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Determination of Dexamethasone Disodium Phosphate using Potentiometric Sensors based on Molecularly Imprinted Polymer in Flow Injection and Batch Systems

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Abstract- Dexamethasone disodium phosphate (DSP) is one of the medications used for treatment of inflammatory diseases. In this work, a potentiometric sensor, based on a non-covalent imprinted polymer, was fabricated for the determination of DSP in pharmaceutical formulations. The molecularly imprinted polymer (MIP) was synthesized by precipitation polymerization using DSP as a template molecule, methacrylic acid as a functional monomer and ethylene glycol dimethacrylate as a cross-linking agent. The sensor was developed by dispersing the DSP imprinted polymer particles in *o*-nitrophenyl octyl ether plasticizer and embedding in poly(vinyl chloride) matrix. MIP was used as a suitable ionophore for the preparation of novel polymeric membrane (PME) and coated graphite (CGE) DSP-selective electrodes. The electrodes exhibit a Nernstian behavior for DSP ions over wide concentration ranges (1.0×10^{-7} - 1.0×10^{-1} M for PME and 1.0×10^{-8} - 1.0×10^{-1} M for CGE, Nernstian response (27.25 mV/decade for PME and 28.11 mV/decade for CGE), very low limits of detection (6.8×10^{-8} M for PME and 6.3×10^{-9} M for CGE), fast response time (~15 s for PME and ~10 s for CGE) and a satisfactory long-term stability (3 months) are characterizations of the proposed sensor. The sensor showed a high selectivity and a sensitive response to the template in aqueous system. The CGE was used in flow injection potentiometry and resulted in well-defined peaks for DSP ions with stable baseline, excellent reproducibility and very high sampling rate of 180 injections per hour. The proposed FIP system was used for the determination of trace DSP in pharmaceutical formulations.

Keywords- Molecularly imprinted polymer; Flow injection poetntiometry; Coated graphite electrode; Dexamethasone disodium phosphate

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1. INTRODUCTION

Dexamethasone disodium phosphate (DSP), (Fig. 1) is a glucocorticoid which is widely used in inflammatory diseases. Its chemical name is 9-fluoro-11b, 17, 21-trihydroxy-16α-methylpregna-1, 4-diene-3, 20-dione 21-(dihydrogen phosphate) disodium salt [1]. DSP is co-formulated with antibiotics in several anti-infective eye preparations for treatment of acute and sub-acute conjunctivitis caused by susceptible strains of aerobic gram positive and negative bacteria [2]. A polarographic method was applied for the trace determination of DSP [3]. The simultaneous determination of chloramphenicol (CPL) and DSP has been accomplished using HPLC methods [4,5]. One method was reported for determination of DSP by DSP selective electrode in its pharmaceutical formulations [6]. Few chromatographic methods were also given for the simultaneous determination of DSP along with other drugs such as ciprofloxacin and ofloxacin [7-12]. Two spectrophotometric methods were reported for determination of CPL and DSP in presence of tetryzoline HCl and benzalkonium chloride (BNZ) [13,14]. Reviewing the reported methods, several ones were reported for determination of DSP by HPLC [15,16] and GC [17]. Additionally, stability-indicating method with degradation studies was reported for simultaneous determination of CPL and DSP in bulk and formulations [18]. Some of them have either a very high limit of quantification (LOQ) or are too much complex, which limits its application for a larger number of samples.

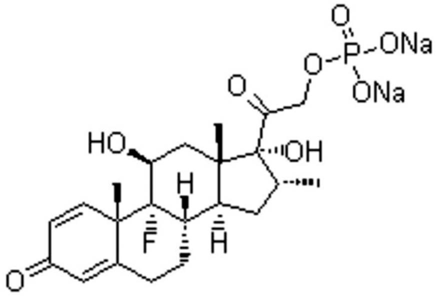

Fig. 1. Chemical structures of Dexamethasone disodium phosphate (DSP)

Different articles were reported as analytical method using MIP based potentiometric sensors for quantitation of drug content in different pharmaceutical formulations and/or biological fluids. Molecular imprinting has now become a well-known process and has also been applied in different areas of biomedical and analytical chemistry. MIPs have the ability to act as receptor [19], enzyme mimics [20], antibody [21] and recently as drug delivery systems (DDS) [22]. Nowadays, one of the most interesting research fields in sensors' field is electrochemical sensors. MIPs were integrated as recognition receptors in potentiometric ISEs for quantitative determination of different organic and inorganic ions [23-29].

There are three important factors should be considered before construction of an electrochemical sensor: i) Good selection of the recognition element receptor; ii) choice of the transducer; and, iii) the integration of both elements. Molecular imprinted polymers (MIPs) are the most promising materials in the preparation of artificial molecular recognition systems. The advantages of using of molecular imprinted technologies that they have high selectivity and sensitivity, which can be used in combination with suitable transducer for desired application [30]. The theory behind this method involves moulding a fabric (with the required chemical recognition properties) around individual molecules (template). By removal of the molecular template, the material preserves its molded shape to fit with that of the template molecules. Thus, molecular imprinting can provide materials that can selectively bind to molecules of interest [30].

The technology of molecular imprinting had increased interest in the recent years, covered the applications of MIPs as recognition element in pharmaceutical, environmental and food quality control connected with various transducers [31].

Different parameters, such as amount and nature of monomer, cross-linker, and porogenic solvent, have to be optimized to obtain the final desired characteristics of the prepared MIPs in terms of affinity, capacity, and selectivity for the target analyte. Thus, different trials should be carried out to reach the optimum mixture of cross-linker and functional monomer. This is to minimize the nonspecific binding as possible. Essentially, proper molar ratios of functional monomer to template are very important to achieve the specific affinity of prepared polymers and number of recognition sites in MIPs.

