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Synthesis of Ibuprofen-Molecularly Imprinted Polymers Used as Sensors to Determine Drug in Pharmaceutical Preparations

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Two molecularly imprinted polymers (MIPs) were prepared by using ibuprofen as the template as well as methacrylic acid (MAA) or acrylamide (ACY) as monomer, used ethylene glycol dimethacrylate (EGDMA) or trimethylol propane triacrylate (TMPTA) as cross linker and benzoyl peroxide as initiator. Non-imprinted polymers (NIPs) prepared by using the same composition of MIPs except the template (ibuprofen). The membrane of MIPs and NIPs were prepared by using dibutyl phthalate (DBPH), oleic acid (O.A), paraffin (PRF) and *o*-nitro phenyloctyl ether (ONPOE) as plasticizers in PVC matrix. The slope, detection limit, linearity range of electrodes MIPs from (-54.25, -45.23) mV/decade, (1.09×10^{-6} M to 9×10^{-6} M) and (1×10^{-5} to 1×10^{-1}), respectively. Stable response of pH range from (3.5-7.5) and the selectivity with inorganic ions (Na^+, Li^+, Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+}) show good selectivity.

Keywords: Molecularly imprinted polymers, Ibuprofen, Ion selective electrode, Different plasticizers.

INTRODUCTION

Ibuprofen, 2-[4-(2-methyl propyl)phenyl]propionic acid is a type of drugs known as NSAIDs. Ibuprofen has anti-inflammatory properties may be weaker than some other NSAIDs. It is used in the management of mild to moderate pain and inflammation in conditions such as dysmenorrhea, headache including migraine, postoperative pain, dental pain, musculoskeletal and joint disorders such as ankylosing, spondylitis, osteoarthritis and rheumatoid arthritis including juvenile idiopathic arthritis, peri-articular disorders such as bursitis and tenosynovitis and soft-tissue disorders such as sprains and strains; it is also used to reduce fever [1,2].

Various methods have been used for the determination of ibuprofen in pharmaceutical and biological samples. Until now, chromatographic methods (HPLC, GC, HPTLC and TLC) [3-10] electrophoretic methods [11-16], spectrophotometric methods [17,18] and titrimetric methods with visual and potentiometric indications [19] are the major technique for the determination of ibuprofen. The aim of this work was the development and validation of a simple, rapid and selective method for the analysis of ibuprofen in commercial pharmaceutical formulations. Molecularly imprinted polymer (MIP) is a process in which functional and cross-linking monomers are copolymerization in the presence of the target analyte (the imprint molecule), which act as a molecular template. The functional monomers initially form a complex with the imprinted molecule [20,21]. The synthesis of MIPs usually involves a parallel process involving synthesis of a non-imprinted polymer (NIP) under conditions identical to those of MIP except that the template is absent. In principle, the NIP is entirely analogous to the MIP except that any binding sites within its porous structure are non-selective. The NIP can therefore be used as a benchmark for assessing the selectivity of the MIP such as recovery and breakthrough as reported [22]. The main techniques for generation MIP can be divided into two groups, namely, covalent and non-covalent on the basis of type of binding between the template molecule and functional groups of the monomers in the polymerization step [23,24].

EXPERIMENTAL

An expandable ion analyzer (WTW model, Germany), a pH meter (WTW model pH 720, Germany) and a saturated calomel electrode (Gallenkamp, USA) were used in this work. All potentiometric measurement was made at room temperature. The potentiometric measurement was recorded using the ibuprofen-MIP sensor in conjunction with secondary calomel electrode as a reference electrode. Construction of the electrode body and immobilization of ibuprofen-MIP in PVC matrix membrane were done using the method given by Craggs *et al.* [25].

