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A Novel Membrane Sensor for Batch and Flow Injection Potentiometric Determination of Cefazolin Sodium in Pharmaceutical Preparations

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A novel potentiometric sensor was prepared, characterized and utilized for static and continuous determination of cefazolin sodium (CFZN). Several metal-ion complexes and anion exchangers were tested as electroactive materials in plasticized polymeric membranes for selective detection of CFZN. Among different electroactive species tridodecyl methyl ammonium chloride (TDMAC) doped membrane electrode was found to exhibit optimal response characteristics. The optimized membrane sensor exhibited near-Nernstian responses (-55.64 mV decade^{-1}) over CFZN concentration range of 0.41 – 10 mM as measured in 50 mM acetate buffer, pH 5.5. The proposed sensor offers the advantage selectivity, does not require pre-treatment and possible interfacing with computerized and automated systems. It is worth noting that the developed membrane electrode exhibited good selectivity toward CFZN over other cephalosporins such as; cefradine, ceftazidime, cefadroxil, cefaclor and cefoperazone, as well as some additives encountered in the pharmaceutical preparations and so these sensors were successfully used for determination of CFZN. A tubular-type detector incorporating a TDMAC based membrane sensor was prepared and used under hydrodynamic mode of operation for continuous CFZN quantification. The tubular-type detector exhibited a concentration range from 0.5 – 10 mM with a near-Nernstian response (-53.91 mV decade^{-1}). Continuous monitoring of CFZN offers the advantages of simple design, ease of construction and possible applications to small volumes of drug solutions without pre-treatment. The developed sensor was utilized successfully in static and continuous modes of operation for the determination of CFZN in dosage forms. The results obtained were in good agreement with the standard method of CFZN analysis.

Keywords: cefazolin, membrane electrodes, flow analysis, potentiometry, ISEs.

1. INTRODUCTION

Since the discovery of penicillin in 1928, β-lactam antibiotics have been used. Cephalosporins have been used since 1948 and assumed a prominent role in modern antimicrobial therapy due to enhanced intrinsic microbiological activities and favourable safety profile [1]. Cefazolin sodium (CFZN) ($C_{14}H_{14}N_8NaO_4S_3$) (Fig 1), a member of the first generation cephalosporins, is active mainly against gram-positive bacteria and to a relative extent against gram-negative organisms and mainly used to treat bacterial infections of the skin. CFZN can also be used to treat moderately severe bacterial infections involving the lung, bone, joint, stomach, blood, heart valve and urinary tract [2]. Moreover, CFZN is clinically effective against infections caused by staphylococci and streptococci of Gram-positive bacteria. In addition CFZN is used as prophylaxis antibiotic before wide range of surgical operations [1].

Several analytical methods have been developed for CFZN determination in pharmaceutical formulations including spectrophotometry [3-6], spectrofluorimetry [7,8], high performance liquid chromatography (HPLC) [9–11], densitometry [12,13] and chemiluminescence [14]. Few reports were found in the literature concerning the electrochemical behaviour of CFZN by means of voltammetry in Britton–Robinson buffers (pH 2.0–11.0) at the mercury electrode [15]. However, the reported detection methods for CFZN were time-consuming and tedious and often suffer from different disadvantages, such as tedious pre-treatment procedures and expensive instrumentations.

Numerous solvent/polymeric membrane ion-selective electrodes (ISEs) have been proposed for quantitating both cationic and anionic drug species, primarily for quality control of pharmaceutical preparations. Because of their simplicity, sensitivity and selectivity, ion selective electrodes (ISEs) are among the most commonly used electrochemical devices applicable for direct and rapid measurement of various cations, anions, some gases, polyions and drugs [16-23]. However, only two records were found about using liquid polymeric membrane electrodes for cephalosporins determinations [24, 25]. Those electrodes, however, were characterized non-Nernstian slopes (-44.4 mV/decade), poor reproducibility, and high detection limit.

In this study, we demonstrated that membrane electrodes containing tridodecyl methyl ammonium chloride (TDMAC) as an anion-exchanger exhibited potentiometric selectivity toward CFZN over other cephalosporins, common ionorganic anions, and drug addtivies. The developed sensor was applied for accurate determination of CFZN in pharmaceutical preparations under static and hydrodynamic modes of operation (FIA). To the best of our knowledge this is the first report on development of membrane electrodes for determination of CFZN.

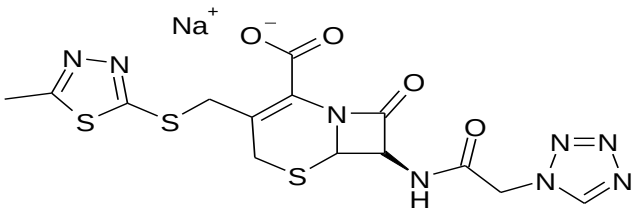

Figure 1. Chemical structure of cefazolin sodium (CFZN).

2. EXPERIMENTAL

2.1. Apparatus

All potentiometric measurements were made at ambient temperature with eight-channel electrode-computer interface (Nico2000 Ltd., UK) controlled by Nico-2000 software. A double junction Ag/AgCl reference electrode (Sentek, UK) was used for all mV measurements and combination glass electrode (Sentek, UK) was used for all pH measurements. The outer compartment of the reference electrode was filled with 1.0 M lithium acetate. The components of the FIA system were similar to those used previously [19]. The flow injection analysis (FIA) system manifold (Fig.2) consisted of a two channel Ismatech MS- REGLO model, peristaltic pump, polyethylene tubing (0.71 i.d.), and an Omni fit injection valve (Omni fit, Cambridge UK) with a sample loop of 150 µl volume. The potential signals were recorded using the data acquisition system described above. Spectronic^{TM} Genesys^{TM}, ultraviolet-visible spectrophotometer (Milton Roy Co., USA) with matched 1 cm quartz cells connected to an IBM computer loaded with the Winspec^{TM} application software was used for the determination of the CFZN using the standard method.