The synthesis of MIPs was done by three different imprinting methods [32], as follows: i) The non-covalent approach, ii) The covalent approach, iii) The semi-covalent approach. The non-covalent imprinting protocol is still the most important method used method to prepare MIP referring to its advantage over the covalent approach of practical simplicity. The non-covalent approach is considered the most widely used method of imprinting due to its relative simplicity on experimental level. The complexation step throughout the synthesis is achieved by mixing the template with the appropriate functional monomer (s), in a suitable solvent that is called Porogen [33]. After preparation, the template is removed from the formed polymer mainly by washing and extracting it with a solvent or a mixture of solvents. The rebinding step is based on non-covalent interactions between the prepared MIPs and the template.

In general, the production of molecular-sensing polymer includes the following steps: (i) Selection and preparation of required monomer(s), cross-linker, porogen (ii) Synthesis of the MIPs, (iii) Washing of MIPs and template removal (iv) Optimization to achieve molecular selectivity, and (v) construction of MIP-based membrane sensors using the prepared polymers [34].

Potentiometric technique is also well-known versatile, simple, rapid and inexpensive method for determination of target ion (molecule). Potentiometric sensors are considered the most widely used analytical technique in many fields, including clinical and environmental analysis and process control. The imprinted polymers become point of interest for scientists focused in the development of the electrochemical sensor [30,35,36]. Using MIPs offers many benefits such as their better stability, low cost, high selectivity, and ease of preparation. Moreover, MIPs are much more stable to organic solvents, pH, high temperature, and pressure than ordinary membranes. The cost of producing MIPs is relatively low, and they can be stored in the dry state at room temperature for long periods of time.

With the increasing of number of MIP based electrochemical sensors with different electrochemical transducers (capacitive, conductometric, amperometric, and voltammetric) [23-25], it is observed that only a few MIP-based sensors have been reported using a potentiometric transducer (in spite of the relatively simple transduction of the potentiometric signal) [23-29,37]. Especially, the areas of environmental monitoring and food and drug analysis require analytical tools facilitate detection of chemicals with high molecular specificity, considering complex biological fluids that resemble high interference. The possibility of incorporating tailor-made, highly selective artificial MIPs makes these synthetic polymers the ideal recognition elements in electrochemical sensors [38,39].

Wide applications using potentiometric ion selective electrodes (ISEs) have been observed in drug pharmaceutical analysis. Incorporation of MIPs in the membrane of ISEs combines the advantages of MIPs of selectivity and specificity to template. These are needed in quantitative analysis of the analyte in different complex matrices that represent high degree of interference.

MIPs play an outstanding role when associated with potentiometric sensors. These electrodes utilize membranes to enable the recognition of a specific ion by transferring it (selectively) across the interface between the sample and membrane phase. This transfer across generates a potential difference that indicate the activity of the transferred ion. Regarding sensor assembly, there are various MIP-based potentiometric sensors that are described by dispersing MIP particles in plasticizer and embedding in PVC matrix [40,41]. In MIP-based potentiometric sensors, incorporation of the imprinted polymer as the active ingredient in a membrane of an ISE provides new electrochemical transduction by chemical recognition which can be practical in analytical objectives.

This purpose can be best served by the designing of DSP ion-selective electrodes based on MIP suitable for use in flow injection potentiometry. There is no previous report on the design of suitable ion-selective electrodes based on MIP for use in flow injection potentiometric determination of DSP.

The advantages of flow injection potentiometry (FIP) by ion selective electrodes, such as low cost, simple instrumentation, rapid response, high sampling rate, wide linear response and high selectivity have well been recognized over the last three decades [42–47]. Moreover, the transient nature of the signal in flow injection analysis (FIA) may help to overcome the effects of interfering ions if the electrode’s response to these ions is slower than that to the target analyte [44], and the lifetime of electrodes may be extended as the surface is predominantly exposed to carrier solution. However, selectivity and fabrication/packaging are still seen as two of the key aspects in the design of potentiometric sensors [48,49].

In this paper, we first report on the preparation of a PVC membrane (PME) and coated graphite electrodes (CGE) based on a molecularly imprinted polymer, which exhibits significantly high selectivity to DSP ions. Then, the successful use of the prepared proper detection system in flow-injection potentiometry (FIP) of DSP ions is discussed.

2. Experimental

2.1. Reagents and materials

All reagents were of the highest grade commercially available and were used without further purification. Reagent grade Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), 2,2′-azobisisobutyronitrile (AIBN), o-nitrophenyloctyl ether (*o*-NPOE), benzyl acetate (BA), dioctyl phthalate (DOP), dibutyl phthalate (DBP), high relative molecular weight poly(vinyl chloride) (PVC) powder, hexadecyltrimethylammonium bromide (HTAB), tetrahydrofuran (THF)), inorganic salts and other solvents were obtained all from Merck or Fluka with highest purity. The sodium or potassium salts of the anions used (from Merck or Aldrich) were of the highest purity available. Doubly distilled water was used throughout. Dexamethasone disodium phosphate was kindly supplied by Alborz daroo pharmaceutical company (Industrial City, Qazvin, Iran). Dexamethasone disodium phosphate's tablets were purchased from a local pharmacy. Standard solutions were prepared freshly with bidisitilled water.

2.2. Synthesis of molecularly imprinted (MIP) or non-imprinted (NIP) polymer particles

The molecular imprinted polymer particles for DSP were prepared from a reagent mixture obtained by mixing 78.0 μL (0.915 mmol) of methacrylic acid, 721.5 μL (3.82 mmol) of ethylene glycol dimethacrylate, 102.248 mg (0.198 mmol) of DSP and 14.5 mg (0.088 mmol) of AIBN in 40 mL chloroform and methanol. The mixture was uniformly dispersed by sonication. After sonication it was purged with N_2 for 10 min and the glass tube was sealed under this atmosphere. It was, then, stirred in a water bath maintained at 60 °C for 20 h. The produced polymer was filtered using a Whatman filter number 1 and washed with acetone and methanol before the template removal. The template was removed by washing the MIP successively in 15 mL of a methanol/acetic acid solution (10:1, v/v, of 98% methanol and pure acetic acid) for three times, each time for 1 h, and then twice in 15 mL of pure water for 1 h. The template extraction of the polymer created the cavities, leading to the specific sorption of the template. In addition, the removal of other materials from the polymer took place (e.g. residual monomers or oligomers and initiator fragments). The non-imprinted polymers were also synthesized following exactly the same procedure, but excluding the template DSP from the formulation.