All of the chemical reagent used with highest purity. Methacrylic acid (MAA) (99 %), acrylamide (ACY) (99 %),

benzoyl peroxide (78 %), ethylene glycol dimethacrylate (EGDMA) ((98 %) and trimethylol propane triacrylate (99 %). Plasctizeser dibutyl phthalate (DBPH), oleic acid (O.A), *o*-nitro phenyl octyl ether (ONPOE) and paraffin (PRF) were obtained from Fluka AG. Other chemicals and reagents of analytical grade quality were obtained from Fluka, BDH and Aldrich.

Ibuprofen standard and profedin tablets (200 mg ibuprofen) were a gift from the state company of drug industries and medical appliances (IRAQ-SDI-Samara). Apifen tablet, 400 mg profen (Ajanta Pharma, India) were obtained from local pharmacies.

The stock standard solution of 0.1 M ibuprofen was prepared by dissolving 2.062 g of standard ibuprofen in ethanol and diluted to 100 mL, (ultrasonicator) equipment was used to assist the dissolving of the drug, several 100 mL standard solutions ranged from 10^{-6} to 10^{-1} M were freshly prepared.

0.1 M stock solution of each of interfering ions; LiCl, NaCl, $CaCl_2$, $MgCl_2$, $Al(NO_3)_3{\cdot}9H_2O$, $Fe(NO_3)_3{\cdot}9H_2O$ and NH_4OH were prepared. The other diluted solutions were prepared in the range needed similar to that present in blood or serum by serial dilution of the appropriate stock solutions.

Synthesis of imprinted polymer: In glass test tube 50 mL, 3 mmol of the monomer (MAA or acrylamide), 15 mmol of the cross-linker (EGDMA or TMPTA), 0.5 mmol of drug as template, 0.3 mmol of initiator (benzoyl peroxide) and 5 mL of chloroform were mixed well to dissolve all the composition. The solution degasses for 30 min with nitrogen gas and cured to 60 °C for 30 min. The polymer left for 24 h to dried and after that was crushed and washed with (1:9) (methanol: acetonitrile) to remove template and repeated wash to be sure all drugs was removed the polymer was dried at 60 °C for 24 h. The polymer was grounded and sieved by mortar the particles with size less than 150 μm was collecting and used in preparing sensing. The non-imprinted polymer (NIP) was prepared with same method but without drug (template).

Synthesis of membrane and electrode construction: The PVC membrane was prepared by mixing 0.17 PVC, 0.4 (DBPH, oleic acid, paraffin and ONPOE as plasticizers) and 0.02 g of the MIP these composition were dissolved in 4 mL of THF. The mixture was poured in (30-35) cm diameter glass ring and allowed to evaporate for 24 h. The electrode was made by attaching a circular disk (10 mm in diameter) of PVC membrane to the end of tygon tube using a THF/PVC as adhesive. The other end of tygon was fixed to a glass tube into which silver wire coated with silver chloride was inserted and filled with 0.1 M of ibuprofen. The electrodes were preconditioned by soaking for 2 h in 0.1 M of ibuprofen solution prior to use.

Preparation of pharmaceutical samples: We used pestle and mortar to grind the tablets to a fine powder. Amounts equivalent to one tablet were weighed and taken into 100 mL volumetric flasks. Samples were mixed by magnetic stirrers for 30 min and filtered through 0.45 nm cellulose filter paper. Then aliquots of filtrates were diluted to get concentrations of 1.0×10^{-3} M profen.

