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A Novel Sensor for Determination of Dexamethasone Disodium Phosphate in Different Pharmaceutical Formulations

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Abstract- A novel electrode was developed for potentiometric determination of dexamethasone sodium phosphate (DSP) using tetraheptyl ammonium bromide (THB) as an anionic exchanger in polyvinyl chloride (PVC) matrix and 2-nitrophenyl octyl ether (2-NPOE) as a plasticizer. Linear responses of 1×10^{-5} to 1×10^{-2} M with slope of -26.50±0.39 mV/decade within working pH range 8-12 were achieved. The percentage recovery of determination of DSP by the proposed DSP selective electrode was 99.96±0.95. Determination of DSP in its pharmaceutical formulations by the proposed electrode revealed its applicability for determination. Moreover, the electrode exhibits good selectivity for DSP with respect to a large number of interfering substances and co-formulated drugs. The fabricated sensor was validated according to ICH guidelines and successfully applied for determination of the studied drug in pure form and pharmaceutical formulations without any interference from additives either labeled or non-labeled. The obtained results have been statistically compared to that of an official spectrophotometric method to give a conclusion that there is no significant difference between the proposed methods and the official one with respect to accuracy and precision.

Keywords- Dexamethasone sodium phosphate, 2-nitrophenyl octyl ether, Potentiometry, Polyvinyl chloride, Tetraheptyl ammonium bromide

1. INTRODUCTION

Dexamethasone sodium phosphate (DSP), (Fig. 1a) is a highly selective glucocorticoid which is widely used in ocular 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].

Reviewing the reported methods, several ones were reported for determination of DSP by HPLC [3,4] and GC [5]. Few chromatographic methods were also given for the simultaneous determination of DSP along with other drugs such as ciprofloxacin and ofloxacin [6-11]. The simultaneous determination of chloramphenicol (CPL) and DSP has been accomplished using HPLC methods [12,13]. Two spectrophotometric methods were reported for determination of CPL and DSP in presence of tetryzoline HCl and benzalkonium chloride (BNZ) [14,15]. Additionally; stability-indicating method with degradation studies was reported for simultaneous determination of CPL and DSP in bulk and formulations [16]. A polarographic method was applied for the trace determination of DSP [17].

Modern ion selective electrodes (ISEs); based on material transport across a specific membrane, are now widely used in determination of trace amounts of analytes. The high selectivity of these electrodes imparts a great advantage over other techniques [18,19].

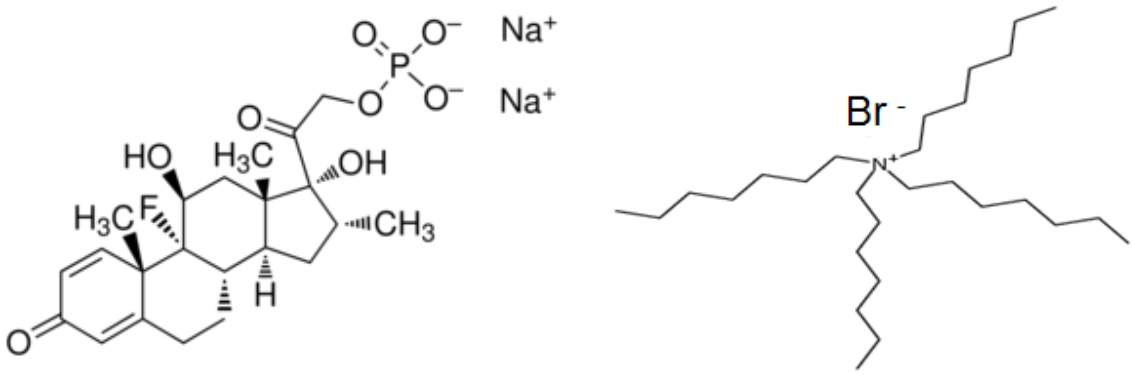
Analytes in colored, turbid or viscous samples could be determined accurately by this technique. These electrodes show a rapid response for concentration changes.

Furthermore, they are used for measurement over a wide concentration range. Ion selective electrodes (ISEs) are generally tolerant for small changes in pH. Further advantage is that they are relatively simple and cheap to develop, set up and run. Moreover, the chemical design of electrodes has been developed to give superior selectivity and response [20,21].

Although chromatographic methods are highly selective, however, their requirements of cleaning up samples and sophisticated instrument preclude their use in routine analysis.