2.3. Electrode preparation

The general procedure to prepare the PVC membrane was to mix thoroughly 29 mg of powdered PVC, 61 mg of plasticizer *o*-NPOE, 3 mg of additive HTAB, and 7 mg of ionophore of MIP or NIP in a glass dish of 2 cm diameter. The resulting mixture was then adequately mixed for 20 min with a magnetic stirrer in 5 mL of THF. The solvent was evaporated slowly until an oily concentrated mixture was obtained. A Pyrex glass tube (5 mm i.d. on top) was dipped into the mixture for 10 s so that nontransparent PVC based polymer membranes with thickness of 0.3 mm thickness were formed. The tube was then pulled out from the mixture and kept at room temperature for 1 h. The membranes were glued to one end of a Pyrex glass tube using a viscous solution of PVC in THF as an adhesive. The tube was then filled with an internal solution (1.0×10^{-3} M DSP). The electrode was finally conditioned for 12 h in a 1.0×10^{-3} M DSP solution. The solutions for the calibration were prepared by consecutive dilution of 0.1 M DSP with bidisitilled water. A silver/silver chloride electrode was used as the internal reference electrode.

To prepare the coated graphite electrodes, spectroscopic grade graphite rods 10 mm long and 3 mm in diameter were used. A shielded copper wire was glued to one end of the graphite rod, and the electrode was sealed into the end of a PVC tube of about the same diameter with epoxy resin. The working surface of the electrode was polished with fine alumina slurries on a polishing cloth, sonicated in distilled water and dried in air. The polished graphite electrode was dipped into the membrane solution mentioned above, and the solvent was evaporated. A membrane was formed on the graphite surface, and the electrode was allowed to stabilize overnight. The electrode was finally conditioned by soaking in a 1.0×10^{-2} M DSP solution for 48 h.

2.4. Emf measurements

The performance of the sensor was investigated by measuring the emf values of various DSP solutions. Potentiometric evaluation of the electrodes was carried out using the following cell:

Ag–AgCl | internal solution, 1.0×10^{-3} M DSP | PVC membrane | test solution | Hg–Hg_2Cl_2, KCl (sat'd).

Potentiometric measurements were carried out with a digital mili voltmeter (Hioki, Model 3256-50) at laboratory ambient temperature. The reference electrode was obtained from Azar Electrode Company (Urmia, Iran). A digital pH meter (Metrohm Model 827) was used for measuring pH. The calibration graphs were constructed by plotting the potential, E, versus the logarithm of Dexamethasone phosphate ion concentration.

2.5. Apparatus

The emf measurements with the PME and CGE were carried out in cell assemblies reported in the previous publications [50-52].

A schematic diagram of the flow injection potentiometric analysis system for the determination of DSP is shown in Fig. 2. The flow cell was made from polyamide in our laboratory.

The cell contained a coated graphite DSP ISE based on MIP with effective surface area of 4.90 mm^2 and a commercial Ag/AgCl reference electrode with a double junction having a terminal tube diameter of 2.5 mm. It was contained a cylindrical solution path of 2.5 mm in diameter and of 1.5 cm in length; the effective volume of the cell was 74 mL. The dead volume of the designed cell was found to be negligible. A 12-channel peristaltic pump (Desaga) was used to continuously draw solution through the cell. A low-pressure rotary injection valve (model 5020 Rheodyne four-way rotary valve, USA) was used in the flow system.

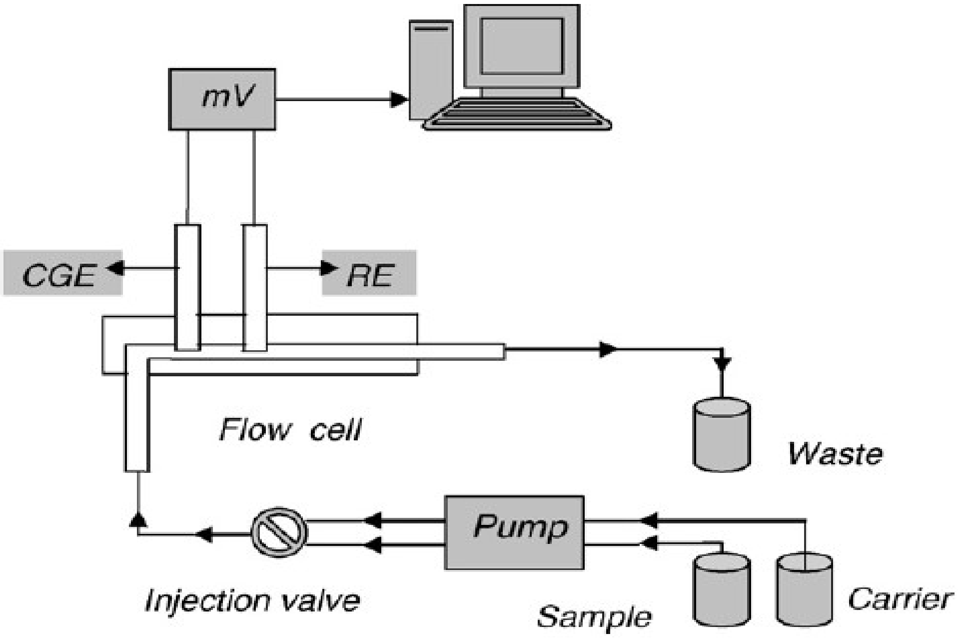

Fig. 2. Manifold of the flow injection potentiometric system

2.6. FIP measurements

Sample solutions were prepared in water at a pH of 6-7. The emf measurements were made at room temperature with a digital millivoltmeter and recorded by a personal computer. The activity coefficients were calculated according to the Debye-Huckel procedure, using the equation $\log \gamma = -0.511z^2 [I^{1/2}(1 + 1.5I^{1/2}) - 0.2I]$ [53], where I is the ionic strength and z the valency.