RESULTS AND DISCUSSION

Four electrodes of (MIP-ibuprofen) (A1, A2, A3, A4) based on using ibuprofen (IBP) template, methacrylic acid (MAA) or acrylamide as monomers, ethylene glycol dimethacrylate (EGDMA) or trimethylol propane triacrylate (TMPTA) as cross-linker and benzoyl peroxide as initiator, used four plasticizers such as: dibutyl phthalate (DBPH), oleic acid, paraffin and *ortho*-nitrophenyloctyl ether (ONPOE) with PVC matrix were studied, respectively. All electrodes show near-Nernstian slopes were obtained for electrodes based on DBPH, oleic acid, paraffin and ONPOE (membranes A1, A2, A3 and A4). The slopes are -52.64, -45.32, -54.25 and -53.61 mV/decade with correlation coefficients of 0.9982, 0.9925, 0.9968 and 0.9888, respectively. The linear range for these electrodes 1×10^{-5} to 1×10^{-1}, 1×10^{-5} to 1×10^{-1}, 1×10^{-5} to 1×10^{-2} and 1×10^{-5} to 1×10^{-2} M with detection limits of 1.09×10^{-6} M, 2.5×10^{-6} M, 9×10^{-6} M and 5.65×10^{-6} M, respectively. The results and other parameters are given in Table-1 and Figs. 1 & 2.

Effect of pH: The effect of pH on the electrode potentials for (ibuprofen) selective membrane electrode (A1) was examined by measuring the e.m.f. of the cell in (ibuprofen) solutions at three different concentrations (10^{-4}, 10^{-3}, 10^{-2}) M in which the pH ranged from (1.0-11.0). The pH adjusted by adding appropriate amounts of hydrochloric acid and/or sodium hydroxide solution. The results are shown in Table-2 and Fig. 3.

Interference studies: In this work the selectivity coefficient was determined by using separate solution method (SSM) (Tables 3 and 4) and match potential method (MPM). The potential was measured for two solutions, one containing Ibuprofen and another contain different ions like (Na^+, Li^+, Ca^{2+}, Mg^{2+}, Al^{3+} and Fe^{3+}). In order to investigate the selectivity of the proposed membrane (A1) and (A3) and the selectivity coefficient measured by equation [26].

$$\log K_{pot} = [(EB - EA)/(2.303RT/zF)] + (1 - zA/zB) \log aA \quad (1)$$

where, EA, EB; zA, zB; and aA, aB are the potentials, charge numbers and activities for the primary A and interfering B ions, respectively at aA = aB.

The selectivity coefficients were also measured by the match potential method according to the equation [27].

TABLE-1
CHARACTERISTICS OF THE IBUPROFEN-MIP ELECTRODES BASED ON DIFFERENT FUNCTIONAL MONOMERS AND PLASTICIZERS

Membrane composition	Ibuprofen-MAA + EGDMA + DBPH	Ibuprofen-MAA + EGDMA + DBPH	Ibuprofen-MAA + EGDMA + DBPH	Ibuprofen-MAA + EGDMA + DBPH
Slope (mV/decade)	-52.64	-45.32	-54.25	-53.61
Linearity range (M)	1×10^{-5} to 10^{-1}	1×10^{-5} to 10^{-1}	1×10^{-5} to 10^{-2}	1×10^{-5} to 10^{-2}
Correlation coefficient	0.9982	0.9925	0.9968	0.9888
Detection limit (M)	1.09×10^{-6}	2.50×10^{-6}	9.00×10^{-6}	2.65×10^{-6}
Life time (day)	~26 day	~11 day	~12 day	~19 day

