Construction and Performance Characteristics of New Fentanyl-Selective Plastic Membrane Electrode

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The construction and performance characteristics of an ion-selective electrode for fentanyl-drug cation, based on an ion-pair complex with tetrakis[3,5-bis-(trifluoromethyl)phenyl]borate anion in a PVC matrix were studied. A linear response for 1×10^{-5} mol dm⁻³ to 1×10^{-2} mol dm⁻³ drug with a slope of 57.9 ± 0.5 mV/decade was established. The optimum pH range was 2 to 6. The lower detection limit was 6.29×10^{-6} mol dm⁻³ fentanyl citrate ($2.1165 \mu\text{g cm}^{-3}$ fentanyl). There were negligible interferences from a number of inorganic cations, structural analogues, and some common drug additives 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.

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Introduction

Fentanyl, *N*-(1-phenethyl-4-piperidyl)propionanilide (Fig. 1), is a synthetic opiate analgesic, which is 50 to 100 times more potent than morphine. It acts in the central nervous system to relieve pain and is widely used in surgical anesthesia as the citrate salt at doses ranging from 2 to 50 $\mu\text{g/kg}$. Meanwhile, fentanyl is also an analogue of illicit drugs, such as heroine, and is highly addictive for abusers. It is significantly more potent than heroine ($\sim 40\times$). It has been sold on the street as heroine, causing some deaths. Illicit use of pharmaceutical fentanyls first appeared in the mid-1970s in the medical community and continues to be a problem in the United States of America. Therefore, various methods have been developed for its quantitative determination: *e.g.*, radioimmunoassay,^{1-5,11,16,28} radioreceptor assay,⁶⁻⁸ enzyme-linked immunosorbent assay,⁹⁻¹³ fluoroimmunoassay,¹⁴ gas chromatography,¹⁵⁻²⁰ capillary gas chromatography,²¹⁻²³ gas chromatography-mass spectrometry,^{4,21,24-28} thin-layer chromatography,²⁶ gas liquid chromatography,²⁹ high-performance liquid chromatography,^{30-36,38} micellar electrokinetic capillary chromatography,³⁷ and electroencephalography,³⁸ *etc.*, all with the disadvantage of tedious sample preparation.

Potentiometric methods with ion-selective membrane electrodes can provide valuable means of monitoring fentanyl in pharmaceutical preparations because of their low cost, ease of use and maintenance, and the simplicity and speed of assay procedure. 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 fentanyl-tetrakis[3,5-bis-(trifluoromethyl)phenyl]borate ion-pair complex for the determination of fentanyl citrate in injections. The

results agreed fairly well with those obtained by the United States Pharmacopoeia (USP) standard procedure.⁴³

Experimental

Reagents

All chemicals were of analytical reagent grade and solutions were prepared with deionized water (conductivity $>1 \mu\text{S cm}^{-1}$). Potassium tetrakis[3,5-bis-(trifluoromethyl)phenyl]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 local chemical suppliers. 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 cm³ 0.1 mol dm⁻³ citrate-NaOH buffer (pH 4.0). By appropriate dilution with the citrate-Na₂HPO₄ 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.

Fig. 1 The structure of fentanyl citrate. C₂₂H₂₈N₂O·C₆H₈O₇, Mol. Wt. 528.61.

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Table 1 Response characteristics of the fentanyl-selective PVC membrane electrode based on fentanyl-tetrakis[3,5-bis(trifluoromethyl)phenyl]borate ion-pair complex with DBP plasticizer

Parameter	Response
Slope (mV/decade) ^a	57.9 ± 0.5
Intercept, <i>E</i> (mV) ^b	441.4 ± 1.3
Correlation coefficient, <i>r</i>	0.9979
Linear range (mol dm ⁻³)	1 × 10 ⁻⁵ – 1 × 10 ⁻²
Lower detection limit (mol dm ⁻³)	6.29 × 10 ⁻⁶

Measurements were made in 0.1 mol dm⁻³ citrate-NaOH buffer at pH 4.0, 25°C.