3. RESULTS AND DISCUSSION

Several parameters were investigated in order to evaluate the performance of the DSP ion-selective electrodes based on MIP (PME and CGE) in terms of membrane composition, calibration curve slopes, linear range, limit of detection, response time, selectivity and sample analysis. Besides of the critical role of the nature of the ionophore in preparing PVC membrane electrodes, it is well understood that the performance characteristics for the ionophore-incorporated PVC membrane may also be very dependent on electrode composition and the nature of the solution of which the electrodes are composed [54-60].

3.1. Effect of membrane composition

Literature reports show that the response behavior of the sensor depends on various features of membranes such as the properties of the plasticizer, nature and amount of ion recognizing material used [61-66]. Thus, different aspects of the composition of membranes based on MIP for DSP ion were optimized, and the results are summarized in Table 2.

The plasticizer to be used in membrane should exhibit high lipophilicity, have high molecular weight, low tendency for exudation from the polymer matrix, low vapor pressure and high capacity to dissolve the substrate and other additives present in the membrane [54-66].

Table 1. Optimization of membrane ingredients for the DSP sensor based on MIP

No.	Membrane composition (%w/w)								Linear range	Slope[a]
	PVC	MIP	AP	BA	DOP	*o*-NPOE	DBP	Additive	[M]	(mV/decade)
1	32	0.0[b]	-	-	-	61	-	-	-	3.2
2	34	5	-	-	-	61	-	-	$1.0\times10^{-1} – 1.0\times10^{-6}$	12.2
3	32	7	-	-	-	61	-	-	$1.0\times10^{-1} – 5.0\times10^{-6}$	18.6
4	30	9	-	-	-	61	-	-	$1.0\times10^{-1} – 5.0\times10^{-6}$	16.1
5	29	7	-	-	-	64	-	-	$1.0\times10^{-1} – 1.0\times10^{-6}$	21.4
6	26	7	-	-	-	67	-	-	$1.0\times10^{-1} – 1.0\times10^{-6}$	20.1
7	29	7	-	-	64	-	-	-	$1.0\times10^{-1} – 3.0\times10^{-6}$	14.0
8	29	7	-	64	-	-	-	-	$1.0\times10^{-1} – 1.0\times10^{-6}$	14.8
9	29	7	64	-	-	-	-	-	$1.0\times10^{-1} – 5.0\times10^{-6}$	10.8
10	29	7	-	-	-	-	64	-	$1.0\times10^{-1} – 1.0\times10^{-6}$	14.6
11	28	7	-	-	-	64	-	1 (HTAB)	$1.0\times10^{-1} – 1.0\times10^{-7}$	23.9
12	***26***	***7***	***-***	***-***	***-***	***64***	***-***	***3 (HTAB)***	$\boldsymbol{1.0\times10^{-1} – 1.0\times10^{-7}}$	***27.2***
13	24	7	-	-	-	64	-	5 (HTAB)	$1.0\times10^{-1} – 1.0\times10^{-7}$	24.3

[a] Mean values of slopes for three replicate measurements.

[b] Containing 7% NIP

Additionally, its viscosity and dielectric constant should be adequate. The nature of a plasticizer or membrane solvent greatly affects all the electrochemical characteristics including potentiometric selectivity because it influences both the dielectric constant of the membrane and the mobility of the molecule or ion in the membrane.

Therefore, several membrane compositions were investigated by varying the amount and nature of plasticizer (Table 1). The influence of the nature of plasticizer on the DSP response was studied on electrodes containing five types of plasticizers having different dielectric constants, namely, BA, DOP, DBP, AP and O-NPOE. As shown in Table O-NPOE with the highest dielectric constant in the series resulted in the best sensitivity of the potential responses. Among five different plasticizers employed, the plasticized membrane with O-NPOE appeared to be more compatible with the MIP as a homogenous and clear membrane could be formed. It was found that the membrane with O-NPOE provided a better slope with a wider linear response range (Fig. 3). It was noticed that MIP based membranes were found to be brittle in the absence of plasticizer and sensor performance could not be checked.

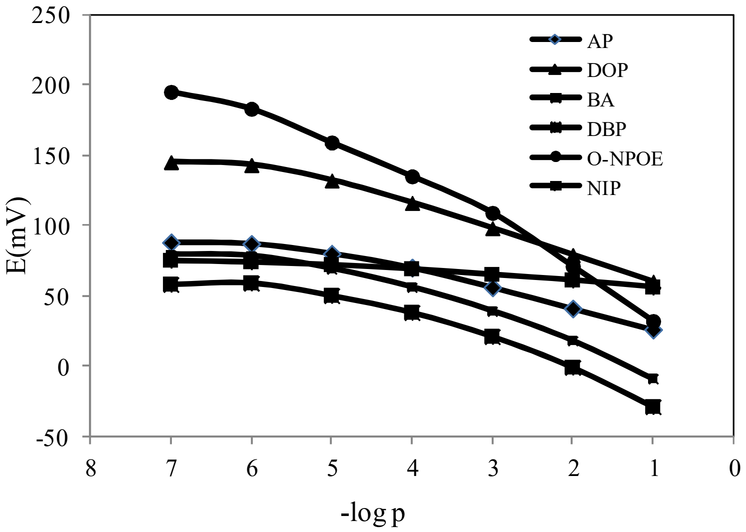

Fig. 3. Potential response of MIP-based DSP potentiometric sensor fabricated with different plasticizers

The ratio of ionophore to PVC influences the working concentration range, slope and response time in case of conventional ionophore-based sensors [57]. In case of imprinted polymer ion selective electrodes, it has been observed that the ratio of PVC to MIP particles was found to play a key role in the sensor performance since the amount of MIP particles determines the number of binding sites available for selective rebinding of DSP [59]. The results in Table 1 show that the membrane having the weight of PVC to MIP particles in the percent ratio of 29:7 gave the best performance.