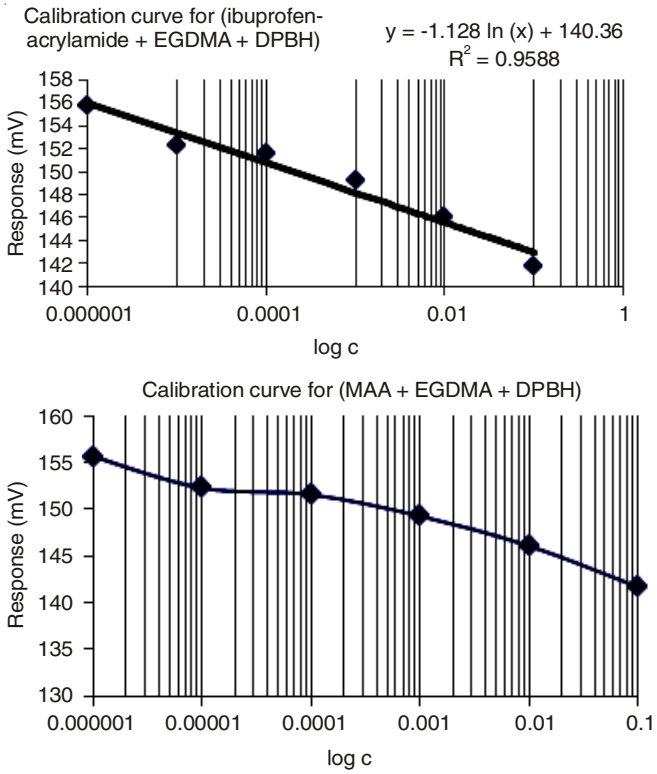

Fig. 1. Calibration curve of ibuprofen-MIP based on acrylamide as monomers and dibutyl phthalate as a plasticizer

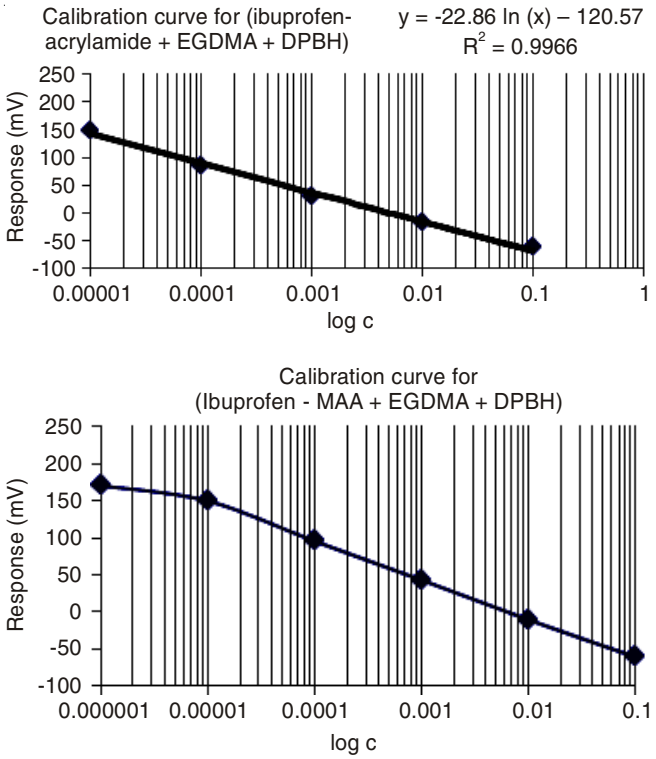

Fig. 2. Calibration curve of ibuprofen-NIP based on acrylamide as functional monomer and dibutyl phthalate as a plasticizer

TABLE-2
WORKING pH RANGES FOR IBUPROFEN SELECTIVE ELECTRODES

Membrane	Membrane composition	pH range		
		1×10^{-2}	1×10^{-3}	1×10^{-4}
A1	Ibuprofen-Acrylamide + EGDMA + DBPH	3.5-6.5	4.0-7.0	4.0-7.0
A2	Ibuprofen-Acrylamide + EGDMA + Oleic acid	4.0-8.0	4.0-7.5	4.0-7.5
A3	Ibuprofen-Acrylamide + EGDMA + Paraffin	4.0-7.5	3.5-7.5	4.0-7.0
A4	Ibuprofen-Acrylamide + EGDMA + ONPOE	4.0-7.0	4.0-7.5	4.0-7.5

TABLE-3
SELECTIVITY COEFFICIENTS OF ELECTRODE AT DIFFERENT CONCENTRATION OF IBUPROFEN OF A1 (IBUPROFEN-ACRYLAMIDE + EGDMA + DBPH) ELECTRODE