2.2. Materials and reagents

High molecular weight poly(vinyl chloride) (PVC) was obtained from Poly sciences (Warrington, PA). Dichloro-tin(IV)-tetraphenylporphorin (Sn TPP-Cl_2),copper-octaethylporphorin (Cu OEP), zinc-octaethylporphorin (Zn OEP), tridodecylmethylammonium chloride (TDMAC), tetradodecylmethylammonium bromide (TDMAB), bis(2-ethylhexyl) sebacate (DOS), benzyltributylammonium bromide (BTBAB), o-nitropheynloctylether (o-NPOE), ethylenebis-triphenylphosphine bromide (EBTPPB) and 2-fluorophenyl-2-nitrophenylether (FPNPE) were obtained from Fluka (Ronkonkoma NY). Tetrahydrofuran (THF) was purchased from Fisher (Fairlawn, NJ). Tris-(hydroxymethyl)aminomethane (TRIS) was obtained from Sigma (St. Louis, MO).Dichlorobis(triphenyl-phosphine)palladium(II) (Pd TPP-Cl_2) was obtained from Aldrich (Milwaukee, WI). The sodium salts of thiocyanate and sulfate were obtained from Matheson (Cincinnati, OH). Sodium salts of iodide and nitrate were purchased from J.T. Baker (Philipsburg, NJ).Sodium salt of acetate was obtained from Sigma (St. Louis, MO).Sodium chloride was obtained from Fisher Scientific (Cincinnati, OH). Cefazolin sodium (Bristol-Myers Squibb Pharmaceutical Co., Cairo, Egypt). Cefaclor monohydrate and cefradine anhydrous (Sigma Chemical Co., St. Louis, USA),cefadroxil monohydrate (Amoun Pharmaceutical Industries Co., APIC, Cairo, Egypt) and cefoperazone sodium (Pfizer Co., Egypt) were obtained as gifts and were used as supplied.Pharmaceutical formulations containing the studied drugs were purchased from the local market. All other reagents used were of analytical-reagent grade. All experiments were performed using doubly distilled water.

Acetate buffer was prepared by titrating 50 mM solution of the acid form with concentrated sodium hydroxide to a pH-value of 5.5 ± 0.01. Phosphate buffer was prepared by titrating 50 mM solution of the acid form with concentrated sodium hydroxide to a pH-value of 7.0 ± 0.01. Tris buffer

was prepared by titrating 50 mM of the base form with concentrated hydrochloric acid to a pH-value of 7.4 ± 0.01.

A stock solution (10 mM) of CFZN at pH 5.5 was prepared by dissolving 47.7 mg of CFZN in 10 ml acetate buffer pH 5.5. Dilute solutions of CFZN were prepared by diluting the stock solution using acetate buffer (pH 5.5). The stock and working standard solutions of CFZN were freshly prepared.

2.3. Polymer membrane electrode preparation

Polymer membrane electrodes were prepared according to the literature procedures [18, 21, 23]. Membrane cocktails were prepared by dissolving appropriate amounts of electro-active materials, different plasticizers, PVC and various mole percentages of additives (relative to the ionophore weight) in ~ 2 mL of THF (see Tables 1 for membrane compositions). A homogeneous mixture was obtained after complete dissolution of all membrane components. Then the mixture was poured into a 22 mm i.d. glass ring placed onto a glass plate and the ring was covered with a filter paper till complete evaporation of THF and formation of a transparent membrane (average thickness of ~ 0.2 mm). Smaller discs of ~ 5 mm diameter were cut out from this master membrane with a cork borer and glued to the end of a PVC tube (8 mm diameter and 3 cm length) by means of THF. The sensor body consisted of a 1-mL pipette tip attached to the PVC tube. An internal filling solution composed of an equal volume of 1 mM CFZN and sodium chloride solutions was used. Ag/AgCl wire (0.3 mm diameter) was used as an inner reference electrode.

Prior to use, membrane electrodes were conditioned for ~24h in a solution having the same composition as the internal filling solution and were stored in the 1 mM NaCl solution when not in use. Potentiometric responses of the prepared membrane electrodes were determined by recording cell potential as a function of CFZN concentrations at ambient temperature. The potentials readings were recorded at a constant stirring when stabilized to ±0.2 mV, and the emf was plotted as a function of logarithm [$CFZN^-$] concentration. A double junction Ag/AgCl reference electrode was utilized in all measurements.

2.4. Preparation of tubular detector and FIA set-up

Flow Injection set-up and the tubular sensor used therein were constructed according to the literature procedures [21]. Casting solution for such electrode was prepared by dissolving 1.0 wt% TDMAC, 66 wt% o-NPOE, 33 wt% PVC in ~ 1 mL of THF. The casting solution was deposited using a micro dropper into a hole (3mm wide x 5 mm length) made in the middle of a 5 cm Tygon tube. After each casting the THF was allowed to evaporate. This casting process was repeated 5 times. The final ISE tube was allowed to dry completely for an additional 1 h. The tube was then inserted into a pipette tip which was sealed in place (to prevent leakage of the internal reference solution). The electrode assembly was completed by filling the inside of the tip with a filling solution (sufficient to cover the tubular electrode) and inserting Ag/AgCl reference wire. The tubular sensor was inserted into

the flow injection system and 50 mM acetate buffer, pH 5.5 was used as a carrier solution at a flow rate of 2 ml min-1. The tubular electrode was located at a distance of about 30 cm from the injection valve and at a distance about 20 cm from the waste container. A double junction Ag/AgCl reference electrode was placed downstream. The detector was calibrated at 25^{o}C under hydrodynamic mode of operation by injection of CFZN standard solutions in the range 0.5-10 mM through a valve loop of about 150 µl volume in the carrier stream. After the baseline was reached the potential signals were recorded using the data acquisition system described above.

2.5. Determination of CFZN in pharmaceutical preparations

The optimized TDMAC-based membrane electrode was applied for the analysis of the cited drug (CFZN) in its pharmaceutical preparations. CFZN is found in the market in the form of injections (Zinol® 0.5 gm and Zinol® 1 gm) for intravenous and intramuscular injections. An accurately weighed amount the drug was transferred into a 10-mL volumetric flask, dissolved in about 5 mL of 50 mM acetate buffer, pH 5.5, diluted to the mark with the same buffer, mixed well and filtered. The first portion of the filtrate was rejected. Further dilutions with the same buffer were made and then the general procedures of static and continuous determination of the investigated drug were followed (see below).