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).

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 an 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 measurements.

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 injections of fentanyl citrate by USP standard procedure.⁴³

Construction of the electrode

The fundamental principle of the fentanyl-selective membrane electrode construction has been described elsewhere. The PVC-membrane composition was 2.0% w/w tetrakis[3,5-bis(trifluoromethyl)phenyl]borate, 49.0% w/w plasticizer and 49.0% w/w PVC.⁴⁴⁻⁴⁶ 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), and the inner reference electrode was Ag/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[3,5-bis(trifluoromethyl)phenyl]borate to form stable ion-pair complexes within the membrane. Each 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 continuously used. 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/Hg₂Cl₂ | 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.

Standard preparation

We dissolved an accurately weighed quantity of fentanyl citrate reference standard in deionized water, and diluted it quantitatively with deionized water to obtain a solution having a known concentration of about 80 µg cm⁻³.⁴³

Fig. 2 Effect of pH on the response of the PVC fentanyl-selective membrane electrode based on fentanyl-tetrakis[3,5-bis(trifluoromethyl)phenyl]borate ion-pair complex for 1 × 10⁻⁶ mol dm⁻³, 1 × 10⁻⁴ mol dm⁻³, 1 × 10⁻² mol dm⁻³ fentanyl citrate solutions.

Assay preparation of injection

We diluted the injection with deionized water so that each cm³ contained the equivalent of about 50 µg of fentanyl.⁴³

Direct potentiometry

Aliquots of 10 cm³ of 1 × 10⁻⁷ mol dm⁻³ to 1 × 10⁻² mol dm⁻³ fentanyl citrate standard solutions were transferred into 25 cm³ beakers. The PVC fentanyl-selective membrane electrode based on fentanyl-tetrakis[3,5-bis(trifluoromethyl)phenyl]borate ion-pair complex in conjunction with a double-junction saturated calomel electrode were placed into well-stirred 10 cm³ volumes of standard solutions in the order 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. With the mean potential of five measurements, the unknown concentration could be derived from the regression equation of the calibration graph.

Standard addition method

A PVC fentanyl-selective membrane electrode based on fentanyl-tetrakis[3,5-bis(trifluoromethyl)phenyl]borate ion-pair complex in conjunction with a double-junction saturated calomel electrode were immersed into a sample of 10 cm³ with unknown concentration (*ca.* 10⁻⁴ mol dm⁻³) for 30 s and the equilibrium potential of *E*₁ was recorded. Then 0.1 cm³ of 1 × 10⁻² mol dm⁻³ of fentanyl citrate standard was added into the testing solution and the equilibrium potential of *E*₂ was obtained after 30 s. From the change of Δ*E* (*E*₂ – *E*₁) one can determine the concentration of the testing sample.⁴⁷

HPLC measurement

The USP measurement was performed with a Bondapak C18 column (4.6 × 250 mm). Equal volumes (about 25 mm³) of the standard preparation and the assay preparation of 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 (C₂₂H₂₈N₂O) in each cm³ of the injection was calculated by the formula:

Table 2 Potentiometric selectivity coefficients K_{ij}^{Pot} for the PVC fentanyl-selective membrane electrode with DBP plasticizer

Interferent	K_{ij}^{Pot}	Interferent	K_{ij}^{Pot}
NaHSO ₃ ^a	1.08×10^{-2}	DL-Alanine ^e	1.26×10^{-2}
Na ₂ CO ₃ ^b	5.62×10^{-3}	Potassium oxalate ^f	5.41×10^{-3}
Benzoic acid ^b	8.26×10^{-3}	NaCl ^g	7.20×10^{-2}
Picric acid ^c	7.13×10^{-3}	Thebaine ^g	2.58×10^{-1}
(NH ₄) ₂ SO ₄ ^c	5.01×10^{-3}	Caffeine ^g	8.64×10^{-3}
Hg(NO ₃) ₂ ^c	2.14×10^{-4}	Cinchonine ^g	7.70×10^{-2}
Urea ^d	7.69×10^{-3}	Theophylline ^g	8.63×10^{-3}
Glucose ^d	8.33×10^{-3}	Morphine HCl ^g	7.72×10^{-3}
DL-Cystine ^e	1.07×10^{-2}	Procaine ^g	2.67×10^{-2}

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.