Ions	Concentration 10^{-1} M		Concentration 10^{-2} M		Concentration 10^{-3} M		Concentration 10^{-4} M	
	E_B (mV)	$K_{A,B}$	E_B (mV)	$K_{A,B}$	E_B (mV)	$K_{A,B}$	E_B (mV)	$K_{A,B}$
Li^+	17.1	2.135×10^{-1}	13.9	1.863×10^{-1}	56.3	5.750×10^{-1}	91.2	7.284×10^{-1}
K^+	12.6	4.793×10^{-2}	39.8	6.417×10^{-2}	63.1	1.055×10^{-1}	97.4	1.921×10^{-1}
Ca^{2+}	-1.2	3.034×10^{-2}	53.1	2.595×10^{-3}	88.6	3.582×10^{-3}	143.2	5.327×10^{-4}
Mg^{2+}	-23	3.096×10^{-2}	43.1	4.291×10^{-3}	78.7	5.894×10^{-3}	122.5	1.508×10^{-3}
Fe^{3+}	37.1	3.011×10^{-3}	97.3	1.304×10^{-4}	147.1	5.974×10^{-5}	183.7	1.496×10^{-5}
Al^{3+}	21.2	6.700×10^{-3}	61.7	-7.815×10^{-4}	93.8	8.721×10^{-4}	156.7	5.820×10^{-5}

TABLE-4
SELECTIVITY COEFFICIENTS FOR ELECTRODES AT DIFFERENT CONCENTRATIONS OF IBUPROFEN FOR A3 (IBUPROFEN-MAA + TMPTA + PARAFFIN) ELECTRODE

Ions	Concentration 10^{-1} M		Concentration 10^{-2} M		Concentration 10^{-3} M		Concentration 10^{-4} M	
	E_B (mV)	$K_{A,B}$	E_B (mV)	$K_{A,B}$	E_B (mV)	$K_{A,B}$	E_B (mV)	$K_{A,B}$
Li^+	86.1	5.135×10^{-1}	149.2	3.641×10^{-1}	171.5	5.750×10^{-1}	91.2	7.284×10^{-1}
K^+	81.8	6.470×10^{-1}	156.1	2.513×10^{-1}	172.3	2.540×10^{-1}	209.7	5.780×10^{-1}
Ca^{2+}	101.2	7.214×10^{-2}	157.3	2.356×10^{-2}	189.5	3.187×10^{-3}	241.3	1.058×10^{-3}
Mg^{2+}	119.6	6.184×10^{-2}	177.7	1.901×10^{-2}	202.7	1.568×10^{-3}	271.9	4.871×10^{-4}
Fe^{3+}	121.7	1.633×10^{-2}	193.1	1.597×10^{-3}	241.2	6.265×10^{-5}	296.3	1.186×10^{-5}
Al^{3+}	137.3	1.659×10^{-2}	179.3	3.353×10^{-3}	231.5	1.055×10^{-4}	299.1	1.021×10^{-5}

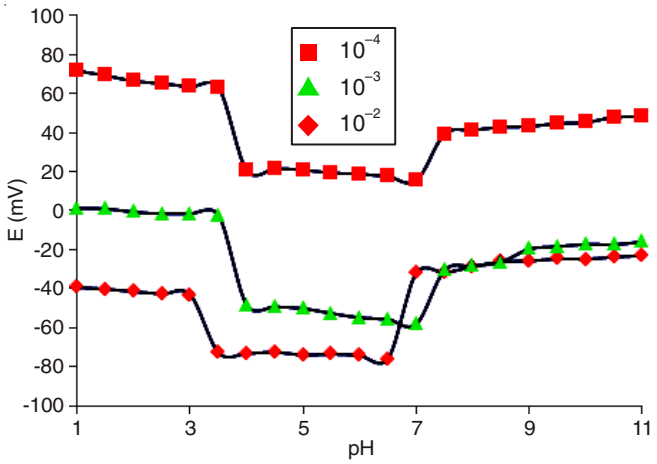

Fig. 3. Effect of pH on the potential of the ibuprofen electrode A1 (ibuprofen-acrylamide + EGDMA + DBPH) at concentration