The amount of ionophore was found to affect the PVC membrane sensitivity. The calibration slope increased with increasing MIP content until a value of 7% was reached. However, further increase in the amount of ionophore resulted in a diminished response slope of the electrode, most probably due to some inhomogeneity and possible saturation of the membrane [61].

Since in preparation of many PVC membrane-selective electrodes a plasticizer/PVC ratio of about 2 resulted in very suitable performance characteristics [60-62], this ratio was kept almost constant in the optimization of the ingredients of the membrane.

It is well-known that the incorporation of lipophilic additives can significantly influence the performance characteristics of a membrane sensor [62-65]. The presence of additives not only improves the response characteristics and selectivity, but also may catalyze the exchange kinetics at the sample-membrane interface. Lipophilic salts and ionic additives can decrease the membrane resistance, reduce cation interference, improve selectivity and sensitivity of the electrode and enhance the response behavior. Their main role is attributed to the inducing permselectivity to some PVC membrane selective electrodes. In this work, the effect of addition of HTAB on the performance of MIP based sensor for DSP was examined as suitable lipophilic additives, on the response characteristics of the proposed PVC membrane, and the results are also included in Table 1. The data given in Table 1 indicate that, in the absence of a proper additive, the sensitivity of the PVC membrane based on MIP is quite low. However, the presence of 3% HTAB, as suitable lipophilic additives, will improve the sensitivity of the DSP sensor considerably (with a slope of 27.2 mVdecade^{-1}).

The addition of HTAB to MIP based membrane responds to DSP in the range 1.0×10^{-7} to 1.0×10^{-1} M. We found out that the membrane with HTAB addition gives better performance over other membranes with respect to working concentration range and slope.

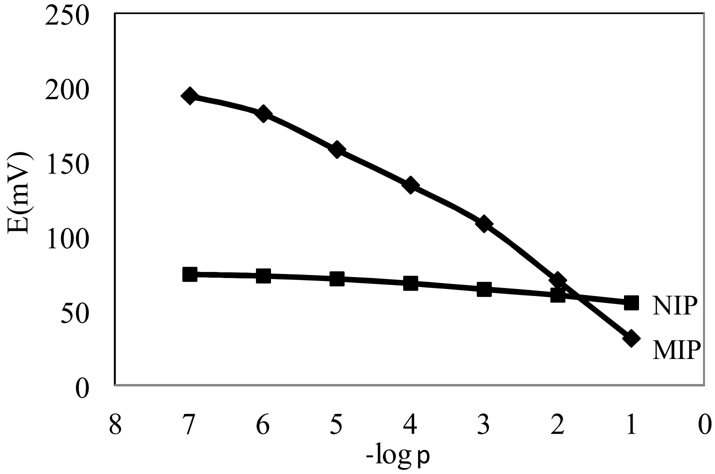

Fig. 4. The potential response curves of MIP and NIP-based membranes

3.2. Calibration Curve

As is obvious from Table 1, membrane no.12 with a PVC/*o*-NPOE/HTAB/MIP percent ratio of 26: 61: 3: 7 resulted in the Nernstian behavior of the membrane electrode over a wide concentration range.

The potential response curves of MIP and NIP-based membranes are shown in Figure 4. The MIP-based membrane shows a good Nernstian response and also proves that the ionic species of DSP are mainly responsible for creating the potential responses in the wide concentration range with a good detection limit based on the IUPAC definition. Evidently, it can be demonstrated that the MIP is effective for specific recognition of the DSP.

3.3. Detection limit

By extrapolating the linear parts of the ion-selective calibration curve the detection limit of the ion-selective electrode can be calculated. In practice, detection limits for the most selective electrodes are in the range of 10^{-7}-10^{-8} M for PME and 10^{-8}-10^{-9} M for CGE. In this work the detection limit of the proposed membrane sensor was 6.8×10^{-8} M for PME and 6.3×10^{-9} M for CGE, as determined from the intersection of the two extrapolated segments of the calibration plots (Fig. 5).

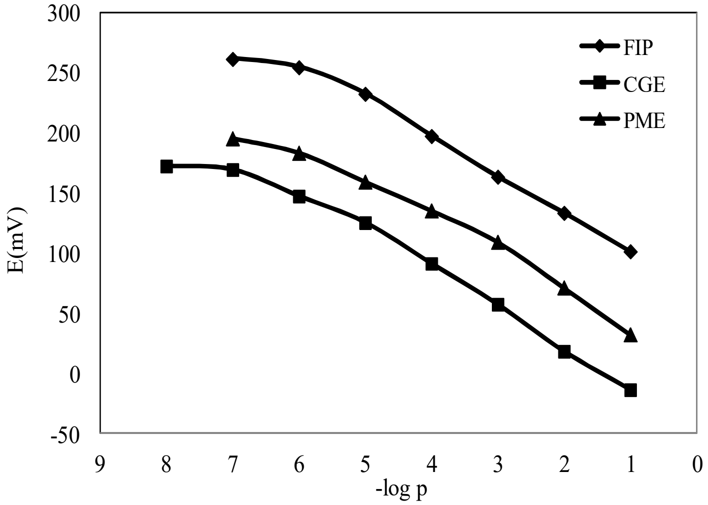

Fig. 5. Calibration graphs for the PME, CGE, and FIP

3.4. pH effect on the electrode response

The influence of pH of the tested solution on the potential response of the membrane PME and CGE was studied under a constant concentration of DSP (1.0×10^{-3} M) and varying the content of the hydrogen ions in the pH range of 1.0-12.0 which was adjusted with HCl or NaOH solution and the emf of the electrode was measured at each pH value, and the results are shown in Fig. 6. As seen, the potentials remained constant from pH 4 to 8, beyond which the potential changed considerably. At higher alkaline media, the potential changed sharply, due to the response of the sensor to both DSP and hydroxide ions. At lower pH than 2.5, due to the protonation of DSP ions, the potential response of the sensor increases. In general, MIP-based sensors in pH 6.0 showed the best analytical performance, with the higher slopes, near-Nernstian behavior and lower detection limits with an average slope of approximately 26.8 mV/decade. Therefore, a pH of 6-7, was chosen for further DSP determination by the proposed sensor.