- a. Anti-oxidants in the normal injections.
 b. Preservative agents in the normal injections.
 c. Precipitating agents of sample preparation.
 d. Endogenous substances of urine.
 e. Amino acids.
 f. Anticoagulants of blood sample preparation.
 g. Structural analogues.

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

in which 336.48 and 528.61 are the molecular weights of fentanyl and fentanyl citrate, respectively; C is the concentration in μg per cm³ of 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

Results and Discussion

The critical response characteristics of a fentanyl-selective PVC membrane electrode based on fentanyl-tetrakis[3,5-bis(trifluoromethyl)phenyl]borate 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 of 1×10^{-5} mol dm⁻³ to 1×10^{-2} mol dm⁻³. The calibration slopes were 57.9 ± 0.5 mV. The lower detection limit observed for the fentanyl-selective membrane electrodes based on fentanyl-tetrakis[3,5-bis(trifluoromethyl)phenyl]borate was determined according to the IUPAC recommendations and was found to be 6.29×10^{-6} mol dm⁻³ fentanyl citrate ($2.1165 \mu\text{g cm}^{-3}$ fentanyl).⁴⁷ The potential readings were stable and consistent to ± 1.1 mV within the same day and were reproducible to within ± 1.6 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.

The pH dependence of the potentials of the electrode was investigated by observing the changes in the potential readings with pH of the unbuffered solutions (1×10^{-6} mol dm⁻³ – 1×10^{-2} mol dm⁻³ fentanyl citrate solution) after addition of small

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 ^a	RSD, %	Recovery ^a	RSD, %
1	98.27	1.05	98.16	0.67
2	98.84	0.83	99.07	0.81
3	99.47	1.09	98.66	0.75
4	98.95	1.08	98.69	0.71
5	100.53	1.01	99.85	0.88

a. % of nominal value. All values were the average of 5 determinations.

volumes of 3 mol dm⁻³ HCl and/or 3 mol dm⁻³ NaOH. It was found that the fentanyl-sensitive membrane electrode based on fentanyl-tetrakis[3,5-bis(trifluoromethyl)phenyl]borate ion-pair complex showed virtually no pH response over the range of 2.0 – 6.0 pH units (Fig. 2). That means the protonation form of fentanyl could be maintained in the range of 2.0 – 6.0 pH units. Decrease in the potentials above pH 6.0 would be presumably due to the formation of the deprotonated fentanyl species and precipitation of free fentanyl base in the test solutions, which were not sensed by the electrode.

The potentiometric selectivity coefficient defines the ability of an ion-selective electrode to distinguish a particular ion from others. It is one of the most important characteristics of an ion-selective electrode, as it often determines whether a reliable measurement in the target sample is possible. If one considers the further utilization of the fentanyl-selective PVC membrane electrode in analyzing biological sample, such as urine and blood, some substances which might exist in the procedure of the sample preparation were selected. The interferences of common inorganic cations, antioxidants and preservative agents in the normal injections, precipitating agents of sample preparation, endogenous substances of urine, amino acids, anticoagulants of blood sample preparation, and some structural analogues on selectivity of proposed electrode were studied by the separated solution method (SSM) recommended by the IUPAC.⁴⁷ 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_{ij}^{Pot}) listed in Table 2 show 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 that caused slight interference.