For batch analysis of CFZN using the optimized membrane sensor, a 10-ml aliquot of the diluted drug solutions were potentiometrically measured using the optimized sensor. The potential readings of the CFZN samples were recorded then CFZN concentrations were calculated using a calibration graph. In case of FIA, at a flow rate 2 ml min^{-1} a flow stream of 50 mM acetate buffer of pH 5.5 carrier solution was allowed to pass through the tubular electrode. The drug test solutions were then injected in triplicates into 150 µl injection loop as described below and the average potential readings were compared with a calibration plot.

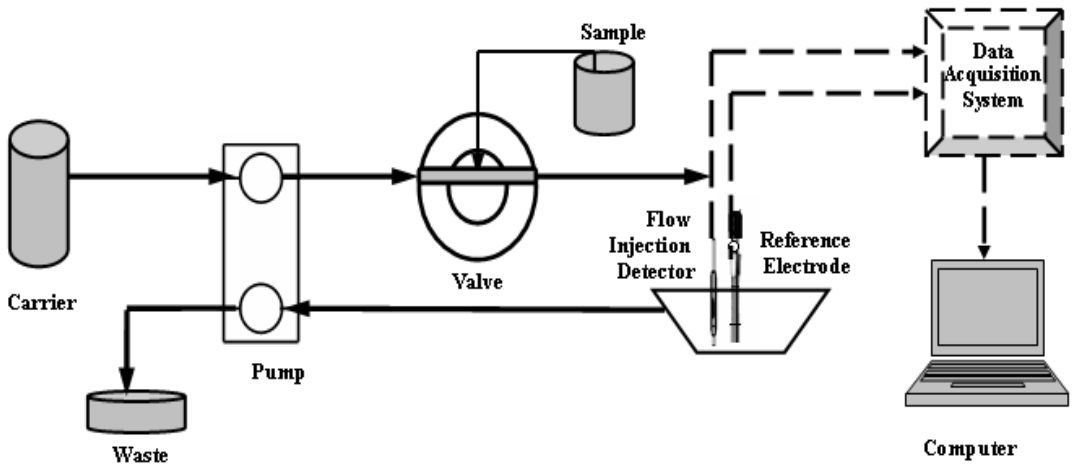

Figure 2. Manifold for the FIA set up used for the determination of CFZN.

3. RESULTS AND DISCUSSION

3.1. Response characteristics of the developed sensors

Plasticized PVC membrane electrodes formulated with different electro-active species were prepared and evaluated (see Table 1 for membrane compostion). Four different types of anion exchangers (TDMAB, TDMAC, BTBAB and EBTPPB) and four types of metal-ion complexes (PdTPP-Cl_2, SnTPP-Cl_2, CuOEP and Zn OEP) were tested.

Figure (3) shows typical potentiometric responses toward CFZN obtained using NPOE plasticized PVC membrane electrodes formulated with different types of anion exchangers (TDMAC, TDMAB, BTBAB and EBTPPB) as measured in 50 mM acetate buffer, pH 5.5. As can be seen in Figure (3) TDMAC based membrane sensor exhibited the best performance characteristics in terms of linear range and slope of the potentiometric response towards CFZN. Table (2) shows the response characteristics of TDMAC based membrane sensor (E1) evaluated according to IUPAC recommendations [26] using data collected over a period of 4 weeks under static conditions. Membrane electrodes formulated with TDMAB ion-exchanger (E4 and E5) exhibited sub-Nernstian slopes and poor reproducibility. Membrane electrodes formulated with BTBAB (E6) and EBTPPB (E7) exhibited no or minimal anionic responses towards CFZN, respectively (see data in Table 1).

Potentiometric responses of membrane electrodes formulated with different metal ion complexes towards CFZN were also investigated. Metal-ion complexes based membrane electrodes are expected to exhibit more selective responses towards anionic drugs compared to anion-exchangers based membrane electrodes [17]. Therefore Cu, Zn, Pd, and Sn metal ion complexes were tested as ionophores for CFZN in polymeric membrane electrodes. Such central metal ions were selected based on the well-known CFZN interactions with those metal ions [27]. Unfortunately, data obtained for metal-ion complexes based membrane electrodes (E8-E11) indicated that such metal ion complexes do exhibit selective interaction with CFZN. For instance, membrane electrodes based on Pd TPP-Cl_2 (E8), and Sn TPP-Cl_2 (E9) exhibited minimal anionic responses towards CFZN. However, membrane electrodes formulated with CuOEP (E10) and ZnOEP (E11) exhibited sub-Nernstian responses of -32.03 and -37.04 mV decade^{-1}, over a concentration ranges of (0.76 -10) mM and (0.31 -10) mM, respectively (Fig.4 and Table 1). The observed anionic responses observed in case of E10 and E11 membrane electrodes are attributed to the presence of 50% TDMAC as an additive in the membrane composition. This notion was based on the observed responses for different anions which showed Hofmeister selectivity pattern (data no shown). The lack of selective and sensitive anionic response of membrane electrodes formulated with metal-ion complexes towards CFZN could be due to the high hydrophilicity of the drug [2] (see the selectivity order based on ion-exchange mechanism below) and more importantly the weak binding ability of CFZN to the central metal ions of the tested complexes. Therefore, membrane electrodes formulated with TDMAC were selected for further investigations, because they exhibited the closest slope to the Nernstian response (-55.64 mV decade^{-1}), widest linear range (0.41-10 mM) and best detection limits.

