RESEARCH ARTICLE

New Membranes for Determination of Norfloxacin in Pharmaceutical Formulations Ad Human Fluids based on Norfloxacin-methyl Orange as Ion Pair

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ABSTRACT

A simple, fast, and easy potentiometric method for the determination of norfloxacin in pharmaceutical formulations and human fluids was constructed. Three PVC membranes for norfloxacin (NFN) were prepared based on the use of ion-pair compound norfloxacin-methyl orange (NFN-MO) as the ion-association substance with different plasticizers: Tris (2-Ethylhexyl)phosphate (TEHP), Di-butyl phthalate (DBPH), and Di-butyl sebeact (DBS). These electrodes gave a Nernstain response equal to 58.09, 55.34, and 55.38 mV/decade with linear ranges 5×10^{-5}–1×10^{-2} M for membranes that depend on TEHP, DBPH and DBS as plasticizers. Detection limits were equal to 4.9×10^{-5}, 4.5×10^{-5}, and 4.0×10^{-5} M, respectively. pH range was also studied at different concentrations of norfloxacin solutions, equally were studied lifetime and selectivity. Potentiometric methods such as direct, standard addition, and multiple standard addition methods were used. The proposed electrode based on TEHP was successfully applied in the determination of norfloxacin in some pharmaceutical formulations and human fluids.

Keywords: Human fluids, Membrane, Norfloxacin, Pharmaceutical formulations, Potentiometric.

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INTRODUCTION

Norfloxacin (NFN) (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)quinoline-3-carboxylic acid,) (Figure 1) is a fluoroquinolone antibacterial agent which exhibits high antimicrobial activity in vitro against a wide variety of gram-negative and gram-positive bacteria, including gentamicin-resistant *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* species.[1] Norfloxacin has a remarkably broad spectrum of activity and excellent pharmacokinetics, allowing for once-daily dosing.[2] Enantiomers microbiological methods.[3,4] or liquid chromatographic techniques[10] with ICP-spectrometry,[5,7,16] fluorescence[8] and/or combination of Ultraviolet,spectrometry detection[11] Potentiometric[17] are analytical procedures suggested for NFN in literature. The former is unsuitable for routine control procedures, as each trial can take several days; laboratories often need adequate facilities for the safe handling of biological compounds. Chromatographic techniques are reliable, accurate, and durable, but they are not as expeditious as is sufficient for routine regulation. They may also lead to the release of highly toxic effluents. The same findings will refer to the electrophoretic procedures mentioned in the literature.[6] Other methods are based on immunoassays;[9] while detailed responses are given, the overall procedure is long and too costly for routine analytical measurements. Alternative and advantageous methods should be focused on speedy and efficient methods which provide highly precise and sensitive measurements. Ion-selective electrodes (ISEs) deliver high accuracy and speed, low analysis costs, improved selectivity and sensitivity over a wide range of concentrations.[13-18] In terms of selectivity and sensitivity, the most important component of these sensing devices is the ionophore or ion carrier because it's binding to the target ion is the molecular-level phenomenon sensed by an ISE. Ion exchangers and neutral macrocyclic compounds have been the "vital elements" of polymeric membrane potentiometric transduction used in the last decades; However, the design of sensing materials complementing the size and charge of a specific ion can lead to very selective

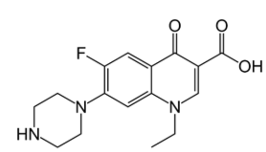

Figure 1: Chemical structure of the norfloxacin

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interactions, thereby improving the sensing unit's selectivity. This research aimed to build up a new ion-selective electrode for the determination of norfloxacin; the sensitivity of this electrode is based on the combination of norfloxacin-methyl orange as the sensing material. The electrode was successfully used for the determination of norfloxacin in pharmaceutical formulations and human fluids.

MATERIALS AND METHODS

Apparatus

Expandable ion analyzer, ORION, model EA940 with calomel reference and Silver-Silver Chloride (Ag/AgCl) electrodes were used in the potentiometry measurements). pH meter (PH700, made in Singapore) was used to measure the pH of the solution and a magnetic stirrer was used to carry out the measurements as shown in Figure 2.

