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Fentanyl-selective polymeric membrane electrode

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A poly (vinyl chloride) membrane electrode with dibutyl phthalate as plasticizer based on a fentanyl-tetrakis(4-chlorophenyl)borate ion-pair complex for the determination of fentanyl citrate in injections is described. A linear response over the range 1×10^{-5} mol · dm^{-3} to 1×10^{-2} mol · dm^{-3} drug with a slope of 59.3 ± 0.6 mV/decade was established. The optimum pH range was 1 to 6. The lower detection limit was 8×10^{-6} mol · dm^{-3} fentanyl citrate (2.7 μg cm^{-3} fentanyl). There were negligible interferences from a number of inorganic cations, structural analogues, and some common drug additives used in injections. The electrode proposed has been successfully applied to determine fentanyl citrate in injections. The results correlated well with those obtained by the United States Pharmacopoeia standard procedure.

1. Introduction

Fentanyl, *N*-(1-phenethyl-4-piperidyl) propionanilide is a synthetic opiate analgesic, which is 50 to 100 times more potent than morphine. Various methods have been developed for its quantitative determination, e.g. radioimmunoassay (RIA) [1–5, 11, 16, 28], radioreceptor assay (RRA) [6–8], enzyme-linked immunosorbent assay (ELISA) [9–13], fluoroimmunoassay [14], gas chromatography (GC) [15–20], capillary gas chromatography [21–23], gas chromatography-mass spectrometry (GC/MS) [4, 21, 24–28], gas liquid chromatography [29], high performance liquid chromatography (HPLC) [30–36, 38], micellar electrokinetic capillary chromatography [37], thin-layer chromatography (TLC) [26], and electroencephalography (EEG) [38], etc. with the disadvantage of tedious sample preparation.

Although ion-selective membrane electrodes (ISME) have been widely used in pharmaceutical analysis [39–42], no electrodes responsive to fentanyl have so far been described. For this reason, we decided to investigate the response characteristics of a poly (vinyl chloride) membrane electrode with dibutyl phthalate as plasticizer based on a fentanyl-tetrakis(4-chlorophenyl)borate ion-pair complex for the determination of fentanyl citrate in injections.

2. Investigations, results and discussion

The critical response characteristics of a fentanyl-selective PVC membrane electrode based on fentanyl-tetrakis(4-chlorophenyl)borate ion-pair complex with DBP plasticizer at 25 °C are given in Table 1. Calibrations were made at a constant pH and ionic strength using 0.1 mol · dm^{-3} citrate-NaOH buffer (pH 4.0). The electrode displayed a linear response for aqueous fentanyl citrate solutions over the concentration range 1×10^{-5} mol · dm^{-3} to 1×10^{-2} mol · dm^{-3}. The calibration slopes were 59.3 ± 0.6 mV. The lower detection limit observed for the fentanyl-selective membrane electrode based on a fentanyl-tetrakis(4-chlorophenyl)borate ion-pair complex was determined according to the IUPAC recommendations and was found to be 8×10^{-6} mol · dm^{-3} fentanyl citrate (2.7 μg × cm^{-3} fentanyl) [44]. The potential readings were stable and consistent to ±1.2 mV within the same day and were reproducible to within ±1.8 mV in a 1×10^{-4} mol · dm^{-3} fentanyl citrate solution for 4 h continuous use. The stability of the electrode response was checked over a period of 3 months. The time required for the electrode to reach 95% of final response was less than 30 s. The electrode response displayed good stability and reproducibility over the test, as shown by the relative standard deviation values in Table 1.

Table 1: Response characteristics of the fentanyl-selective PVC membrane electrode

Parameter	Response
Slope (mV/decade)[a]	59.3 ± 0.6
Intercept E (mV)[b]	507.8 ± 1.6
Correlation coefficient, r	0.9987
Linear range (mol · dm^{-3})	$1 \times 10^{-5} - 1 \times 10^{-2}$
Lower detection limit (mol · dm^{-3})	7.99×10^{-6}

[a] Standard deviation of average slope values for multiple calibration (n = 45)
[b] Standard deviation of values recorded over a period of 3 months (n = 45)
Measurements were made in 0.1 mol · dm^{-3} citrate-NaOH buffer at pH 4.0, 25 °C.

The pH dependence of the electrode potentials was investigated by observing the changes in the potential readings with pH of unbuffered solutions (1×10^{-6} mol · dm^{-3} -1×10^{-2} mol · dm^{-3} fentanyl citrate solution) after addition of small volumes of 3 mol · dm^{-3} HCl and/or 3 mol · dm^{-3} NaOH. It was found that the electrode showed virtually no pH response over the range of 1.0–6.0 pH units. That means the protonated form of fentanyl was stable over the range of 1.0–6.0 pH units. Decrease in the potentials at above pH 6.0 would presumably be due to the formation of the deprotonated fentanyl species and to precipitation of free fentanyl base in the test solutions, which was not sensed by the electrode.