Plasticizer is a very important constituent of polymer membrane electrodes and strongly affects most potentiometric response characteristics such as detection limits [28] sensitivity and more

importantly selectivity [29]. The type of plasticizer controls to much extent the dielectric constants (ε) of the membrane phase, which strongly affects ion-ionophore interactions as well as the partition of ions between aqueous and membrane phases. To investigate the effect of plasticizers on the response characteristics of the CFZN sensitive membrane electrodes formulated with TDMAC three different plasticizers with wide range of Ɛ were used in the formulation of such membrane electrodes. Those plasticizers are *o*-NPOE (Ɛ= 24), FPNPE (Ɛ= 50), and DOS (Ɛ= 3.88) [30]. The performance characteristics of TDMAC based membrane electrodes formulated with the three plasticizers are shown in Figure (5) and depicted in Table (1). As can be seen, membrane electrodes constructed with *o*-NPOE exhibited closest response to the Nernstian slope (-55.64 mV decade^{-1}) compared to those formulated with DOS (-50.9 mV/decade^{-1}) and FPNPE (-42.7 mV decade^{-1}). Therefore membrane electrodes formulated with TDMAC and plasticized with *o*-NPOE were selected for further investigations.

Different buffers (acetate, pH 5.5; phosphate, pH 7.0; and Tris, pH 7.4) to select the buffer system that results in the best performance characteristics for TDMAC based membrane electrodes sensitive towards CFZN. It was found that no responses were obtained using Tris and phosphate buffers. However, in acetate buffer pH (5.5) the electrode response towards CFZN was near-Nernstian response. This may be attributed to fact that upon using acetate buffer pH 5.5 about 99.9% of CFZN was ionized to the anionic form [CFZN$^-$] so maximum response was obtained at this pH and upon increasing the pH, CFZN was chemically degraded (pKa of CFZN; 2.1(COOH)) [31]. Moreover, the deterioration of the response at slightly high pH could be due to the hydroxide ion interference. Therefore acetate buffer was selected for practical application the optimized membrane electrode. The response characteristics (e.g., response time, linear range, detection limit.....etc.) of the optimized membrane electrode are summarized in Table (2).

Table 1. Composition and response characteristics of metallo-porphyrins and ion-exchangers based membrane electrodes for determination of CFZN

Membrane sensor	Plasticizer	Electroactive species, 1 wt%	Additive	Slope	Linear range
E_1	*o*-NPOE	TDMAC	-	-51.6	0.41 – 10 mM
E_2	DOS	TDMAC	-	-50.1	0.41 – 10 mM
E_3	FPNPE	TDMAC	-	-43.0	0.41 – 10 mM
E_4	*o*-NPOE	TDMAB	-	-22.4	0.31 – 10 mM
E_5	DOS	TDMAB	-	-20.6	0.31 – 10 mM
E_6	*o*-NPOE	BTBAB	-	NR	NR
E_7	*o*-NPOE	EBTPPB	-	-45.98	4.3 – 10 mM
E_8	o-NPOE	Pd TPP-Cl_2	-	-7.98	0.31 – 10 mM
E_9	*o*-NPOE	Sn TPP-Cl_2	-	-8.97	0.31 – 10 mM
E_{10}	*o*-NPOE	Cu OEP	TDMAC (50mol%)	-32.0	0.76 – 10 mM
E_{11}	*o*-NPOE	Zn OEP	TDMAC (50mol%)	-37.0	0.31 – 10 mM

All membranes were prepared using 66 wt% plasticizer: 33 wt% PVC.

NR: no response was observed

Table 2. Summary of response characterization of TDMAC based membrane sensor under static mode of operation for determination of CFZN

Parameter	TDMAC *
Slope, (mV per decade)	-55.6
Lower limit of linear range, (mM)	0.41
Response time ($t_{95\%}$): For conc. < 10^{-3} (sec.) For conc. ≥ 10^{-3} (sec.)	 <30 <10
Working range, (pH)	4.6 – 6
Detection limit, (mM)	0.34
Life span, (week)	8

* Average of five measurements.

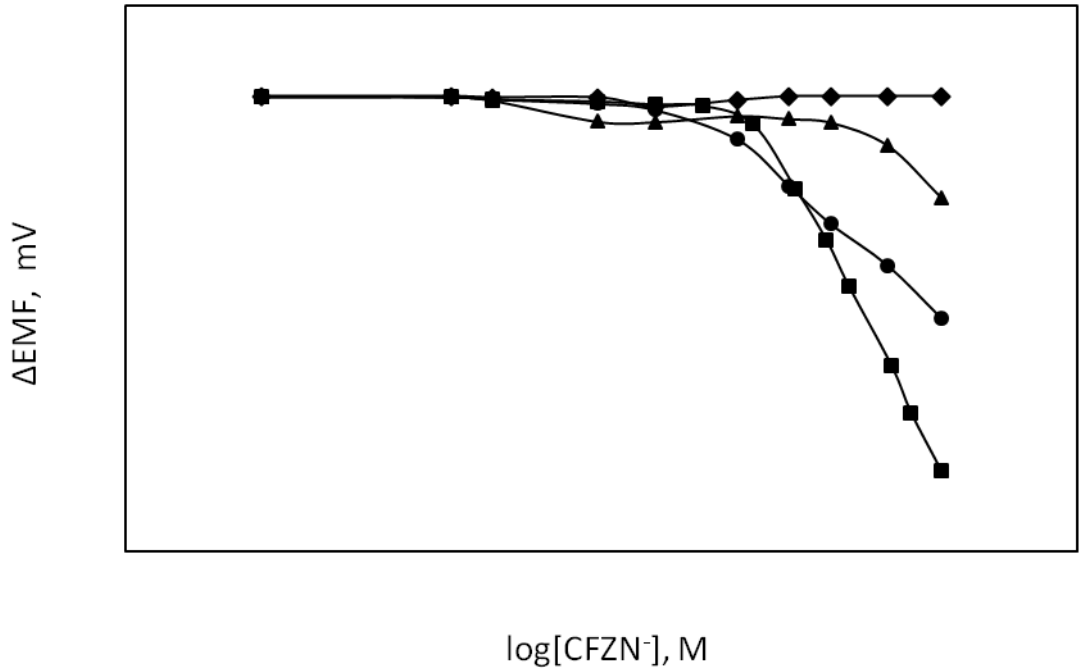

Figure 3. Potentiometric response of various PVC membrane electrodes towards CFZN measured in 50 mM acetate buffer, pH 5.5. The ion-exchangers tested were (■) TDMAC, (●) TDMAB, (♦) BTBAB and (▲) EBTPPB.