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

In conclusion, the fentanyl-selective PVC membrane electrode based on fentanyl-tetrakis[3,5-bis(trifluoromethyl)phenyl]borate ion-pair complex exhibited useful analytical characteristics for the determination of fentanyl citrate in pharmaceutical preparations.

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References

- W. E. Woods, H. H. Tai, C. Tai, T. Weckman, T. Wood, H. Barrios, J. W. Blake, and T. Tobin, *Am. J. Vet. Res.*, **1986**, *47*, 2180.
- R. L. Sliller, A. M. Scierka, P. J. Davis, and D. R. Cook, *Forensic Sci. Int.*, **1990**, *44*, 1.
- G. L. Henderson, M. R. Harkey, and A. D. Jones, *J. Anal. Toxicol.*, **1990**, *14*, 172.
- V. W. Watts and Y. H. Caplan, *J. Anal. Toxicol.*, **1990**, *14*, 266.
- W. L. Wang, E. J. Cone, and J. Zacny, *Forensic Sci. Int.*, **1993**, *61*, 65.
- V. Levi, J. C. Scott, P. F. White, and W. Sadee, *Pharm. Res.*, **1987**, *4*, 46.
- M. E. Alburges, G. R. Hanson, J. W. Gibb, C. O. Sakashita, and D. E. Rollins, *J. Anal. Toxicol.*, **1991**, *15*, 311.
- M. E. Alburges, G. R. Hanson, J. W. Gibb, C. O. Sakashita, and D. E. Rollins, *J. Anal. Toxicol.*, **1992**, *16*, 36.
- T. Tobin, H. H. Tai, C. L. Tai, P. K. Houtz, M. R. Dai, W. E. Woods, J. M. Yang, T. J. Weckman, and S. L. Chang, *Res. Commun. Chem. Pathol. Pharmacol.*, **1988**, *60*, 97.
- F. T. Delbeke and M. Debackere, *J. Vet. Pharmacol. Ther.*, **1989**, *12*, 1.
- T. Tobin, S. Kwiatkowski, D. S. Watt, H. H. Tai, C. L. Tai, W. E. Woods, J. P. Goodman, D. G. Taylor, and T. J. Weckman, *Res. Commun. Chem. Pathol. Pharmacol.*, **1989**, *63*, 129.
- W. Ruangyuttikarn, M. Y. Law, and D. E. Rollins, *J. Anal. Toxicol.*, **1990**, *14*, 160.
- G. S. Makowski, J. J. Richter, R. E. Moore, R. Eisma, D. Ostheimer, M. Onoroski, and A. H. Wu, *Ann. Clin. Lab. Sci.*, **1995**, *25*, 169.
- J. McDonald, R. Gall, P. Wiedenbach, V. D. Bass, B. Deleon, C. Brockus, D. Stobert, S. Wie, and C. A. Prange, *Res. Commun. Chem. Pathol. Pharmacol.*, **1987**, *57*, 389.
- T. J. Gillespie, A. J. Gandolfi, R. M. Maiorino, and R. W. Vaughan, *J. Anal. Toxicol.*, **1981**, *5*, 133.
- R. J. H. Woestenborghs, D. A. Stanski, J. C. Scott, and J. J. P. Heykants, *Anesthesiology*, **1987**, *67*, 85.
- S. Suzuki, H. Tsuchihashi, and H. Arimoto, *J. Chromatogr.*, **1989**, *475*, 400.
- P. Kintz, P. Mangin, A. J. Lungnier, and A. A. Chaumont, *J. Chromatogr.*, **1989**, *489*, 459.
- A. Szeitz, K. W. Riggs, and C. Harvey-Clark, *J. Chromatogr. B: Biomed. Appl.*, **1996**, *675*, 33.