In spite of progress in the design of highly selective electrodes for various ions, there has not been any report on the development of selective and sensitive DSP sensors. This paper describes a novel construction, potentiometric characterization, and analytical application of DSP sensor.

The electrode sensor is responsive to DSP using THB as an anionic exchanger in PVC matrix and 2-NPOE as a plasticizer, (Fig. 1b). The investigated potentiometric method was found to be simple, rapid, low cost, more sensitive than the reported methods, and can be accurately and successfully applied for determination of DSP in pure form and different pharmaceutical formulations in presence of additives either labeled or non-labeled without previous treatment.



(a) **(b)**

Fig. 1. Chemical structures of (a) dexamethasone sodium phosphate (DSP); (b) tetraheptylammonium bromide (THB)

2. EXPERIMENTAL

2.1. Instruments

Potentiometric measurements were carried out using an Ag/AgCl double-junction type external reference electrode (Thermo Scientific Orion 900200, MA, USA; 3.0 M KCl saturated with AgCl as an inner filling solution and 10% KNO_3 as bridge electrolyte) and Jenway digital ion analyzer model 3330 (Essex, UK). A Jenway pH glass electrode (Essex, UK) was used for pH adjustments.

2.2. Materials

2.2.1. Reference sample

Dexamethasone sodium phosphate (DSP) was kindly donated by ADCO Co., Cairo, Egypt. Its purity was found to be 99.82±0.73 %, according to BP method [22].

2.2.2. Pharmaceutical formulations

Spersadex comp.® eye drops were manufactured by Novartis Pharma AG-Switherland; BN.: 420654. Each mL is claimed to contain 5 mg chloramphenicol, 1 mg dexamethasone disodium phosphate and 0.1 mg benzalkonium chloride as preservative.

Dexatobrin eye drops were manufactured by Eipico-Egypt; BN.: 1408543. Each mL is claimed to contain 3 mg tobramycin, 1 mg dexamethasone disodium phosphate and 0.1 mg benzalkonium chloride as preservative.

Isoptomaxidex eye drops were manufactured by Alcon-Egypt; BN: 140514. Each mL is claimed to contain 1 mg dexamethasone disodium phosphate and 0.1 mg benzalkonium

chloride as preservative. These previously mentioned eye drops were all purchased from Egyptian local market.

2.3. Reagents

All chemicals and reagents were of analytical grades.
Bi-distilled de-ionized water was obtained from "Aquatron" Automatic Water Still A4000, Bibby Sterillin Ltd. (Staffordshire-England). Potassium dihydrogen phosphate, potassium chloride, sodium hydroxide, hydrochloric acid and disodium hydrogen phosphate were from ADWIC, El-Nasr Pharmaceutical Chemical Company (Cairo-Egypt). Polyvinyl chloride (PVC), tetraheptyl ammonium bromide (THB), 2-nitrophenyl octyl ether (2-NPOE), and tetrahydrofuran (THF) were from Aldrich-Germany.

2.4. Standard solutions

2.4.1. Stock standard solution of DSP (1×10^{-2} M)

It was prepared by transferring 0.516 g DSP into a 100-mL volumetric flask, dissolved in a sufficient amount of phosphate buffer pH 9 then the volume was completed to the mark with the same solvent.

2.4.2. Working standard solutions of DSP (1×10^{-6} - 1×10^{-3} M)

Different solutions of varying strengths (1×10^{-6}-1×10^{-3} M) were freshly prepared by serial dilutions from the stock solution using phosphate buffer (pH 9) into a series of 25-mL volumetric flasks.

2.5. Procedures

2.5.1. Sensor fabrication

In a glass Petri dish (5-cm diameter), 0.4 mL of 2-NPOE was thoroughly mixed with 190 mg PVC and 10 mg THB. The mixture was dissolved in 10 mL THF by stirring using a glass rod. The Petri dish was then covered with a Whatman No. 3 filter paper and left to stand overnight to allow solvent evaporation at room temperature. A master membrane with a thickness of 0.1 mm was obtained.

2.5.2. Electrode assembly

From each master membrane, a disk (about 8 mm diameter) was cut using a cork borer and was then fixed using THF to a transposable PVC tip clipped into the end of the electrode

glass part. Equal volumes of 1×10^{-2} M DSP and 1×10^{-2} M potassium chloride (prepared in phosphate buffer pH 9) were mixed and used as an internal reference solution. Ag/AgCl wire (1mm diameter) was immersed in the internal reference solution as an internal reference electrode. The sensor was conditioned by soaking in a 1×10^{-2} M DSP stock standard solution for 24 h and storing in the same solutions when not in use.