The potentiometric responses of ISEs are greatly affected by the pH of the medium due to hydroxide/hydronium ion interferences. Consequently, it is important to determine operative pH range of ISEs. The potentiometric responses of TDMAC based membrane sensor were examined at different pH values over a pH range of 3.21-11.28 (Fig.6). This was performed in presence of 1.0 and 10 mM concentrations of CFZN. At each concentration level the pH of CFZN solution spanned over wide pH range by addition of minimal volumes of either hydrochloric acid or sodium hydroxide solutions. The potential of TDMAC membrane electrode was then recorded at each pH value. As can be seen in Figure (6) the potential of the potential readings of TDMAC based membrane sensor is practically

independent of pH in the pH range of 4.5 - 6.0. The potentials of the prepared membrane sensor slightly increased upon pH decrease due to the hydronium ion interference and the decrease on the ionized/non ionized ratio of CFZN. While, upon pH increase over pH 6, the potential readings declined up to pH 8.0 then the readings remained almost stable and reached a plateau. This decrease in potential may be attributed to progressive degradation of CFZN (see above) and the hydroxide ion interference.

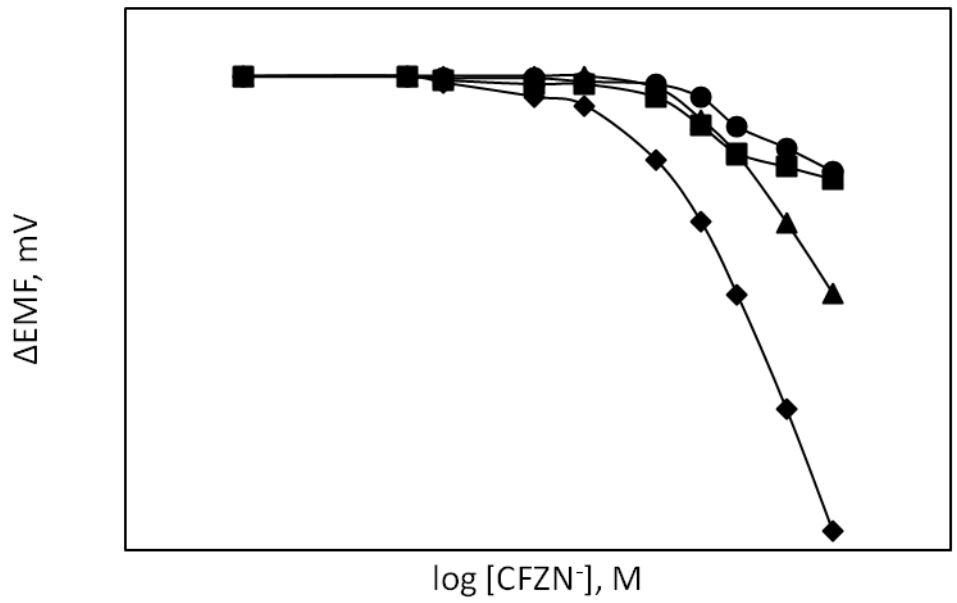

Figure 4. Potentiometric response of various PVC membrane electrodes towards CFZN measured in 50 mM acetate buffer, pH 5.5. The ionophores tested were (■)Pd TPP-Cl_2, (●)Sn TPP-Cl_2, (▲)Cu OEP and (♦)Zn OEP.

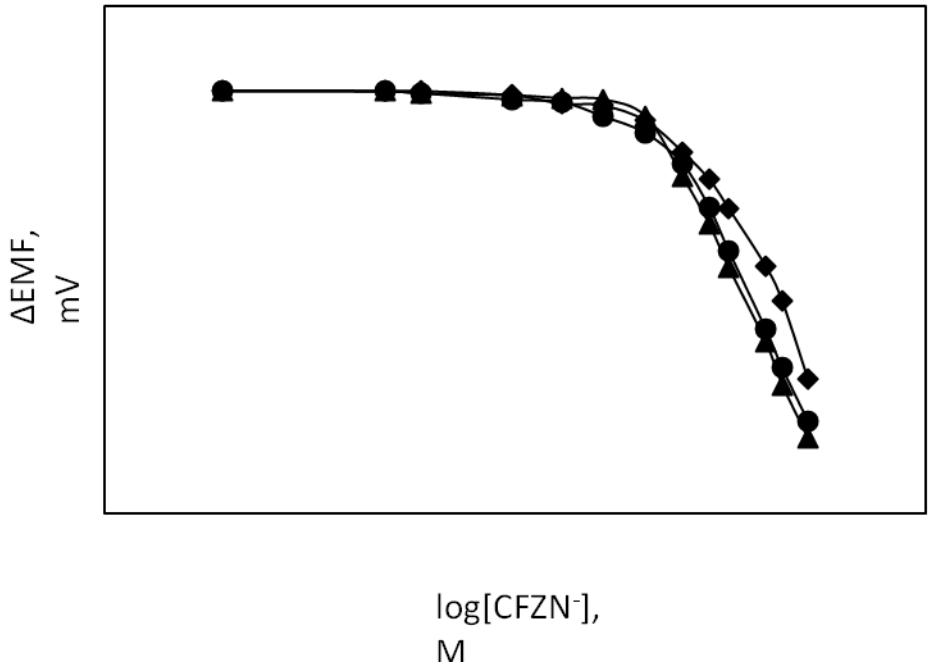

Figure 5. Potentiometric response of TDMAC based membrane electrodes formulated with different solvents mediators; (▲) *o*-NPOE, (●) DOS and (♦) FPNPE.

The dynamic response time of CFZN sensitive membrane electrode was assessed in order to determine its feasibility for automation. The dynamic response time was investigated by recording the potential responses of TDMAC membrane electrode at time intervals of 5 seconds upon changing the concentration level of CFZN. The relationship between the potential readings and the response time was plotted and the time required to attain 95% of the equilibrium ($t_{95\%}$) was determined and found to be less than 10 s for drug concentration ≥ 1.0 mM. These results indicated that the proposed sensor can be applied for CFZN determination in continuous mode of operation (see data in Figure 7).