$$K_{pot} = \Delta a_A/a_B,\ \Delta a_A = -a_A\text{-}a_A \qquad (2)$$

The second method used in measured selectivity coefficient was match potential method (MPM). Selectivity coefficient is given by using eqn. 2 is defined by the ratio of the activity of the primary ion relative to an interfering ion when they generate identical potentials in the same reference solution. In this method both monovalent ions are treated in the same manner and the valence of the ions does not influence the selectivity coefficient. The results of selectivity coefficient are shown in the Tables 5 and 6 were calculated from the concentration of interfering ions which endued the same amount of potential change as that induced by the increase of the concentration of primary ion.

Sample analyses: Three potentiometric techniques were used for the determination of (ibuprofen) *viz.*, direct method, standard addition method (SAM) and titration method. The following equation was used for standard addition method:

$$CU = CS/10\Delta E/S\ [1 + (VU/VS)] - (VU/VS) \qquad (3)$$

TABLE-5
SELECTIVITY COEFFICIENTS FOR IBUPROFEN ELECTRODES (10^{-3}) AND (10^{-1}) M OF INTERFERING-ION DETERMINED BY MATCH POTENTIAL METHOD (MPM)

Membrane composition	Interfering-ion (10^{-1}) M	log K_{pot}	
		ΔE = 40	ΔE = 60
Ibuprofen-Acrylamide + EGDMA + DBPH (A1)	Na^+	3.668×10^{-1}	1.043×10^{-1}
	Ca^{2+}	3.534×10^{-1}	6.520×10^{-1}
	Fe^{3+}	3.361×10^{-1}	1.196×10^{-1}
Ibuprofen-MAA + TMPTA + Paraffin (A3)	Na^+	6.767×10^{-1}	3.678×10^{-1}
	Ca^{2+}	6.561×10^{-1}	3.975×10^{-1}
	Fe^{3+}	5.076×10^{-1}	2.446×10^{-1}

TABLE-6
SELECTIVITY COEFFICIENTS FOR IBUPROFEN ELECTRODES (10^{-4}) AND (10^{-1}) M OF INTERFERING-ION DETERMINED BY MATCH POTENTIAL METHOD (MPM)

Membrane composition	Interfering-ion (10^{-1}) M	log K_{pot}	
		ΔE = 40	ΔE = 60
Ibuprofen-Acrylamide + EGDMA + DBPH (A1)	Na^+	1.562×10^{-1}	2.923×10^{-1}
	Ca^{2+}	1.448×10^{-1}	3.886×10^{-1}
	Fe^{3+}	1.653×10^{-1}	5.689×10^{-1}
Ibuprofen-MAA + TMPTA + Paraffin (A3)	Na^+	4.835×10^{-1}	2.024×10^{-1}
	Ca^{2+}	5.056×10^{-1}	2.197×10^{-1}
	Fe^{3+}	5.147×10^{-1}	2.430×10^{-1}

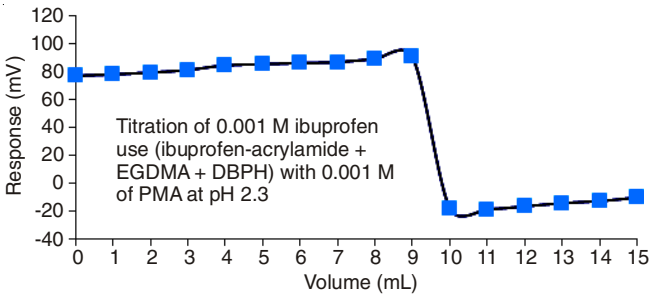

Fig. 4. Titration curve of electrode (ibuprofen-acrylamide + EGDMA + DBPH) for drug solution containing 0.001 M ibuprofen with 0.001 M of PMA as titrant solution