Materials and Reagents

Norfloxacin standard ($C_{16}H_{18}FN_3O_3$) was obtained from Sigma Aldrich. The pharmaceutical formulations Norfloxacin tablets and drop were supplied from Ajanta Pharma Limited, India. Methyl Orange ($C_{14}H_{14}N_3NaO_3S$) was obtained from Fluka. The following plasticizers: di-butyl sebcate $C_{18}H_{34}O_4$, purity (98%), Tris (2-ethyl hexyl) phosphate (TEHP) ($C_{24}H_{51}O_4P$), purity (97%), Di-butyl phthalate (DBPH) ($C_{16}H_{22}O_4$), purity (99%) were obtained from Fluka. Other chemicals such as Tetrahydrofuran (C_4H_8O; FW 72), Polyvinylchloride CH_2-CHCl) n with high molecular weight, hydrochloric acid, and sodium hydroxide were supplied by Fluka., NaCl, KCl, LiCl, $CaCl_2$, $Mg(NO_3)_2$.$6H_2O$, $ZnCl_2$ $AlCl_3.6H_2O$, $Fe(Cl)_3$. $6H_2O$ and $CrCl_3.6H_2O$ were purchased from BDH. chemical solutions used in this work were prepared from distilled water.

Preparation of the Ion Pair

The preparation of (NFN-MO) ion-pair was performed by mixing 100 mL of 0.01 M solution of the NFN with 100 mL of 0.01 M methyl orange with stirring. The resulting precipitate was filtered off, washed with distilled water and then dried for one day.

Membrane Preparation

As described by Davis et al. (Moody and Thomas, 1980), the method of solidifying the norfloxacin into the PVC matrix membrane was developed. A 0.040 g of (NFN-MO) matrix was mixed with 0.360 g of plasticizer and 0.17 g of PVC powder, then added (5.0 or 6.0) mL of tetrahydrofuran (THF) with stirring until the formed viscous solution. The solution was poured into a glass casting ring about 30 mm in length and a diameter of 35 mm. It consists of two pieces; the glass cylinder is one of them, and the glass plate is the other. The two pieces were pasted together using a viscous mixture polyvinyl chloride-tetrahydrofuran (PVC-THF). The top side of the cylinder was covered with filter paper which put a heavyweight (~200 g). The assembly was left 2-3 days to permit graduate solvent evaporation.

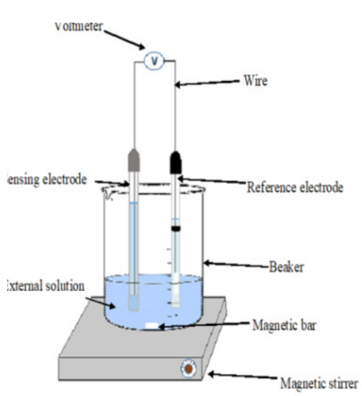

Figure 2: Digrame of ISE cell

Selectivity

Two methods were used to determine the selectivity coefficient of the potentiometric electrodes toward various species: the separate solution method (SSM),[19,] and the match potential method (MPM).[20] In the SSM method, the following equation was used:

$$K_{pot\ A,B} = a_A\ (1 - z_A/z_B)e^{(E_B - E_A)}\ z_A\ F/\ (RT) \qquad (1)$$

Where E_A is the potential of the drug and E_B for the interfering ions. While in the MPM method, the equation (2) was used:

$$K_{pot\ A,B} = (a_A` - a_A)\ /\ a_B \qquad (2)$$

Potentiometric Analysis

Direct Method

In this method, the potential of the sample was measured directly by using the neural foraminal narrowing (NFN) indicator electrode. The concentration was calculated by using the calibration curve of standard NFN.