- H. Ohta, S. Suzuki, and K. Ogasawara, *J. Anal. Toxicol.*, **1999**, *23*, 280.
- V. Watts and Y. Caplan, *J. Anal. Toxicol.*, **1988**, *12*, 246.
- S. Bjokman and D. R. Stanski, *J. Chromatogr.*, **1988**, *433*, 95.
- P. Kintz, A. Tracqui, A. J. Lungnier, P. Mangin, and A. A. Chaumont, *Methods Find. Exp. Clin. Pharmacol.*, **1990**, *12*, 193.
- S. N. Lin, T. P. Wang, R. M. Caprioli, and B. P. Mo, *J. Pharm. Sci.*, **1981**, *70*, 1276.
- T. Goromaru, H. Matsuura, N. Yoshimura, T. Miyawaki, T. Sameshima, J. Miyao, T. Furuta, and S. Baba, *Anesthesiology*, **1984**, *61*, 73.
- P. Lillunde and T. Korte, *J. Anal. Toxicol.*, **1991**, *15*, 71.
- D. P. Kingsbury, G. S. Makowski, and J. A. Stone, *J. Anal. Toxicol.*, **1995**, *19*, 27.
- B. Fryirsa, A. Woodhouse, J. L. Huang, M. Dawsen, and L. E. Mather, *J. Chromatogr., B: Biomed. Appl.*, **1997**, *688*, 79.
- S. R. Kowalski, G. K. Gourlay, D. A. Cherry, and C. F. McLean, *J. Pharmacol. Methods*, **1987**, *18*, 347.
- K. Kumar, D. J. Morgan, and D. P. Crankshaw, *J. Chromatogr.*, **1987**, *419*, 464.
- T. D. Wilson, T. Maloney, and W. B. Amsden, *J. Chromatogr.*, **1988**, *445*, 299.
- M. Tsuchiya, W. Ueda, M. Tomoda, and M. Hirakawa, *Masui*, **1991**, *40*, 644.
- W. M. Dewell, M. Khandaghabadi, M. J. D'souza, and H. M. Solomon, *Am. J. Hosp. Pharm.*, **1993**, *50*, 2374.
- K. Kumar, J. A. Ballantyne, and A. B. Baker, *J. Pharm. Biomed. Anal.*, **1996**, *14*, 667.
- E. J. Portier, K. de Blok, J. J. Butter, and C. J. van Boxtel, *J. Chromatogr. B: Biomed. Sci. Appl.*, **1999**, *723*, 313.
- J. Lambropoulos, G. A. Spanos, N. V. Lazaridis, T. S. Ingallinera, and V. K. Rodriguez, *J. Pharm. Biomed. Anal.*, **1999**, *20*, 705.
- R. Weinberger and I. S. Lurie, *Anal. Chem.*, **1991**, *63*, 823.
- J. C. Scott, K. V. Ponganis, and D. R. Stanski, *Anesthesiology*, **1985**, *62*, 234.
- V. V. Cosofret, *Tr. Anal. Chem.*, **1991**, *10*, 298.
- V. V. Cosofret and R. P. Buck, "Pharmaceutical Applications of Membrane Sensors", **1992**, CRC Press, Boca Raton.
- V. V. Cosofret and R. P. Buck, *Crit. Rev. Anal. Chem.*, **1993**, *24*, 1.
- R.-I. Stefan, G.-E. Baiulescu, and H. Y. Aboul-Enein, *Crit. Rev. Anal. Chem.*, **1997**, *27*, 307.
- United States Pharmacopoeia, **1995**, Vol. XXIII, US Pharmacopoeial Convention Inc., Rockville, MD, 654–655.
- A. Craggs, G. L. Moody, and J. D. R. Thomas, *J. Chem. Edu.*, **1974**, *51*, 541.
- C. R. Martin and H. Fresier, *Anal. Chem.*, **1980**, *52*, 562.
- V. V. Cosofret and R. P. Buck, *Anal. Chim. Acta*, **1984**, *174*, 299.
- R. P. Buck and E. Lindner, *Pure Appl. Chem.*, **1994**, *66*, 2527.