	1.4×10^{-3}	2.9×10^{-3}	2.1×10^{-3}
Al^{3+}	4.7×10^{-4}	3.9×10^{-5}	8.7×10^{-4}
L- Systin	2.9×10^{-3}	1.3×10^{-3}	3.3×10^{-3}
L- Lucien	1.8×10^{-3}	2.4×10^{-3}	3.6×10^{-3}
Starch	7.6×10^{-4}	9.1×10^{-4}	5.8×10^{-4}
Glucose	2.2×10^{-3}	1.5×10^{-3}	1.1×10^{-3}
Lactose	6.4×10^{-3}	5.2×10^{-4}	8.3×10^{-3}
sucrose	4.2×10^{-3}	2.1×10^{-3}	5.6×10^{-3}
Urea	3.7×10^{-3}	2.0×10^{-3}	2.8×10^{-3}
Quinidine	5.7×10^{-4}	8.1×10^{-4}	7.7×10^{-4}
Peudoephedrine HCl	6.3×10^{-4}	9.5×10^{-4}	5.1×10^{-4}
Caffeine	3.0×10^{-4}	2.4×10^{-4}	2.9×10^{-4}
Atropine SO_4	1.2×10^{-3}	1.7×10^{-3}	2.0×10^{-3}
Naloxone HCl	4.0×10^{-1}	5.1×10^{-1}	8.4×10^{-1}
Chlorpromazine HC	5.2×10^{-3}	9.8×10^{-3}	7.7×10^{-3}
Tramadol HCl	2.7×10^{-3}	3.3×10^{-3}	4.1×10^{-3}

Quantification of nalbuphine hydrochloride

Direct potentiometric determination of nalbuphine hydrochloride using nalbuphine-phosphotungstate electrode(s) type I, II and III, was performed and calculated from the calibration curve. The direct potentiometric determination of nalbuphine hydrochloride in pure form using the proposed electrodes gave average recovery % of 99.51±0.4, 99.43±0.4 and 99.48±0.5 for electrode I, II and III respectively. Furthermore, the results obtained were compared with the reference method[11], (UV-detection for nalbuphine hydrochloride using 0.1M hydrochloric acid. The conc. 0.02-0.03 mg/mL and the absorbance measured at 284 nm), and the results are listed in TABLE 4.

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TABLE 4 : Determination of nalbuphine in pure form using nalbuphine-PT sensors in comparison with reference method

Types of electrodes	Types of samples	Statistical parameter	Reference method	Direct potentiometry	
				Calibration method	Standard addition method
Nalbuphine-PT-plastic membrane electrode	Pure sample	Mean±SD	99.22±0.735	99.10±0.560	99.51±0.418
		N	7	7	6
		't'		(0.352)(2.160)*	(0.907)(2.179)*
		F		(1.72)(3.87)*	(3.09)(4.28)*
Nalbuphine-PT-coated wire electrode	Pure sample	Mean±SD	99.56±0.587	99.28±0.385	99.43±0.367
		N	6	7	6
		't'		(0.997)(2.201)*	(0.460)(2.228)*
		F		(2.33)(4.39)*	(2.33)(2.56)
Nalbuphine-PT-coated graphite electrode	Pure sample	Mean±SD	99.35±0.541	99.46±0.617	99.48±0.549
		N	6	6	6
		't'		(0.413)(2.228)*	(0.413)(2.228)*
		F		(1.30)(5.05)*	(1.03)(5.05)*

Validation of the proposed ISE method

Accuracy

The accuracy of the proposed ISE method was investigated by the determination of nalbuphine hydrochloride in spiked placebo samples prepared from serial concentrations of nalbuphine reference standards. The results summarized in TABLE 5, show that the proposed ISE method is an accurate one for the determination of nalbuphine hydrochloride in their pharmaceutical preparations without interfering from the coformulated adjuvants as indicated by the percentage recovery values.

TABLE 5 : Determination of nalbuphine hydrochloride in nalbuphine-spiked placebo samples using nalbuphine-phosphotungstate sensors

Statistical parameters	Sample of ampoules placebo		
	Nalbuphine-PT Plastic membrane electrode	Nalbuphine-PT Coated wire electrode	Nalbuphine-PT Coated graphite electrode
N	9	9	9
Mean	99.35	99.18	99.41
SD	0.358	0.872	0.523
RSD	0.360	0.879	0.526

Linearity

Under the optimal experimental ISE conditions, linear relationships exist between the electrode potential/mV and the logarithm of corresponding concentration of the investigated drug. The regression data, correlation coefficients (r) and other statistical parameter are previously listed in TABLE 2.

Precision

The precision of the proposed ISE method, measured as percentage relative standard deviation (RDS %) was tested by repeating the proposed method for determination of the investigated drug in its pharmaceutical preparations to nine replicates. The RSD% values for the repeated determinations were 0.680 %, 0.598% and 0.439% for determination of nalbuphine hydrochloride in Nalufin® ampoules using electrode I, II and III respectively, and 0.828 %, 0.329 % and 0.777 % in Nubain® ampoules using electrode I, II and III respectively. The above RSD% values are less than 2% indicating good precision.

Robustness and ruggedness

The robustness of the proposed ISE method was tested by investigating the capacity of the method to remain unaffected by a small but a deliberate variation in method parameters and provides an indication of its reliability during normal usage[12]. While the ruggedness of the proposed method was investigating the degree of reproducibility at test results obtained by the analysis of the same samples under a variety of conditions such as different laboratories, analysts and instruments. The results obtained by using another model of pH-meter (Orion 420 A) were compared with those obtained using

model of pH-meter (Jenway 3040). The obtained results are close and also reveal validity of the method. The results previously listed in TABLE 2.