Standard Addition Method

In this method, the sample of (10^{-4}) M with the volume of 10 ml is inserted followed by several additions of 0.1 mL of (1×10^{-2}) M. The potential was calculated before and after each addition. The concentration of the sample is calculated using equation (3).

$$C_U = C_S/_{10}[1+V_U/V_S]-(V_U/V_S) \qquad (3)$$

Where C_U, C_S the concentration of unknown and standard solution, respectively, V_U , V_S are the volume of unknown and standard solution, respectively, S: the slope of electrode, ΔE: the potential difference.

Application to Serum and Urine

A urine or blood sample was obtained from a healthy volunteer and spiked with 1×10^{-2} M NFN standard solution. The synthetic urine or blood sample was centrifuged at 2500 rpm for 10 minutes. Then, the top layer was separated then directly analyzed using the proposed sensors.[21]

RESULT AND DISCUSSION

Effect of Plasticizers

The plasticizer's polarity and chemical composition may significantly affect the ion-selective electrode's sensitivity, stability, selectivity, and dynamic response range. Three plasticizers (DBPH, TEHP, and DBS) were used in the development of three electrodes to choose an appropriate plasticizer for the construction of this electrode (Table 1). The effect of these plasticizers on the response of NFN electrodes was evaluated as shown in Figures 3-5.

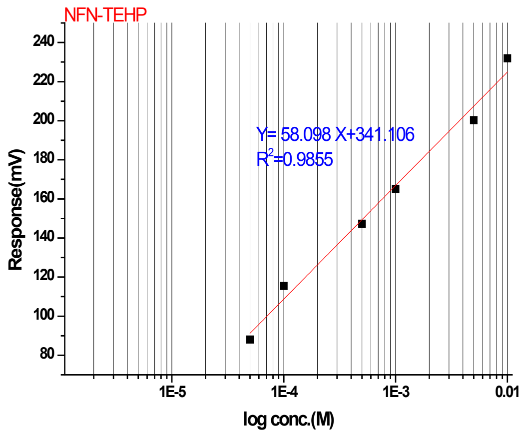

Figure 3: Calibration curve of NFN-TEHP.

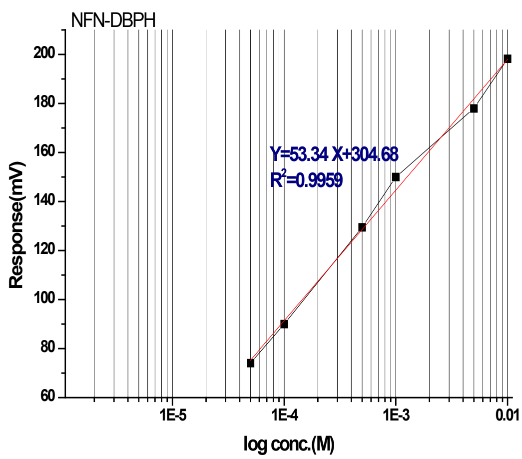

Figure 4: Calibration curve of NFN-DBPH.

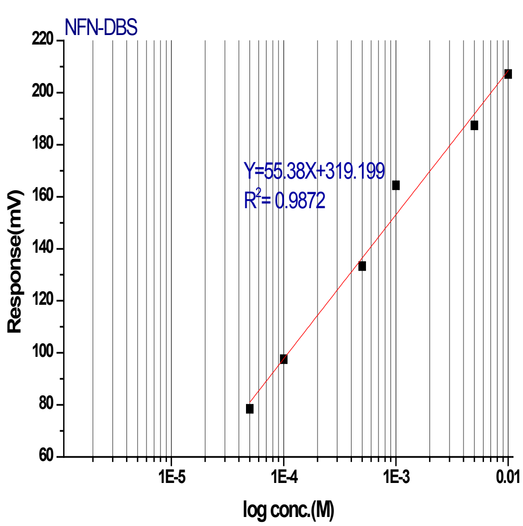

Figure 5: Calibration curve NFN-DBS.