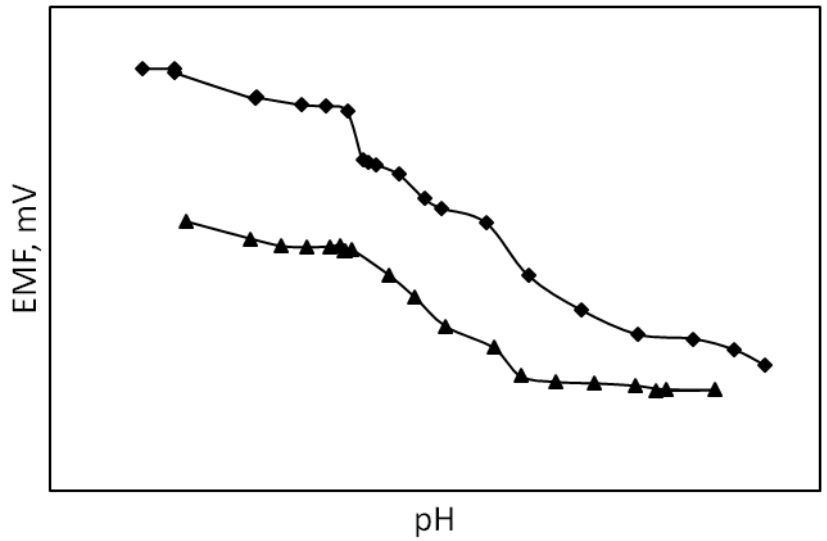

Figure 6. Influence of pH on the potentiometric response of TDMAC based membrane sensor in presence of: (▲) 10 mM and (♦) 1 mM CFZN.

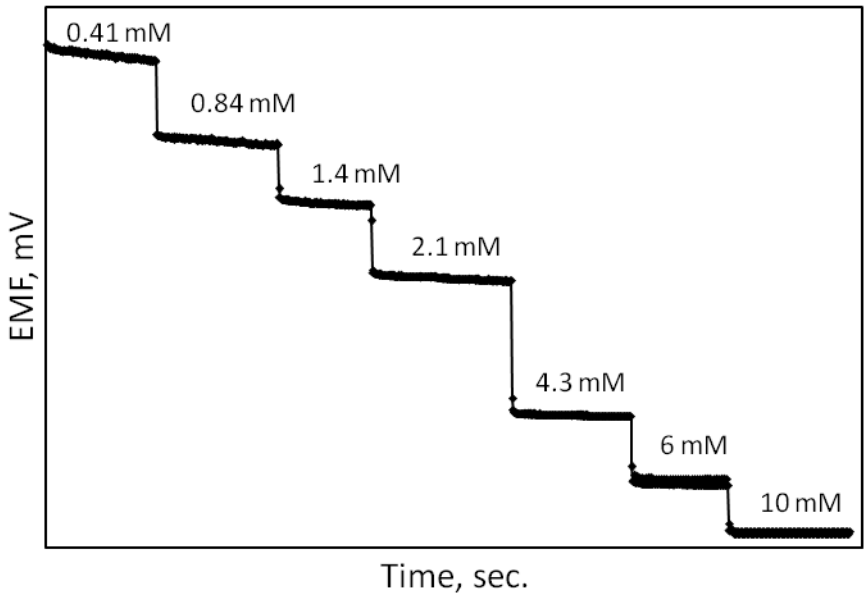

Figure 7. Response time of TDMAC based membrane sensor towards CFZN measured in acetate buffer pH 5.5.

Ideally, ISE do not respond to any other ions from the sample solution except to primary ions. In reality, however, interfering ions compete with primary ions, and they can be extracted into the membrane. The modified separate solution method was used for calculations of unbiased selectivity coefficients following the literature procedure [32]. The potentiometric selectivity coefficients were calculated using the cell EMF values obtained by extrapolating the Nernstian response region to 1 M for both interfering ion and CFZN ion. The selectivity coefficient values were calculated using the following equation (1) [32],

$$K_{CFZN^-,j}^{pot} = \exp\left(\frac{z_{CFZN^-}F(E_j^0 - E_{CFZN^-}^0)}{RT}\right) (1)$$

where, $E_{CFZN^-}^0, E_j^0$ are the potential values of the $CFZN^-$ and interfering ions at 1 M, respectively. z_{CFZN^-} is the charge of the $CFZN^-$ ion. R, T and F have their regular meanings. It should be mentioned that selectivity coefficients were calculated as described above if Nernstian responses were obtained for the primary and the interfering anion. Maximal limiting potentiometric selectivity coefficients of anion that induced very little or sub-Nernstian responses are calculated according to the literature recommendations [32]. If the primary ion response was not Nernstian, theoretical slopes were used in calculation of the selectivity coefficients.

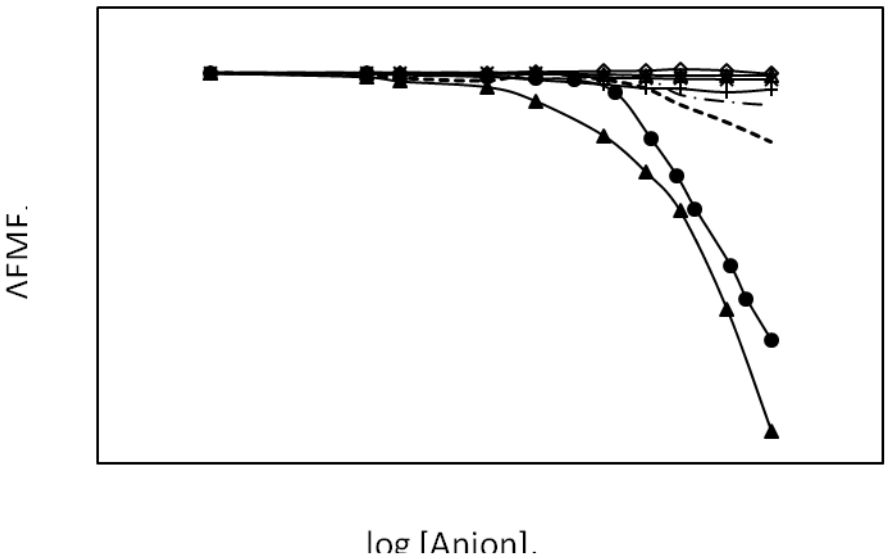

Figure 8. Potentiometric responses of CFZN sensitive electrodes using TDMAC based membrane electrodes, measured in 50 mM acetate buffer pH 5.5, towards various anions, additives and some cephalosporins: (●) $CFZN^-$, (---) Cefadroxil, (◊) Cefoperazone, (….) Cl^-, (Δ) SO_4^{-2}, (▲) NO_3^-, (○) CH_3COO^-, (*) Cefradine, (—|—) Cefaclor, (-.-.-.) Ceftazidime, (…♦…) glucose, (□) fructose and (—) maltose.