TABLE-7
DETERMINATION OF IBUPROFEN-ION SAMPLES BY POTENTIOMETRIC TECHNIQUE

Electrode No.	Concentration (M)			
	Sample	Measurements using potentiometric methods		
		Direct	Standard addition method	Titration
Iibuprofen - Acrylamide + EGDMA + DBPH (A1)	1×10^{-3}	1.013×10^{-3}	0.9964×10^{-3}	0.965×10^{-3}
	RSD %	1.331	0.553	0.798
	RC %	101.3	99.64	96.5
	RE %	1.3	-0.36	-3.5
	1×10^{-4}	1.024×10^{-4}	0.9906×10^{-3}	0.97×10^{-4}
	RSD %	1.212	0.284	1.041
	RC %	102.4	99.06	97
	RE %	2.4	-0.94	-3
Ibuprofen-MAA + TMPTA + paraffin (A3)	1×10^{-3}	0.979×10^{-3}	0.9906×10^{-3}	0.97×10^{-3}
	RSD %	0.118	0.619	1.871
	RC %	97.9	99.06	97
	RE %	-2.1	-0.94	-3
	1×10^{-4}	1.02×10^{-4}	0.9934×10^{-4}	0.965×10^{-4}
	RSD %	0.792	0.338	2.445
	RC %	102	99.34	96.5
	RE %	2	-0.66	-3.5

*Each measurement was repeated three times

TABLE-8
SAMPLE ANALYSES OF PHARMACEUTICAL IBUPROFEN USING A1 (IBUPROFEN-ACRYLAMIDE + EGDMA + DBPH) ELECTRODE

Electrode No.		Direct	Standard addition method	Titration
Apifen (India) (Ajanta)	Concentration prepared	1×10^{-3}	1×10^{-3}	1×10^{-3}
	Found	0.978×10^{-3}	0.982×10^{-3}	0.960×10^{-3}
	Recovery (%)	97.8	98.2	96.0
	RE (%)	-2.2	-1.8	-4
	RSD (%)	0.936	0.572	1.457
	Concentration prepared	1×10^{-4}	1×10^{-4}	1×10^{-4}
	Found	0.97×10^{-4}	0.976×10^{-4}	0.955×10^{-3}
	Recovery (%)	97	97.6	95.5
	RE (%)	-3	-2.3	-4.5
	RSD (%)	0.824	1.1	2.771
Profedin (Iraq) (SDI)	Concentration prepared	1×10^{-3}	1×10^{-3}	1×10^{-3}
	Found	0.983×10^{-3}	0.986×10^{-3}	0.950×10^{-3}
	Recovery (%)	98.3	98.6	95.0
	RE (%)	-1.7	-1.4	-5.0
	RSD (%)	1.631	0.882	2.018
	Concentration prepared	1×10^{-4}	1×10^{-4}	1×10^{-4}
	Found	1.033×10^{-4}	0.987×10^{-4}	0.96×10^{-4}
	Recovery (%)	103.3	98.7	96
	RE (%)	3.3	-1.3	-4
	RSD (%)	1.773	0.275	2.904

where CU, CS, VU and VS; are the concentration of unknown solution, volume of standard solution, volume of unknown and volume of standard solution. The results are listed Table-7.

For potentiometric titration, 10^{-3} M of dodeca-molybdophosphoric acid was used as a titrant. A typical titration plot is shown in Fig. 4.

The electrode (A1) was proved to be useful in the potentiometric determination of ibuprofen in pharmaceutical preparations and the data obtained for pharmaceutical samples are listed in Table-8.