Response Time

The response time was measured for the norfloxacin electrode based on DBPH, TEHP, and DBS for two concentrations (1×10^{-2}, 1×10^{-6}) M as shown in Figures 6, 7, and 8. The values of response time are increasing as the concentration decrease. This is attributed to the need for more time to reach the equilibrium between the ion- pair in the membrane and the external solution when the concentration of the external solution is too low.

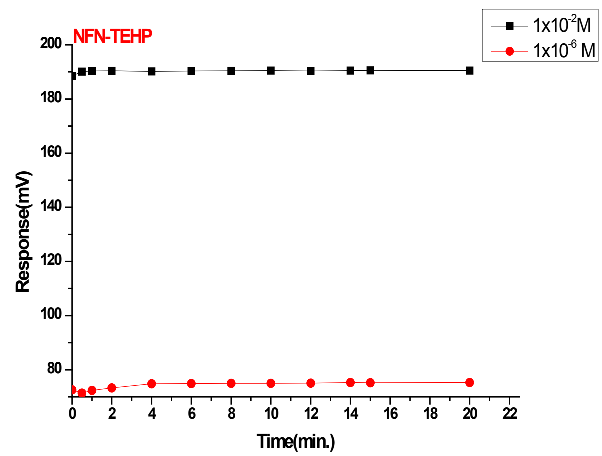

Figure 6: Response time of NFN-TEHP

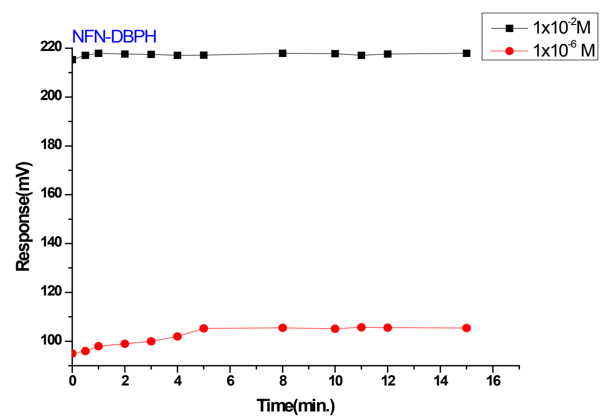

Figure 7: Response time of NFN-DBHP

Table 1: Effect of the plasticizers on the electrode

Parameters	*DBPH*	*TEHP*	*DBS*
Slop (mV/decade)	53.34	58.09	55.38
Detection limit (M)	4.5×10^{-5}	4.9×10^{-5}	4.0×10^{-5}
Linear range (M)	$5*10^{-5}$–$1*10^{-2}$	$5*10^{-5}$–$1*10^{-2}$	$5*10^{-5}$–$1*10^{-2}$
Lifetime (day)	12	30	34
pH	4.0–8.0	4.0–8.0	4.0–8.0
R	0.9979	0.9927	0.9936
Response time (min.)	0.3 at 10^{-2} M 5.0 at 10^{-6} M	0.5 at 10^{-2} M 4.0 at 10^{-6} M	0.4 at 10^{-2} M 2.5 at 10^{-6} M