No correction was made for the slight changes in the liquid junction potential of the cell as a function of increasing the test anion activities. The selectivity coefficients of CFZN sensitive

membrane sensor were at 50 mM acetate buffer, pH 5.5. Potentiometric selectivities of the proposed sensor were for other cepahlosporins, commony inorganic anions and some additives used in the drug formulations. As shown in Figure (8) and data depicted in Table (3), it was found that TDMAC based membrane sensor exhibited good selectivity towards CFZN over other cephalosporins such as; cefradine, ceftazidime, cefadroxil, cefaclor and cefoperazone, as well as other additives found in the pharmaceutical preparations such as; glucose, fructose and maltose. This indicates that the optimized sensor could be used successfully for determination of CFZN in presence of other cephalosporins and other pharmaceutical additives. The selectivity pattern observed was found to follow the Hofmeister series as follows: SCN^- > I^- > NO_3^- > $CFZN^-$ > cefadroxil > ceftazidime > Cl^- > acetate > cefaclor > cefradine > SO_4^{-2} > cefoperazone (see data in Table 3). It is worth mentioning that anions that exhibited interferences (e.g., SCN^-, I^-, NO_3^-) are not present in drug formulations.

Table 3. Potentiometric selectivity coefficients of *o*-NPOE plasticized TDMAC based membrane sensor

Interfering ion	$\log K^{pot}_{CFZN,\beta}$
Cl^-	≤ -2.47
SO_4^{-2}	≤ -2.67
NO_3^-	0.68
I^-	1.69
SCN^-	2.36
CH_3COO^-	≤ -2.58
Glucose	≤ -2.42
Fructose	≤ -2.47
Maltose	≤ -2.50
Cefradine®	≤ -2.65
Cefadroxil®	≤ -1.86
Cefaclor®	≤ -2.57
Cefoperazone®	≤ -2.70
Ceftazidime®	≤ -2.43

[a]maximal selectivity limit (≤) was used for ions that exhibited minimal response towards interferent ions [32].

3.2. Tubular CFZN sensitive membrane electrode

A tubular-type detector (Fig.2) incorporating a TDMAC based membrane sensor was prepared and used under hydrodynamic mode of operation for continuous CFZN quantification. A linear relationship between CFZN concentration and FIA signals was obtained over a concentration range 0.5 – 10 mM as measured in 50 mM acetate buffer, pH 5.5 (Fig.9 inset). The slope of the calibration plot was near-Nernstian (50.1 mV $decade^{-1}$). The relative standard deviation of FIA potential signals was better than ± 2%. Table (4) shows the general response characteristics of the tubular TDMAC based membrane sensor under FIA mode of operation.

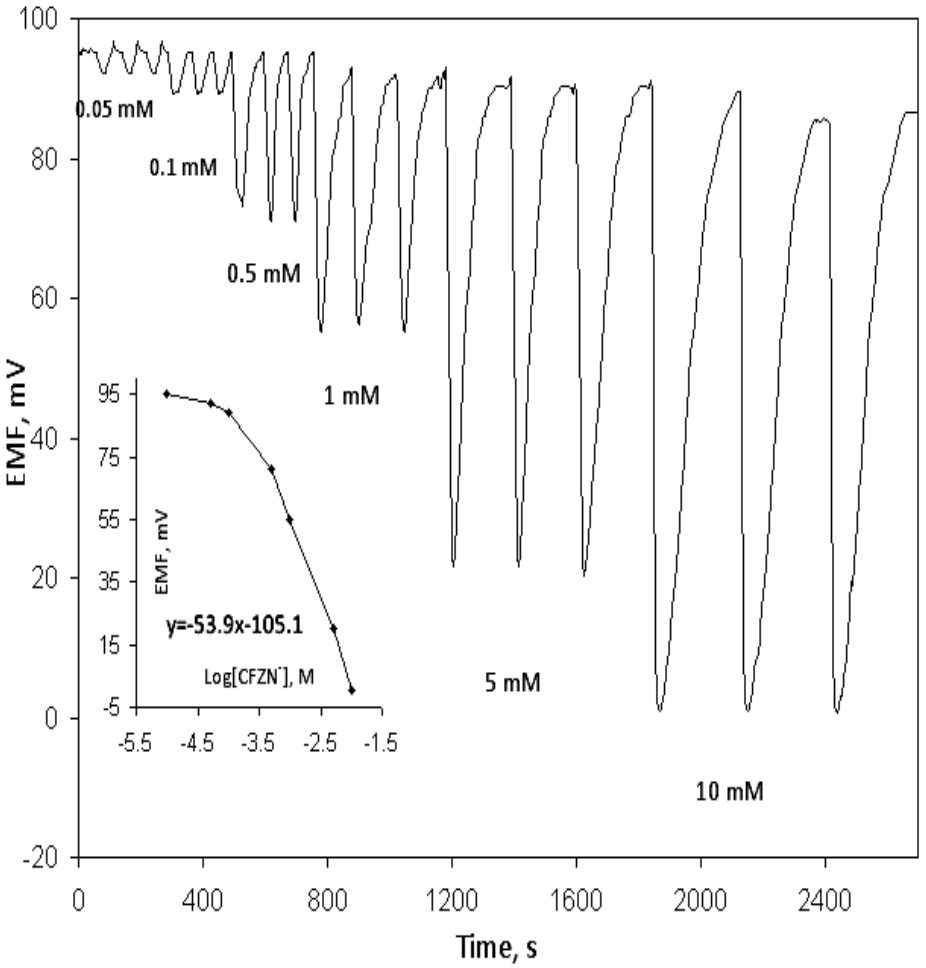

Figure 9. Typical (FIA) peaks produced by injection of standard CFZN through 150 µl valve loop into a stream of 50 mM acetate buffer pH 5.5 using the TDMAC based membrane sensor. The inset shows a FI response curve.