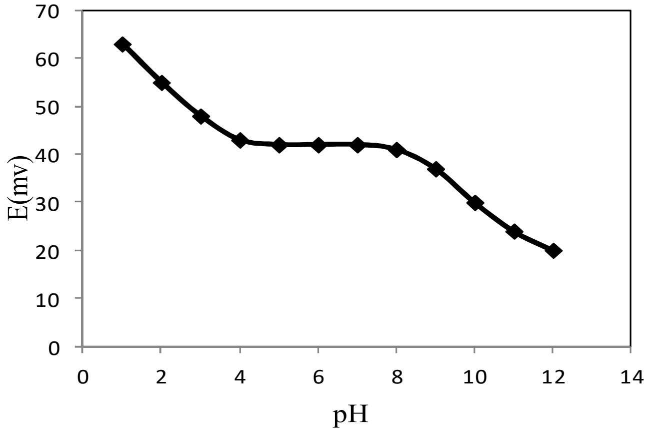

Fig. 6. The effect of pH on the potentiometric response of MIP-based DSP membrane sensor at concentration 1×10^{-3} M

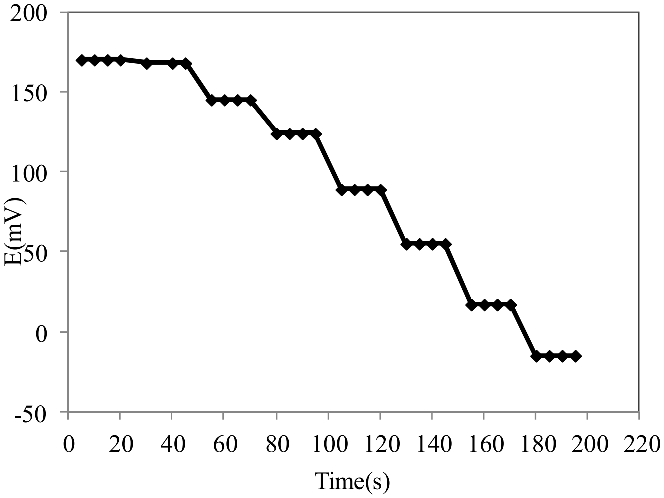

Fig. 7. Dynamic response of the MIP-based DSP potentiometric sensor

3.5. Response time of the electrode

For analytical applications, the response time of a membrane sensor is on important factor. The response time of the MIP based membrane ISE is defined as the average time required for the sensor to reach ±1 mV of the magnitude of the equilibrated potential signal after successively immersing in a series of DSP solutions, each having a 10-fold concentration difference and the results are shown in Fig. 7. As it is seen, in whole concentration ranges, the sensor reaches to the equilibrium response in a short time. (< 15s and < 10s for PME and CGE respectively).

3.6. Stability and lifetime

The stability and lifetime of the DSP sensor were tested over a period of 13 weeks. During this period, the electrode was in daily use over extended period of time (1 h per day). A slight gradual decrease in the slope, from 27.25 to 25.7 mVdecade^{-1}, was observed.

3.7. Selectivity coefficients

The selectivity is clearly one of the most important characteristics of a potentiometric sensor, which represents the preference of a sensor for the response to primary ion over other (interfering) ions that are present in the solution. In this work, the potentiometric selectivity coefficients were determined by matched potential method (MPM). MPM is recommended by IUPAC [66].

In MPM method, the potentiometric selectivity coefficient is defined as the activity ratio of primary and interfering ions that give the same potential change under identical conditions. At first, a known activity (a'_A) of the primary ion solution is added into a reference solution that contains a fixed activity (a_A) of primary ions, and the corresponding potential change (ΔE) is recorded. Next, a solution of an interfering ion is added to the reference solution until the same potential change (ΔE) is recorded. The change in potential produced at the constant background of the primary ion must be the same in both cases.

$$K^{\mathrm{pot}}_{\mathrm{A,B}} = \frac{(a'_{\mathrm{A}} - a_{\mathrm{A}})}{a_{\mathrm{B}}}$$

Selectivity coefficients for the MIP-based electrode are listed in Table 2. As it is obvious from Table 2, when the MIP sensor is applied to determine DSP, all the other substances hardly interfere with the determination. In most cases, the selectivity coefficients were in the order of 10^{-3} and lower. SSM [66–71], recommended to overcome the difficulties associated with the methods based on the Nicolsky–Eisenman equation [71]. According to this method, the emf-pM^{n+} plots were obtained for the DSP and the interfering ion (over a pM^{n+} range of 1–8), separately, using the proposed electrode system. Then by using a pair of values of primary (a_A) and interfering (a_B) ion concentrations at which the electrode takes the same potential in separate solutions (isopotential concentrations) and equation $K^{\mathrm{pot}}_{\mathrm{A,B}}$ = ln $a_A/a_B^{2/z}$ (where z is the charge of interfering ion), the selectivity coefficient $K^{\mathrm{pot}}_{\mathrm{A,B}}$ was determined. The resulting $K^{\mathrm{pot}}_{\mathrm{A,B}}$ values for both the PME and CGE are listed in Table 2.

From the data given in Table 2, it is seen that the PME and, especially, the CGE possess good selectivities for all anions tested. In fact, for the case of all anions used, the selectivity coefficients are in the order of 10^{-4} to 10^{-2}, which clearly indicate that the disturbance produced by these anions in the functioning of the proposed DSP sensors is negligible. Meanwhile, the data given in Table 2 revealed that, in all cases, the selectivity coefficients obtained for the CGE are lower than the corresponding values for the PME, emphasizing the superiority of the former electrode in this respect as well.