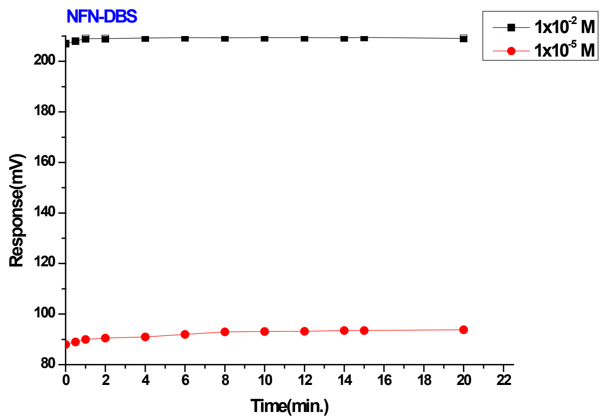

Figure 8: Response time of NFN-DBS

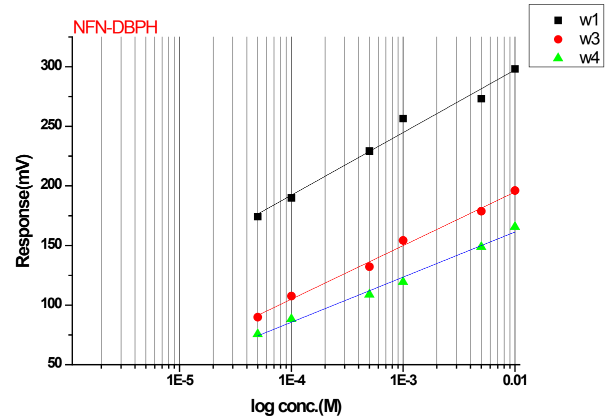

Figure 11: Lifetime of the (NFB-DBPH) electrode.

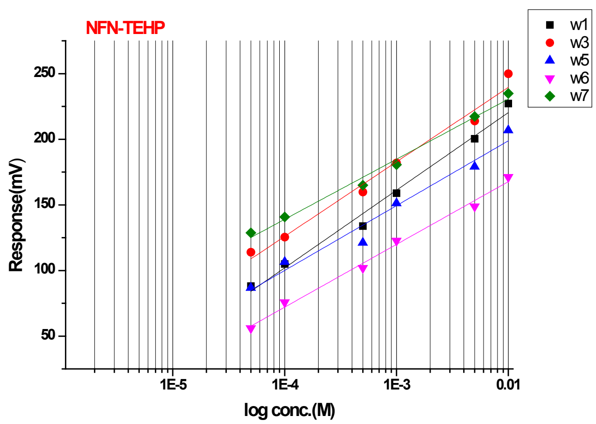

Figure 9: Lifetime of the (NFN-TEHP) electrode.

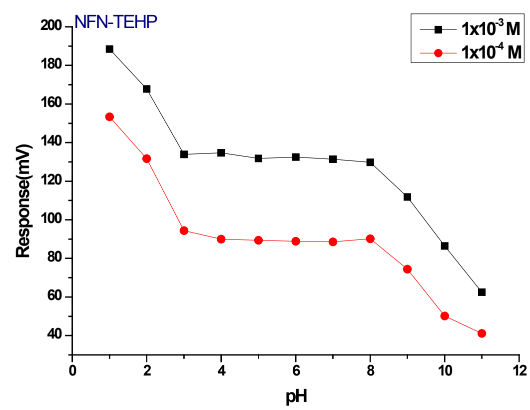

Figure 12: Effect of pH on NFN-TEHP electrode response

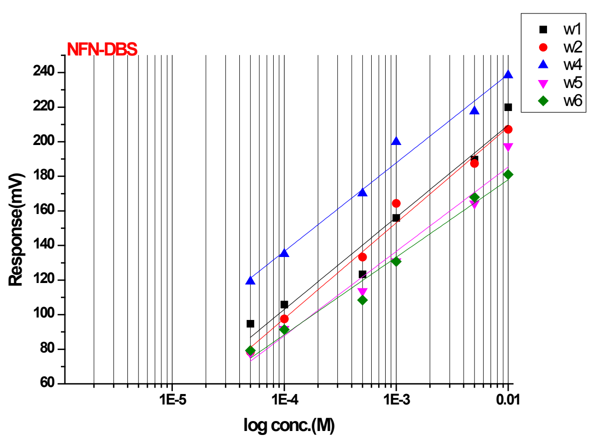

Figure 10: Life time of the (NFN-DBS) electrode.