Interference by common inorganic cations, antioxidants and preservative agents in normal injections, precipitating agents used in sample preparation, endogenous substances in urine, amino acids, anticoagulants used in blood sample preparation, and some structural analogues with the selectivity of the proposed electrode were studied by the separated solution method (SSM) recommended by IUPAC [44]. The concentrations of fentanyl citrate and the interferents were kept at a level of 1×10^{-3} mol · dm^{-3} in solutions of the same pH and ionic strength (0.1 mol · dm^{-3} citrate-NaOH buffer of pH 4.0) at 25 °C. The potentiometric selectivity coefficients ($K^{Pot}_{I,J}$) listed in Table 2 showed that the proposed electrode exhibited reasonable selectivity towards fentanyl citrate. There was no significant interference from most of the tested substances with the exception of thebaine which produced slight interference.

The proposed electrode was used for assay of the fentanyl citrate content of injections by the standard addition method. The results of the potentiometric methods compared with the USP standard procedure are shown in Table 3.

Table 2: Potentiometric selectivity coefficients $K_{I,J}^{Pot}$ for the PVC fentanyl-selective membrane electrode

Interferent	$K_{I,J}^{Pot}$	Interferent	$K_{I,J}^{Pot}$
$NaHSO_3$ [1]	5.03×10^{-4}	DL-Alanine [5]	6.07×10^{-3}
Na_2CO_3 [2]	4.01×10^{-3}	Potassium oxalate [6]	7.71×10^{-3}
Benzoic acid [2]	5.35×10^{-3}	NaCl [7]	2.14×10^{-2}
Picric acid [3]	6.30×10^{-3}	Thebaine [7]	1.79×10^{-1}
$(NH_4)_2SO_4$ [3]	2.04×10^{-3}	Caffeine [7]	5.36×10^{-3}
$Hg(NO_3)_2$ [3]	3.27×10^{-4}	Cinchonine [7]	6.53×10^{-2}
Urea [4]	7.38×10^{-3}	Theophylline [7]	1.57×10^{-2}
Glucose [4]	1.53×10^{-2}	Morphine HCl [7]	2.44×10^{-2}
DL-Cystine [5]	4.84×10^{-3}	Procaine [7]	1.06×10^{-2}

[1] Antioxidants in normal injections [2] Preservative agents in normal injections [3] Precipitating agents in sample preparation [4] Endogenous substances in urine [5] Amino acids [6] Anticoagulants in blood sample preparation [7] Structural analogues
The concentrations of fentanyl citrate and the interferents were kept at a level of 1×10^{-3} mol · dm^{-3} in solutions of the same pH and ionic strength (0.1 mol · dm^{-3} citrate-NaOH buffer of pH 4.0) at 25 °C.

Table 3: Comparison of mean values of potentiometric method and USP standard method for the assay of fentanyl citrate in injection

Sample (0.1 mg/2 cm^3)	Potentiometric method		USP standard method [43]	
	Recovery (% of nominal value)[a]	RSD (%)	Recovery (% of nominal value)[a]	RSD (%)
1	99.29	1.04	98.96	0.90
2	98.51	0.90	98.58	0.81
3	98.41	0.77	98.30	0.62
4	99.82	0.95	98.79	0.55
5	100.26	1.06	99.73	0.74

[a] All values were the average of 5 determinations

As can be seen from Table 3, the results correlate well with those obtained by the USP standard procedure [43].

3. Experimental

3.1. Reagents

All chemicals were analytical reagent grade and solutions were prepared with deionized water (conductivity >1 μS · cm^{-1}). Potassium tetrakis(4-chlorophenyl) borate and poly (vinyl chloride) (PVC, high molecular weight) were obtained from Fluka, dibutyl phthalate (DBP) from Sigma, tetrahydrofuran (THF), ammonium acetate, methanol, acetonitrile, and glacial acetic acid were from a local chemical supplier. The fentanyl citrate reference standard was obtained from the National Anesthesia Drug Laboratory, Beijing, PR China. Its characteristic was consistent with the USP [43]. The fentanyl citrate injections (0.1 mg/2 cm^3) were supplied by the Hubei Yiyao Ltd. Co., Hubei, PR China. A 1×10^{-2} mol · dm^{-3} fentanyl citrate stock solution was prepared by dissolving 2.6431 g of pure anhydrous fentanyl citrate reference standard in 500 cm^3 0.1 mol · dm^{-3} citrate-NaOH buffer (pH 4.0). By appropriate dilution with the citrate-Na_2HPO_4 buffer, a series of standard solutions in the concentration range 1×10^{-7} mol · dm^{-3} to 1×10^{-3} mol · dm^{-3} were obtained.