2.5.3. Direct potentiometric determination of DSP in its pure sample using the fabricated sensor

The Electrode sensor was conjugated with double junction Ag/AgCl reference electrode, calibrated by being immersed in its respective drug solutions (1×10^{-6}-1×10^{-2} M) and allowed to equilibrate while stirring until constant reading of the potentiometer. Then, electromotive forces (e.m.f) were recorded within ±1 mV. The sensor was washed with phosphate buffer pH 9 before and after each run till reaching a constant potential. Calibration graph was plotted relating the recorded electrode potentials obtained by the proposed sensor *versus* log molar concentrations of the studied drug. The corresponding regression equation was computed.

2.5.4. Accuracy

Accuracy of the results was checked by applying the proposed method for determination of different concentrations of DSP. The percentage recoveries revealed good accuracy of the proposed method.

2.5.5. Precision (Repeatability and Intermediate precision)

Three concentrations of DSP (10^{-3}, 10^{-4}, 10^{-5} M) were analyzed three times, each intra-daily on three successive days using the previously mentioned procedures. The percentage recoveries and standard deviations for the studied drug were calculated from the computed regression equation.

2.5.6. Application of the proposed method for determination of DSP in Spersadex comp.®, Dexatobrin and Isoptomaxidex eye drops using the proposed fabricated sensor

Different volumes equivalent to 5.16 mg DSP from each eye drops were accurately transferred into a 100-mL volumetric flask and diluted to the mark with phosphate buffer pH 9. Concentration of this solution was claimed to be 1×10^{-4} M of DSP. The prepared electrode in conjunction with double junction Ag/AgCl reference electrode was immersed in the prepared solution. The resulting potential was recorded and respective concentration was calculated from the corresponding regression equation.

3. RESULTS AND DISCUSSION

Ion selective electrodes (ISEs) have shown both ion exchange and perm-selectivity of sensor ions [23]. It is well known that sensitivity and selectivity depend significantly on its membrane composition and properties of the solvent mediator employed as well as the plasticizer/PVC ratio used [24].

It is rewarding to get new fabricated electrode with competitive properties for determination of pharmaceutical active constituents. Utilizing properties of the composite materials as efficiently as possible to achieve this goal is crucial.

Taking these points in consideration, we have extensively worked in design and optimization of the proposed electrode.

3.1. Sensor fabrication

PVC matrix is a regular support and reproducible trap for ion association complexes in ISEs. Nevertheless, its use creates a need for plasticization and places a constraint on choice of mediator [23]. Membrane constituents were dissolved in THF that was slowly evaporated at room temperature leading to membrane formation.

The solvent mediator, in particular, has a dual function: it acts as a liquifying agent, making the membrane material workable, that is enabling homogenous solubilization and modifying the distribution constant of the ion-exchanger used and sustaining these characteristics on continued use. The proportion of solvent mediator must be optimized in order to minimize the electrical asymmetry of the membrane in order to keep the sensor as clean as possible and to stop leaching to the aqueous phase [25]. In the present investigation, the optimum available mediator for fabrication of sensor was found to be 2-NPOE. It plasticizes the membrane and adjusts its permittivity to provide the highest possible selectivity and sensitivity.

It is well known that lipophilic ionic sites promote the interfacial ion-exchange kinetics and decrease the bulk resistance by providing mobile ionic sites in the electrode matrix [26].

Structural formula of DSP reveals that it has a phosphate moiety with pKa value of 6, thus it behaves as anion in basic medium. Therefore DSP ion selective electrode membrane should exhibit anion exchange capacity. This was achieved by using a newly introduced lipophilic anionic exchanger; tetraheptylammonium bromide (THB) [21], where the membrane was initially conditioned in 1×10^{-2} M DSP for 1 day in order to replace the original exchangeable counter ion (Br^-) of the ion exchange with DSP. Initial trials were done using bi-distilled water but the obtained potentials were less stable with non-reproducible Nernstian slopes in comparison with using phosphate buffer pH 9.

Although, the obtained slope value was -26.50±0.39 mV/concentration decades with correlation coefficient 0.9998. The results obtained by the proposed THB sensor showed great accuracy, higher reproducibility and selectivity for DSP determination.

3.2. Sensor calibration and response time

Electrochemical performance characteristics of the proposed sensor were systematically evaluated according to IUPAC standards [27]. Table 1 shows the results obtained over a period of two weeks for the developed sensor.