Lifetime

The three constructed electrodes required 2 hours for conditioning in 1.0×10^{-2} M norfloxacin solution at room temperature to reach the stable potential. A calibration curve was created for electrode and the slope for the (NFN-TEHP), (NFN-DBS) and (NFN-DBPH) electrodes after 2 hours of soaking were 58.09, 55.38, and 53.34, respectively, but after 5 weeks of soaking the slope become 49.39 for electrode A and after 4 weeks of soaking the slop become 48.82 for electrode B while electrode C the slope become 47.33 after 14 days as shown in Figures 9-11

Effect of pH

The effect of pH on the electrode potential of selective electrode norfloxacin with different plasticizers (TEHP, DBPH, and DBS) was calculated by measuring the cell potential in norfloxacin solutions at two different concentrations 10^{-4}, 10^{-3} M in which the pH was measured from 1–11. pH was modified by adding sufficient quantities of hydrochloric acid and/or sodium hydroxide solution and the obtained results were shown in Figures 12–14. This meant that the proposed electrodes could be used to test a wide variety of norfloxacin solutions without changing the pH. The response of the electrodes has been increased very irregularly at pH values that have very high acidity. This may be due to the reaction of the electrodes to H^+ and analyte ions.

Selectivity

Selectivity behavior is clearly one of the most significant ion-selective electrode characteristics. It's determining whether the proposed electrode can gain a reliable measurement; as a result, the potential response was assessed in the presence of various interfering ions using. An essential characteristic

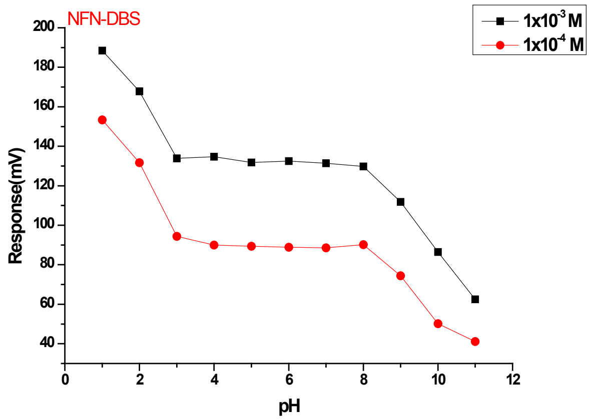

Figure 13: Effect of pH on NFN-DBS electrode response

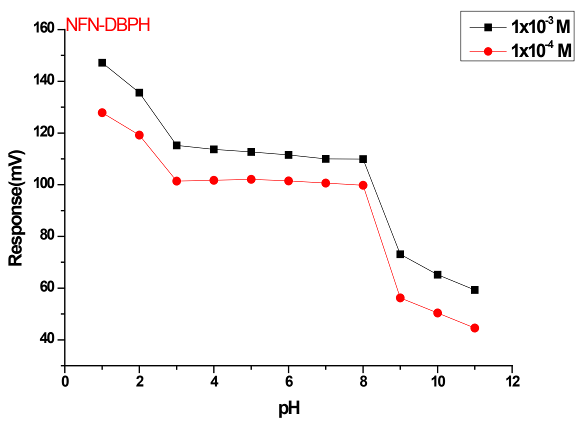

Figure 14: Effect of PH on NFN-DBPH electrode response

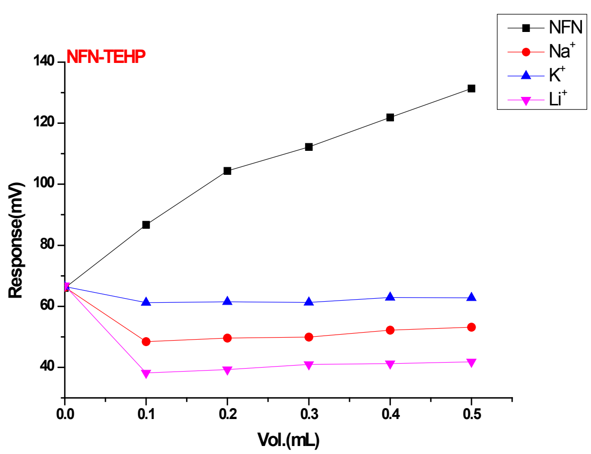

Figure 15: Selectivity For NFN-TEHP for mono-cations by MPM

of an ion-selective electrode was responded to primary ions in the existence of other ions in the same solution; this measurement was expressed in the term of potentiometric selectivity coefficient.[13] The influence of the interfering ions, which are (Li^{+}, Na^{+}, K^{+}, Ca^{+2}, Mg^{+2}, Zn^{+2}, Cr^{+3}, Fe^{+3}, Al^{+3}) on the electrode response using separate solution method (SSM)