REFERENCES

1. Martindale, The Complete Drug Reference, Pharmaceutical Press, London, edn 33, p. 64 (2002).
2. K.M. Algobahi and Y.M.H. Younis, *Int. J. Res. Pharm. Sci.*, **2**, 69 (2012).
3. P.S. Bonato, M.P.F.M. Del Lama and R. de Carvalho, *J. Chromagr. B*, **796**, 413 (2003).
4. R. Canaparo, E. Muntoni, G.P. Zara, C. Della Pepa, E. Berno, M. Costa and M. Eandi, *Biomed. Chromatogr.*, **14**, 219 (2000).
5. S.S. Chitlange, D.M. Sakarkar, S.B. Wankhede and S.G. Wadodkar, *Indian J. Pharm. Sci.*, **70**, 398 (2008).
6. S. Mitic, G. Miletic, A. Pavlovic, B. Arsic and V. Zivanovic, *J. Serb. Chem. Soc.*, **73**, 879 (2008).
7. R. Hamoudová and M. Pospíšilová, *J. Pharm. Biomed. Anal.*, **41**, 1463 (2006).
8. V.V. Dhavse, D.V. Parmar and P.V. Devarajan, *J. Chromatogr. B Biomed. Sci. Appl.*, **694**, 449 (1997).
9. M.B. Lippstone and J. Sharma, *J. Planar Chromatogr. Modern TLC*, **8**, 427 (1995).
10. M.R. Khoshayand, H. Abdollahi, M. Shariatpanahi, A. Saadatfard and A. Mohammadi, *Spectrochim. Acta A*, **70**, 491 (2008).
11. A.B. Thomas, N.G. Dumbre, R.K. Nanda, L.P. Kothapalli, A.A. Chaudhari and D.A. Deshpande, *Chromatographia*, **68**, 843 (2008).
12. A.L. Huidobro, F.J. Rupérez and C. Barbas, *J. Chromatogr. A*, **1119**, 238 (2006).
13. J. Sádecká, M. Cakrt, A. Hercegová, J.L. Polonský and I. Skacáni, *J. Pharm. Biomed. Anal.*, **25**, 881 (2001).
14. W. Wei, X.D. Yu and H.X. Ju, *J. Chromatogr. Sci.*, **42**, 155 (2004).
15. R.K. Nema, M. Verma and M. Kumar, *Int. J. Chem. Sci.*, **6**, 1638 (2008).
16. S. Bilge, Innovation in Chemical and Biology, Springer, Determination of ibuprofen, pseudoephedrine HCl, chlorpheniramine maleate and nipagen by liquid chromatography and fractional factorial design, Vol. 10, pp. 285-290 (2009).
17. European Pharmacopoeia, Council of Europe, Strasbourg, edn 5 (2002).
18. British Pharmacopoeia 98/34/EEC, The Stationery Office, London, p. 1024 (2005).
19. The United States Pharmacopoeia, USP-27/NF-22, Authority of the United States Pharmacopoeia Convention, Inc, Rockville, p. 987 (2004).
20. K. Haupt, Molecular Imprinting, Springer-Verlag Heidelberg (2012).
21. H.X. Ju, Z. Xueji and J. Wang, NanoBiosensing: Principles, Development and Application, Springer Science+Business Media, LLC, p. 268 (2011).
22. N. Harun, Section of Forensic Medicine and Science, Division of Cancer Sciences and Molecularly Pathology, Faculty of Medicine, University of Glasgow, February, pp. 88-89 (2010).
23. S. Li, Y. Gei, S.A. Piletsky and J. Lunec, Molecularly Imprinted Sensors, Elsevier B.V., pp. 36-41 (2012).
24. V.E. Pakade, Ph.D. Thesis, University of Witwatersrand, Johannesburg, South Africa, pp. 10-14 (2012).
25. A. Graggs, G.J. Moody and J.D.R. Thomas, *J. Chem.*, **51**, 541 (1974).
26. Y. Umezawa, K. Umezawa and H. Sato, *Pure Appl. Chem.*, **67**, 507 (1995).
27. K. Tohda, D. Dragoe, M. Shibata and Y. Umezawa, *Anal. Sci.*, **17**, 733 (2001).