Table 2. Selectivity coefficients of DSP ion-selective electrodes for different anions

Interfering	PME	CGE	FIP
CO_3^{2-}	3.8×10^{-2}	9.5×10^{-3}	2.5×10^{-3}
SCN^-	9.7×10^{-3}	6.1×10^{-3}	7.9×10^{-4}
ClO_4^-	8.1×10^{-3}	5.5×10^{-3}	6.5×10^{-4}
IO_3^-	7.5×10^{-3}	2.7×10^{-3}	5.7×10^{-4}
CN^-	6.6×10^{-3}	8.5×10^{-4}	4.4×10^{-4}
$S_2O_3^{2-}$	5.9×10^{-3}	7.5×10^{-4}	3.7×10^{-4}
SO_3^{2-}	4.6×10^{-3}	5.9×10^{-4}	3.1×10^{-4}
SO_4^{2-}	2.5×10^{-3}	5.1×10^{-4}	2.8×10^{-4}
NO_3^-	8.5×10^{-4}	4.3×10^{-4}	2.8×10^{-4}
NO_2^-	6.5×10^{-4}	3.5×10^{-4}	$<10^{-5}$
Cl^-	6.1×10^{-4}	$<10^{-5}$	$<10^{-5}$
HCO_3^-	4.9×10^{-4}	$<10^{-5}$	$<10^{-5}$

3.8. Internal solution effect

The influence of the concentration of internal solution of the PVC electrode was studied as follows. Three similar membranes were prepared under optimal membrane composition, and each electrode was filled with an internal solution of varying DSP concentrations of 1.0×10^{-2}, 1.0×10^{-3}, and 1.0×10^{-4} M. The electrodes were then conditioned for 24 h by soaking in a 1.0×10^{-3} M DSP solution. Finally, the emf versus p DSP plot for each electrode was constructed in a p DSP range of 1-7.

Based on the generally adopted ion-selective response formalism, the internal solution may affect the electrode response when the membrane internal diffusion potential is appreciable. Thus, the proposed sensor was examined at different concentrations of inner reference solution. It was found that the variation of the concentration of the internal solution (in the range of 1.0×10^{-2} –1.0×10^{-4} M DSP) does not cause any significant difference in the corresponding potential response, except for an expected change in the intercept of the resulting Nernstian plots. A 1.0×10^{-3} M concentration of the reference solution is quite appropriate for smooth functioning of the electrode system.

4. Flow-injection potentiometry with the CGE

In the next step, the proposed DSP-selective CGE was successfully used as a suitable indicator electrode in the flow injection system shown in Fig. 2. In order to achieve the best FIP response, several flow injection parameters including tubing length, flow rate sample volume, composition of carrier solution and sampling rate were thoroughly investigated.

The length of tubing from injection valve port to cell was made as small as practical to minimize dispersion and dilution.
Thus, for the proposed electrode, 5 cm was selected for tubing length with respect peak heights.

The home-made flow cell used in this study made it possible to work at flow rates higher than those previously reported in the literature [72-74]. The dependency of the peak heights and peak width (and time to recover the base line) with flow rate was studied using the electrode response to a 1.0×10^{-2} M solution of DSP ion (Fig. 8).

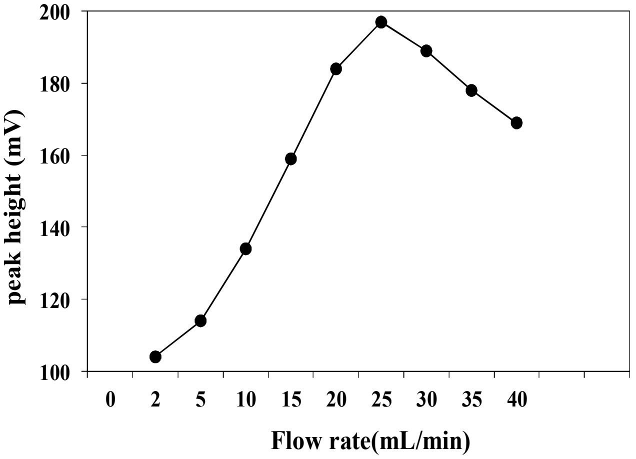

Fig. 8. Dependency of peak height on flow rate. Carrier contains 1.0×/10^{-3} M KCl and Sample contains 1.0×/10^{-2} M DSP

As the flow rate increased, the peaks became narrower and increased in height to a nearly plateau at a flow rate of 25 mL min^{-1}. However, the peak width increased considerably at flow rates higher than 25 mL min^{-1}. Thus, a flow rate of 25 mL min^{-1} was selected as an optimum value for further studies. This flow rate is higher than those of previous reports [72-74] and it increases sampling rate and decreases the total time of analysis.

In general, the peak heights increased with the increasing sample volume, although the effect was less marked at higher concentrations [72,73]. For the proposed sensor, different sample volumes from 50 to 500 μL were studied; the peak height reached nearly 100% of steady-state at 350 μL injected. Thus, this sample volume was selected as an optimum amount.

It is well-known that, in FIP, the composition of the carrier solution also affects the response behavior of ion-selective electrode in terms of the base line stability [75-77]. In the proposed flow system, a hydrochloric acid solution of pH 4.5 was used as carrier, which resulted in quite stable base lines, when samples were injected in the concentration range of 1.0×10^{-7} to 1.0×10^{-1} M.

In the analytical flow systems, the sampling rate (sample throughput) is an important factor representing the capability of system in online analysis. The proposed potentiometric FIA system revealed sampling rates higher than 180 injections per hour.

In Fig. 9 are shown the duplicate peaks from the proposed FIP system obtained under optimal experimental conditions for varying concentrations of DSP solutions in the range of 1.0×10^{-7} to 1.0×10^{-1} M. The corresponding emf-p DSP plot is included in Fig. 5. As seen, the calibration curve is quite linear over a wide concentration range of 1.0×10^{-7} to 1.0×10^{-1} M with a slope of 27.7 mV decade^{-1} and very low LOD of 3.4×10^{-8} M.