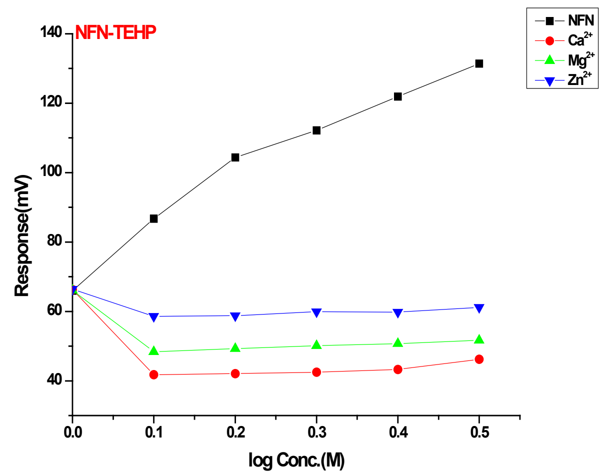

Figure 16: Selectivity For NFN-TEHP for Di-cations by MPM

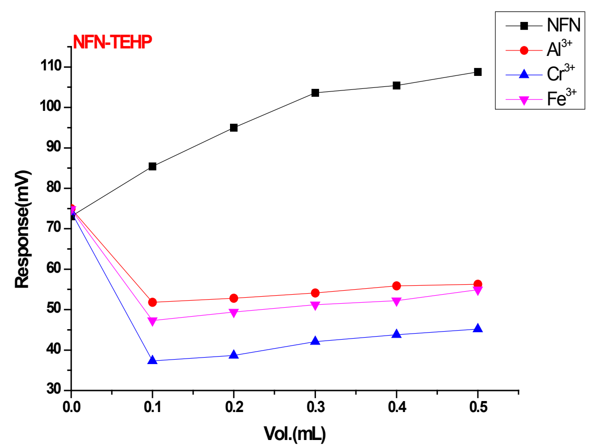

Figure 17: Selectivity For NFN-TEHP for tri-cations by MPM

and match potential method (MPM) were examined, as shown in Figures 15, 16, and 17. The selectivity coefficient values of the SSM are listed in Table 2

From Table 2, low values of selectivity coefficients were obtained, which means no interfering of these cations on the electrode (NFN-MO+TEHP) response, and Figures 15-17 show no interferences of the cations on Gatifloxacin at concentrations 10^{-4} M. Therefore; the selectivity coefficients cannot be determined because there is no difference in potential between the drug solution and interfering cation even at 5 mV or 10 mV

Analytical Application

In investigating electrode to the determination of norfloxacin in pharmaceutical preparations (NOR eye drops) using direct and standard addition methods.[15] The results were summered up in Tables 3-5. The suggested electrode was demonstrated to be useful in the potentiometric determination of NOR in pharmaceutical products and human fluids by direct, standard addition, and multi-standard addition methods.

Table 2: Selectivity coefficient values for the electrode ((NFN-MO+TEHP) at different concentration in the presences of some cations