Typical calibration plot is shown in Fig. 2. Slope of the calibration plot was computed from the linear part of the calibration graph and found to be -26.50±0.39 mV/concentration decades.

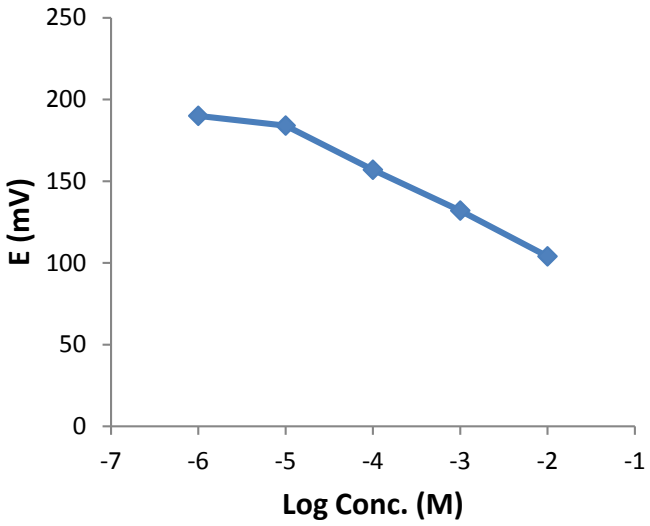


Fig. 2. Profile of the potential in mV/ Log DSP molar concentration using the proposed sensor [1×10^{-6}-1×10^{-2} M]

The sensor displayed constant potential readings within ±2 mV from one day to another. The required time for the sensor to reach values within ±1 mV of the final equilibrium potential after increasing drug concentration 10-folds was found to be 10 seconds for DSP electrode. Slope values of the proposed electrodes were about 30; the typical value of divalent substances.

Linearity was assessed and mean percentage recoveries are given in Table 1. To evaluate precision, three concentrations within the linear range (10^{-5}, 10^{-4} and 10^{-3} M solutions of DSP) were chosen. Three solutions of each concentration were prepared and analyzed in triplicate (repeatability assay). This assay was repeated on three different days (intermediate precision assay), Table 1.

3.3. Effect of pH and temperature

For quantitative measurements with ISEs, effect of pH on the response of the proposed sensor was studied over pH ranges of 2–12 to reach the optimum experimental conditions. This was manipulated by adding drops of diluted hydrochloric acid and sodium hydroxide solutions (about 0.1N) on the 1×10^{-4} M and 1×10^{-3} M solutions of DSP. The potential obtained at each pH value was recorded. Fig. 3 shows potential-pH profile for 10^{-3} and 10^{-4} M DSP for the proposed sensor.

It is apparent that sensor responses are fairly constant in solutions of a pH value within the ranges 8-12, in these pH ranges DSP is completely ionized and thus are sensed.

Below pH 8 there was a slight gradual increase in the potential with decreasing pH without a well-defined constant region, this could be explained that below pH 8 the medium is not basic enough to cause complete dissociation and ionization of the phosphate group of DSP and also the membrane may extract H^+ leading to the neutralization of O^- of DSP which in turn leads to weak unstable response.

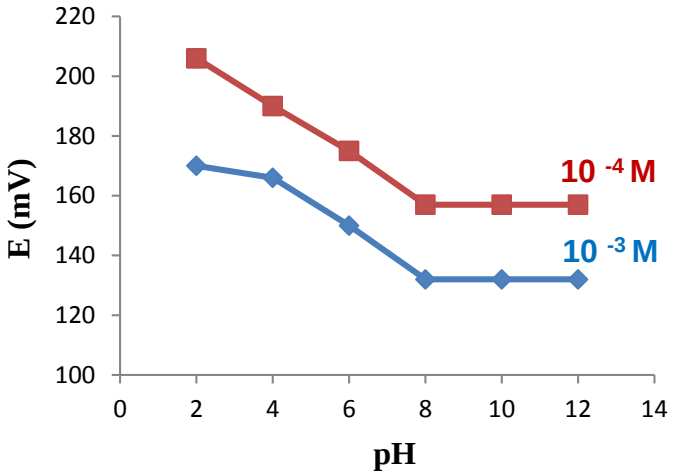


Fig. 3. Effect of pH on the response of the proposed sensor for DSP [working pH range: 8-12]