Table 4. Response characteristics of tubular TDMAC based membrane detectors under hydrodynamic (FIA) mode of operation for determination of CFZN

Parameter	TDMAC – PVC membrane
Slope, (mV per decade)	-53.91
Lower limit of linear range, (mM)	0.5
Lower limit of detection, (mM)	0.38
Optimum flow rate, (ml min^{-1})	2
Carrier 50 mM acetate buffer, (pH)	5.5

* Average of three measurements.

3.3. Batch and flow injection applications of CFZN sensitive membrane electrode

Commercially available pharmaceutical dosage forms of CFZN were analysed utilizing the optimized TDMAC based membrane electrode as well as by using the standard spectrophotometric method [4]. The proposed potentiometric method was applied successfully for determination of the

CFZN in its pharmaceutical dosage forms under static mode of operation with good accuracy and precision. The results obtained, using the batch method, were found to be comparable with those measured using the reported method [4] (Table 5). No significant difference was found by applying *t*- and *F*-tests at 95% confidence level indicating good accuracy and precision. *F*-test revealed that there was no significant difference between the means and variances of the two sets of results.

Table 5. Determination of CFZN in its pharmaceutical formulations by the proposed potentiometric method under static mode of operation and the reported method

Pharmaceutical product	Recovery % ± SD[a,b]		
	Batch	FIA	Reported method [c]
Zinol® vials[d] 500 mg of cefazolin sodium/vial (IV, IM)	99.68 ± 1.16 *t* = 1.45 *F* = 2.34	99.26 ± 0.55 *t* = 0.31 *F* = 3.56	99.14 ± 0.98
Zinol® vials[d] 1000 mg of cefazolin sodium/vial (IV, IM)	99.67 ± 0.77 *t* = 1.41 *F* = 2.52	98.11 ± 0.61 *t* = 0.71 *F* = 2.82	98.67 ± 0.82

[a] Each value is the mean of five determinations.

[b] The tabulated values at 95% confidence limits are t = 2.78 and F = 6.39, respectively.

[c] Reference [4].

[d] Pharco Pharmaceuticals, Alexandria, Egypt.

Table 6. Standard addition method for the assay of CFZN in its pharmaceutical dosage forms using TDMAC based membrane sensor under static and continuous modes of operation

Pharmaceutical formulation	Authentic drug added (mol L^{-1})	Batch		FIA	
		Authentic drug found (mol L^{-1})	Recovery (%) ± SD[a]	Authentic drug found (mol L^{-1})	Recovery (%) ± SD[a]
Zinol® vials 500 mg of cefazolin sodium/vial (IV, IM)	1.0×10^{-5}	0.95×10^{-5}	95.00±1.4	0.93×10^{-5}	93.00±1.5
	5.0×10^{-5}	4.81×10^{-5}	96.20±0.9	4.74×10^{-5}	94.80±0.6
	7.0×10^{-5}	6.73×10^{-5}	96.14±1.1	6.77×10^{-5}	96.71±0.9
Zinol® vials 1000 mg of cefazolin sodium/vial (IV, IM)	1.0×10^{-5}	0.98×10^{-5}	98.00±0.5	0.91×10^{-5}	91.00±1.2
	5.0×10^{-5}	4.86×10^{-5}	97.20±0.9	4.91×10^{-5}	98.20±0.7
	7.0×10^{-5}	6.57×10^{-5}	93.86±0.8	6.72×10^{-5}	96.00±0.9

[a] Average of six determination.

Recovery studies were also carried out by applying standard addition method under static mode of operation. Recovery experiments were made to evaluate the accuracy of the proposed potentiometric methods on both the pure authentic and the dosage forms. Different concentrations of the authentic drug being determined were added to the sample preparation which was then analysed for the total amount of the drug present. The difference between the analytical results of the samples with

and without the added drug gave the recovery of the amount added drug. The results clearly proved the accuracy of the proposed method for selective determination of the investigated drug without interference from common excipients. The results in Table (6) indicate good recoveries and confirm the absence of interference due to common excipients. This together with being simple, rapid, economic, sensitive, precise and accurate allows its use for quality control of the studied drug.

4. CONCLUSIONS

A novel potentiometric sensor selective for cefazolin sodium (CFZN) is prepared, characterized and successfully used for static and continuous drug determination. Four different types of anion exchangers (TDMAB, TDMAC, BTBAB and EBTPPB) and four types of metal-ion complexes (PdTPP-Cl_2, SnTPP-Cl_2, CuOEP and Zn OEP) were tested. Among different electroactive species tridodecyl methyl ammonium chloride (TDMAC) doped membrane electrode was found to exhibit optimal response characteristics. The optimized membrane sensor exhibited near-Nernstian responses (-55.64 mV decade^{-1}) over CFZN concentration range of 0.41 – 10 mM as measured in 50 mM acetate buffer, pH 5.5. The developed sensor offered high selectivity towards CFZN compared to other cephalosporins such as; cefradine, ceftazidime, cefadroxil, cefaclor and cefoperazone, as well as other additives found in the pharmaceutical preparations such as; glucose, fructose and maltose. This indicates that the optimized sensor could be used successfully for determination of CFZN in presence of other cephalosporins and other pharmaceutical additives. The drug is determined in pure powders and in dosage forms. The developed sensor offers the advantages of fast response times, reasonable selectivity, elimination of drug pre-treatment or separation steps, low cost and possible interfacings with computerized and automated systems. The tubular-type detector exhibits a concentration range from 0.5 – 10 mM with a near-Nernstian response (-53.91 mV decade-1). Continuous monitoring of CFZN offers the advantages of simple design, ease of construction and possible applications to small volumes of drug solutions without pre-treatment. The developed sensor was utilized successfully in static and continuous modes of operation for the determination of CFZN in dosage forms. The results obtained were in good agreement with the standard method of CFZN method of analysis.

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