Conc. (M)	$K_{A,B}$								
	Na^{+}	K^{+}	Li^{+}	Ca^{+2}	Mg^{+2}	Zn^{+2}	Al^{+3}	Cr^{+3}	Fe^{+3}
$1*10^{-2}$	0.017	0.010	0.016	$3.07*10^{-3}$	$2.27*10^{-3}$	$2.64*10^{-3}$	$9.48*10^{-4}$	$1.89*10^{-3}$	$2.19*10^{-3}$
$5*10^{-3}$	0.041	0.057	0.036	$5.79*10^{-3}$	$5.21*10^{-3}$	$6.06*10^{-3}$	$2.36*10^{-3}$	$2.82*10^{-3}$	$4.45*10^{-3}$
$1*10^{-3}$	0.264	0.316	0.297	$1.00*10^{-2}$	$1.30*10^{-2}$	0.012	$5.93*10^{-3}$	$3.37*10^{-3}$	$5.07*10^{-3}$
$5*10^{-4}$	0.312	0.455	0.520	$1.00*10^{-2}$	$1.50*10^{-2}$	$6.12*10^{-3}$	$5.66*10^{-3}$	$4.57*10^{-3}$	$4.39*10^{-3}$
$1*10^{-4}$	0.459	0.512	0.773	$6.77*10^{-3}$	$6.07*10^{-3}$	$2.65*10^{-3}$	$1.71*10^{-3}$	$1.61*10^{-3}$	$1.73*10^{-3}$
$5*10^{-5}$	0.642	0.889	0.431	$7.83*10^{-3}$	$9.12*10^{-3}$	$3.9*10^{-3}$	$2.41*10^{-3}$	$2.17*10^{-3}$	$2.11*10^{-3}$
$1*10^{-5}$	0.263	0.410	0.496	$1.40*10^{-3}$	$1.63*10^{-3}$	$1.05*10^{-3}$	$3.29*10^{-4}$	$3.65*10^{-4}$	$0.47*10^{-4}$
$5*10^{-6}$	0.344	0.384	0.467	$7.64*10^{-4}$	$1.04*10^{-3}$	$7.54*10^{-4}$	$1.15*10^{-4}$	$1.65*10^{-4}$	$1.42*10^{-4}$
$1*10^{-6}$	0.966	0.981	0.992	$1.01*10^{-3}$	$1.01*10^{-3}$	$8.57*10^{-4}$	$1.06*10^{-4}$	$1.30*10^{-4}$	$9.09*10^{-5}$

Table 3: Estimation of the pharmaceutical application and human fluids by a direct method

Drug	*Original conc.(M)*	*Found conc.(M)*	*RSD% n = 3*	*RC%*	*RE%*
Standard of norfloxacin	1×10^{-4}	1.03×10^{-4}	0.77	103	3
Norfloxacin (tablet)	1×10^{-4}	1.02×10^{-4}	0.75	102	2
Drop	1×10^{-4}	0.98×10^{-4}	0.63	98	-2
Urine	1×10^{-4}	0.97×10^{-4}	1.08	97	-3
Serum	1×10^{-4}	1.04×10^{-4}	1.04	104	4

Table 4: Estimation of the pharmaceutical application and human fluids by standard addition Method.

Drug	*Original conc.(M)*	*Found conc.(M)*	*RSD% n= 3*	*RC%*	*RE%*
Standard of norfloxacin	1×10^{-4}	1.02×10^{-4}	0.35	102	2
NORfloxacin (tablet)	1×10^{-4}	0.99×10^{-4}	0.10	99	-1
Drop	1×10^{-4}	0.96×10^{-4}	0.04	96	-4
Urine	1×10^{-4}	1.01×10^{-4}	1.11	101	1
Serum	1×10^{-4}	0.99×10^{-4}	0.70	97	-1

Table 5: Estimation of the pharmaceutical application and human fluids by mulit standard addition method

Drug	*Original conc. (M)*	*Found conc. (M)*	*RC%*	*RE%*
Standard of norfloxacin	$1*10^{-4}$	0.99×10^{-4}	99.0	-1.0
Norfloxacin (tablet)	$1*10^{-4}$	0.97×10^{-4}	97.0	-3.0
Drop	$1*10^{-4}$	0.99×10^{-4}	99.0	-1.0
Urine	$1*10^{-4}$	0.99×10^{-4}	99.0	-1.0
Serum	$1*10^{-4}$	0.98×10^{-4}	98.0	-2.0

CONCLUSIONS

New norfloxacin selective electrodes based on ion-pair complex of NFN-MO with different plasticizers were constructed. The best norfloxacin electrode was based on TEHP. This electrode was used for drug determination in pharmaceutical preparations and human fluids. The electrode based on TEHP gave excellent electrode parameters and no interference with several cations. The proposed analytical method is proved to be simple and rapid, with good accuracy

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