3.4. Sensors selectivity

The potential response of the studied sensor in presence of the drug and a number of related substances was studied, and potentiometric selectivity coefficient, -log (K^{Pot} $_{Primary\ ion,\ interferernt}$) was used to evaluate the extent to which a foreign ion would interfere with the response of the electrodes to its primary ion. Selectivity coefficients were calculated by the separate solutions method (SSM) [28], where potentials were measured for 10^{-3} M drug solution and then for 10^{-3} M interferent solution, separately, then potentiometric selectivity coefficients were calculated using the following equation:

$$- \log (K^{pot}{}_{\text{primary ion interferent}}) = E_1 - E_2 / S$$

Where E_1 is potential measured in 10^{-3} M solution of 1^{ry} ion solution, E_2 potential measured in 10^{-3} M solution of interferent and S is slope of the sensor.

The response of the proposed sensor was assessed in presence of interfering substances expected to be present in different commercially available eye drops.

Benzalkonium chloride (preservative) in addition to co-formulated drugs; such as chloramphenicol and tobramycin were tested as potential interferents. Results of the calculated selectivity coefficients showed that the proposed sensor displayed high selectivity and no significant interference was observed from the susceptible interfering species, Table 2.

3.5. Potentiometric determination of DSP in Spersadex comp.®, Dexatobrin and Isoptomaxidex eye drops using the proposed fabricated sensor

The results obtained for determination of DSP in Spersadex comp.®, Dexatobrin and Isoptomaxidex eye drops using the proposed sensor proved applicability of the method without prior treatment or separation, Table 3.

Table 1. Response characteristics of the investigated ion selective electrode and validation parameters of the response and the regression equation

Parameter	**Sensor**
Range (M)	1×10^{-5}-1×10^{-2}
Regression parameters	
Slope (mV/ decade)[a] ± SE of slope	-26.50±0.39
Intercept (mV)[a] ± SE of intercept	51.50±1.42
Correlation coefficient (r)	0.9998
Working pH range	8-12
Response time (s)	10
Recovery % Mean ± SD	99.96 ± 0.95
Precision	
Repeatability (RSD %)	0.74
Intermediate precision (RSD %)	1.05
LOD [b]	6.31×10^{-6}

[a] Average of three determinations.

[b] LOD (Limit of detection) was measured by interception of the extrapolated arms of Fig. 2.

The obtained results have been statistically compared to that of an official spectrophotometric method [22]. The calculated t and F values were less than the theoretical ones indicating that there was no significant difference between the proposed and official method with respect to accuracy and precision, Table 4.

Table 2. Potentiometric selectivity coefficients ($K^{pot}_{DSP\ I}$) of the proposed sensor using separate solutions method (SSM)

Interfering Substances *	**Selectivity Coefficient**
Chloride	5.4×10^{-3}
Chloramphenicol	2.3×10^{-3}
Tobramycin	2.2×10^{-3}

* Chloride is the inorganic moiety of benzalkonium chloride which is used as a preservative while chloramphenicol and tobramycin are the co-formulated drugs in the commercially available eye drops.

Table 3. Application of the proposed sensor for determination of DSP in different pharmaceutical formulations

Pharmaceutical Formulation	**Found [a] % ± SD**
Spersadex® (BN.: 420654)	98.74 ± 1.27
Dexatobrin® (BN.:1408543)	102.52 ± 1.08
Isoptomaxidex® (BN.:140514)	103.77 ± 1.56

[a] Average of three determinations

Table 4. Statistical analysis of results obtained by the proposed method as compared with the official method for analysis of DSP in their pure powdered form

Item	**Sensor**	**Official method** [22] **
Mean	99.96	99.82
S.D.	0.95	0.73
n	4	6
Variance	0.90	0.54
Student's t test *	0.26 (2.31)	
F test *	1.67 (5.41)	

* The figures in parenthesis are the corresponding theoretical values at P=0.05.
** The official method used is spectrophotometry at 241.5 nm, using distilled water as a blank.

4. CONCLUSION

As for electrochemical determination of DSP, responses of the fabricated sensor are sufficiently precise, accurate and prove great selectivity for quantitative determination of DSP in pure form and in different pharmaceutical formulations. Moreover, usage of the proposed sensor compromises a great advantage of eliminating any need for drug pretreatment or separation steps. Therefore, it can be used for routine analysis of DSP in quality control laboratories. In general, ISEs proposed here offer simplicity in design and a very low limit of detection as well as being rapid, inexpensive, portable and could compete with many sophisticated methods currently available.

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