3.2. Apparatus

All EMF measurements were made with a pHS-3C Digital pH meter (Kai Li Scientific Instrument Ltd. Co., Xiaoshan, PR China). The external reference electrode was a model 801 double-junction saturated calomel electrode with outer bridge electrolyte (Jiangsu Electroanalytical Instrument Factory, Jiangsu, PR China). A model 65-1 glass electrode (Kangling Optical & Electrical Tech. Ltd. Co., Shanghai, PR China) was used for pH measurement.
ALC/GPC model 201 HPLC (Waters, USA) and DL-800 Chromatographic Working Station (Dalian Elite Scientific Instruments Co. Ltd., Liaoning, PR China) were employed for the determination of fentanyl citrate in injections by the USP standard procedure [43].

3.3. Construction of the electrode

The fundamental principles of construction of the fentanyl-selective membrane electrode been described elsewhere [45–47]. The PVC-membrane composition was 2.0% w/w tetrakis(4-chlorophenyl) borate, 49.0% w/w plasticizer and 49.0% w/w PVC. The electrode body was filled with a 10^{-4} mol · dm^{-3} fentanyl citrate solution of pH 4.0 (citrate-NaOH buffer solution, saturated with AgCl). The electrode was pre-conditioned for 24 h by soaking it in a 10^{-2} mol · dm^{-3} fentanyl citrate solution. Fentanyl cation reacted with tetrakis(4-chlorophenyl) borate, to form a stable ion-pair complex within the membrane. The complex was obtained in situ by soaking the PVC membrane in 10^{-2} mol · dm^{-3} fentanyl citrate solution. The electrode should be washed with deionized water before measurement. It could be kept in air when used continuously. The inner filling solution should be removed when not in use for a long time. All potentiometric measurements were performed using the following cell assembly: Hg/Hg_2Cl_2 | KCl (satd.) ‖ salt bridge ‖ sample solution | membrane | 10^{-4} mol · dm^{-3} fentanyl citrate ‖ Ag/AgCl. The electrode was washed with deionized water and blotted with tissue paper between measurements.

3.4. Direct potentiometry

Aliquots of 10 cm^3 of 1×10^{-7} mol · dm^{-3} to 1×10^{-2} mol · dm^{-3} fentanyl citrate standard solutions were transferred into 25 cm^3 beakers. The PVC fentanyl-selective membrane electrode based on a fentanyl-tetrakis(4-chlorophenyl)borate ion-pair complex in conjunction with a double-junction saturated calomel electrode was placed into the well stirred 10 cm^3 aliquots of standard solutions with concentrations of 1×10^{-7} mol · dm^{-3} to 1×10^{-2} mol · dm^{-3} and potentials were recorded. The measured potential was plotted against the logarithm of the fentanyl citrate concentration. Using the mean potential of five measurements an unknown concentration could be derived from the regression equation of the calibration graph.

3.5. Standard addition method

A membrane electrode a in conjunction with a double-junction saturated calomel electrode was immersed in a sample of 10 cm^3 with unknown concentration (ca. 10^{-4} mol · dm^{-3}) for 30 s and the equilibrium potential of E_1 was recorded. Then 0.1 cm^3 of 1×10^{-2} mol · dm^{-3} fentanyl citrate standard was added to the test solution and the equilibrium potential of E_2 was obtained after 30 s. From the change of ΔE ($E_2 - E_1$) one can determine the concentration of the test sample [44].

3.6. HPLC measurement

The USP measurement was performed with a μBondapak C18 column (4.6 × 250 mm). Equal volumes (about 25 mm^3) of the standard preparation and the assay preparation of the injection were injected into the chromatograph separately. The chromatograms were recorded and the responses for the major peaks were measured. The quantity, in μg, of fentanyl in each cm^3 of the injection was calculated by the formula:

$$(336.48/528.61)\,CD\,(r_u/r_s)\,,$$

in which 336.48 and 528.61 are the molecular weights of fentanyl and fentanyl citrate, respectively, C is the concentration, in μg · cm^{-3}, of the fentanyl citrate reference standard in the standard preparation, D is the dilution factor used to obtain the assay preparation, and r_u and r_s are the peak responses for the fentanyl peak obtained from the assay preparation and the standard preparation, respectively [43].

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