Detection limit

The detection limit of the investigated drug was calculated according to IUPAC recommendation which stated that the detection limit is the concentration at which the measured potential differs from that predicted by the linear regression by more than 18 mV. The values were previously reported in TABLE 2; indicate that the proposed ISE method is sensitive for detection of very small concentrations of nalbuphine hydrochloride.

Analytical Applications of the proposed method
Application to pharmaceutical preparations
Nalbuphine hydrochloride ampoules

The proposed ISE method was applied to determination of nalbuphine hydrochloride in their dosage forms. The mean % recovery found and RSD%, indicate that the proposed validated method could be adopted for the determination of the investigated drug in its pharmaceutical preparations without interference from the coformulated adjuvants. TABLE 6, shows the results obtained from the determination of nalbuphine hydrochloride in its ampoules in comparison with reference method[11].

TABLE 6 : Comparative analytical results of the proposal and reference method for the tested drug in some pharmaceutical preparations

| Types of electrodes | Types of samples | Statistical parameter | Reference method | Direct potentiometry | |
				Calibration method	Standard addition method
Nalbuphine-PT-plastic membrane electrode	Nalufin®20 mg/mL	Mean±SD	99.50±0.447	99.20±0.407	98.94±0.430
		N	6	7	6
		't'		(1.258)(2.201)*	(2.211)(2.228)*
		F		(1.19)(4.39)*	(1.08)(5.05)*
	Nubain®20 mg/mL	Mean±SD	98.98±0.385	98.99±0.498	99.11±0.396
		N	6	6	6
		't'		(0.039)(2.228)*	(0.576)(2.228)*
		F		(1.68)(5.05)*	(1.06)(5.05)*
Nalbuphine-PT-coated wire electrode	Nalufin®20 mg/mL	Mean±SD	99.59±0.474	99.11±0.332	99.08±0.956
		N	5	6	6
		't'		(1.906)(2.262)*	(1.149)(2.262)*
		F		(2.05)(5.19)*	(4.06)(5.19)*
	Nubain®20 mg/mL	Mean±SD	99.12±0.626	98.96±0.895	98.92±0.439
		N	6	6	6
		't'		(0.359)(2.228)*	(0.640)(2.228)*
		F		(2.04)(5.05)*	(2.03)(5.05)*
Nalbuphine-PT-coated graphite electrode	Nalufin®20 mg/mL	Mean±SD	99.02±0.624	98.89±0.437	98.92±0.817
		N	7	6	6
		't'		(0.439)(2.201)*	(0.245)(2.201)*
		F		(2.04)(4.39)*	(1.71)(4.39)*
	Nubain®20 mg/mL	Mean±SD	99.32±0.582	99.10±0.657	98.99±0.663
		N	6	6	6
		't'		(0.614)(2.228)*	(0.915)(2.228)*
		F		(1.27)(5.05)*	(1.30)(5.05)*

Content uniformity assay of nalbuphine ampoules

The proposed ISE method described good accuracy and precise for the quality control tests, the content uniformity assay showed that the $(R.S.D < 2\%)$,

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with mean standard deviation 99.67±0.7, 99.42±0.9 and 99.17±0.6 for electrode I, II and III respectively.

Application to serum and urine

The proposed ISE method was applied to determination of nalbuphine hydrochloride in biological fluids as human serum and urine. The results obtained were summarized in TABLE 7.

TABLE 7 : Determination of nalbuphine hydrochloride in human serum and urine

Types of sensors	Statistical parameter	Direct potentiometry	
		Calibration method	Standard addition method
Nalbuphine-PT plastic membrane electrode	Serum sample		
	Mean±SD	99.03±0.447	98.91±0.580
	N	7	6
	Variance	0.200	0.336
	SE	0.169	0.237
	RSD	0.451	0.586
	Urine sample		
	Mean±SD	99.08±0.549	99.18±0.588
	N	6	6
	Variance	0.301	0.346
	SE	0.224	0.240
	RSD	0.554	0.593
Nalbuphine-PT coated wire electrode	Serum sample		
	Mean±SD	98.92±0.727	98.84±0.703
	N	7	6
	Variance	0.529	0.494
	SE	0.275	0.287
	RSD	0.735	0.711
	Urine sample		
	Mean±SD	98.85±0.743	99.16±0.358
	N	6	6
	Variance	0.552	0.128
	SE	0.303	0.146
	RSD	0.744	0.361
Nalbuphine-PT coated graphite electrode	Serum sample		
	Mean±SD	99.07±0.591	98.78±0.475
	N	7	6
	variance	0.349	0.226
	SE	0.223	0.194
	RSD	0.597	0.481
	Urine sample		
	Mean±SD	99.04±0.837	98.98±0.463
	N	6	6
	variance	0.701	0.214
	SE	0.342	0.189
	RSD	0.845	0.468

CONCLUSION

The proposed potentiometric procedures based on the construction of different types of electrodes, plastic membrane, coated wire and coated graphite might be useful analytical tools for the determination of nalbuphine hydrochloride in different real samples. The present electrodes show high sensitivity, reasonable selectivity, fast static response, long term stability and applicability over a wide range. The previously described procedures for nalbuphine determination were highly specific and advantageous over other reported methods, since no interferences from the recipients, impurities, or other accompanying drug components. The proposed procedures were accurate, precise for the determination of the drug in pharmaceutical dosage forms as well as biological fluids.

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