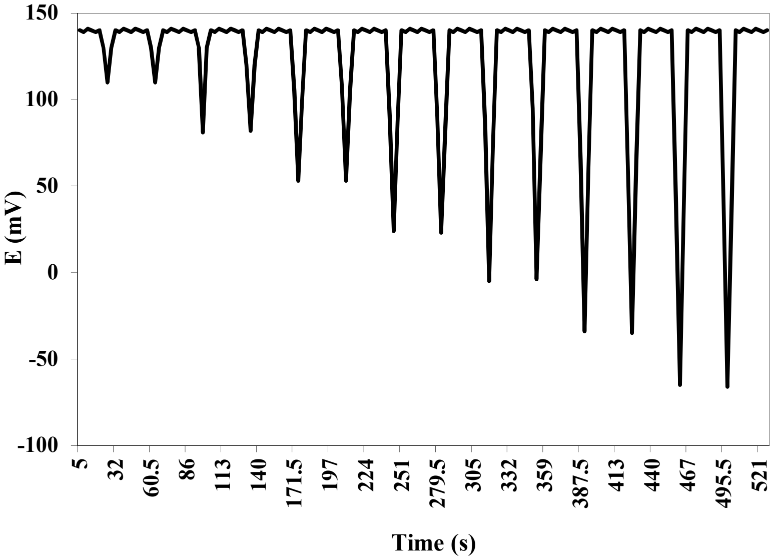

Fig. 9. Potentiometric peaks for two repetitive measurements of different DSP ions concentrations (10^{-7}-10^{-1}M). These peaks were obtained from the electrode response when 350 ml of a number of DSP solutions at a flow rate of 25 ml/min were injected.

The repeatability of the proposed CGE in the flow-injection system under the optimized conditions was checked by 10 repetitive injections of a 1.0×10^{-2} M solution of DSP ion; the results are shown in Fig. 10. The relative standard deviation of the peak heights (%RSD) for 10 replicate injections of 1.0×10^{-2} M solutions of DSP was found to be 1.5%.

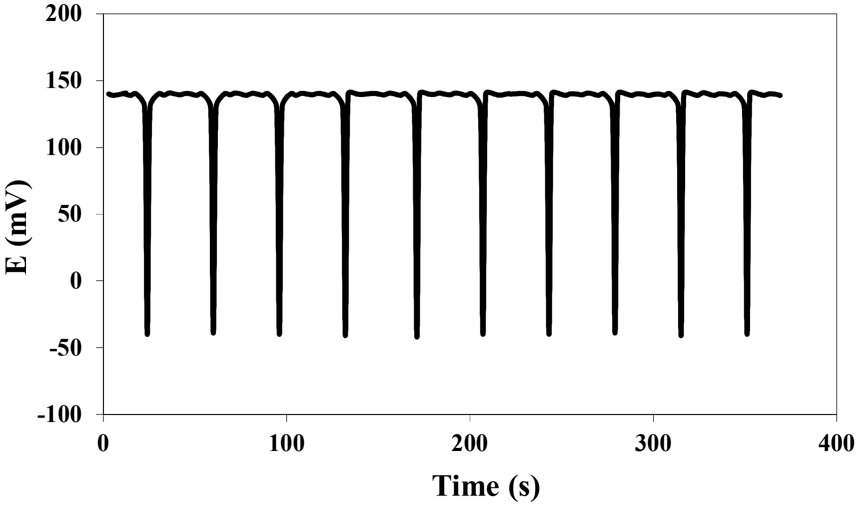

Fig. 10. Reproducibility of flow injection signals for 10 repetitive injections of a 0.01 M DSP

5. Determination of DSP in pharmaceutical formulations

The potentiometric DSP membrane sensors can be used for routine analysis and quality control/quality assurance during manufacture of DSP. The use of the sensor in a FIA mode of operation shorten the assay time, allow the use of little sample quantities for drug detection in both parent and related pharmaceutical preparations. Potentiometric determination of DSP in drug formulations under static mode of operation was carried out using both direct potentiometry and the standard addition (spiking technique).

Table 3. Determination of DSP in some pharmaceutical preparations using MIP based membrane sensor

		Recovery found * (%)			
Sample	Labeled, mg tablet-1	Direct potentiometry	Standard addition	FIA	Spectrophotometry
1*	100	98.4±1.3	96.7±0.8	97.8±1.4	98.3±1.1
2**	100	97.2±0.9	98.1±0.7	97.2±1.3	98.7±1.4

* 1 Januvia® tablets (Merck Sharp and Dohme Co., Pavia, Italy)
**2 Janumet® tablets (Merck Sharp and Dohme Co., Cairo, Egypt)

With the direct potentiometric technique, the recoveries were 98.3±1.1and 97.2±0.9%. The standard addition method showed results with recoveries of 97.8±1.4 and 98.1±0.7% (Table 3). With the flow injection technique, the recoveries were 96.7±0.8% and 97.2±1.3%. These data were compared with results obtained by spectrophotometric method [18].

An F test showed no significant difference at 95% confidence limit between the means and variances of the results. The calculated F values (n=10) of the results obtained by the present sensor and different potentiometric techniques (Table 3) for drug tablets were less than 2.19, compared with the theoretical tabulated value (F=3.18).

4. CONCLUSIONS

The proposed FIP system for Dexamethasone phosphate ion determination was found to work well under laboratory conditions. A DSP potentiometric sensors were fabricated from an MIP based on the use of MAA as a functional monomer exhibited excellent potentiometric performances such as fast response, a wide working pH range, high sensitivity, long-term stability, good selectivity and automatic feasibility. The use of these sensors as detectors for the continuous monitoring of Dexamethasone phosphate ion offered an advantage of simple design, low cost, ease of construction and possible application in the routine control of Dexamethasone phosphate ions samples. Optimization and full validation of the assay method enable accurate, precise and rapid measurements of as low as 6.8×10^{-8} mol L^{-1} Dexamethasone phosphate ions in different samples for MIP membrane based sensors and eliminates the prior separation steps that are usually necessary in the determination of DSP in pure solutions and in pharmaceutical preparations.

Application to DSP evaluation in the routine control of pharmaceutical drug solutions revealed good results. The results are favorably compared with data obtained using the standard method.

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