Preparation and Study of Cephalexin Selective Electrodes and Their Application in Pharmaceutical Drugs

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الخلاصة

حضرت أقطاب إنتقائية للسيفاتكسين بتحضير المادة الفعالة (السيفالكسين- فوسفوتنكستيت) Ceph-PT مع الملدنات المختلفة وهي داي بيوتيل فوسفيت (DBP) ، داي بيوتيل فثاليت (DBPH) ،داي اوكتيل فثاليت (DOP) ،ثراي بيوتيل فوسفيت (TBP) و اورثو نيترو فنيل إيثر (NPOE) في غشاء اصله من بولي فاينيل كلورايد (PVC). الدراسة شملت خواص ألأقطاب كتأثير الدالة الحامضية (pH) ، المحلول الداخلي للأقطاب، الأنتقائية وحد التحسس ومدى الخطية .

إن أفضل الأقطاب المحضرة هي باستعمال الملدنات (DBPH, DOP).

والمحلول الداخلي (10^{-3}M Ceph+10^{-3}M NaCl) حيث ان الميل لمنحني المعايرة هو (58.5mV/decade و59.5) وبحدود تحسس($3x10^{-5}$ M , $5x10^{-5}$) على التوالي و مدى خطية متساوي 10^{-2} M – 10^{-4} ولهما ثبوتية وتكرارية جيدة.

إن هذه الأقطاب أستخدمت لتقدير السيفالكسين في المستحضرات الدوائية الكبسول والمعلق الفموي وان معدل الأسترجاع والانحراف المعياري كالأتي : , (100.344) ± 0.120) (100.116 ± 0.393). تمت مقارنة النتائج المستحصلة من الأقطاب مع نتائج المشتقات الطيفية حيت تطابقت نتائج المشتقة الطيفية الأولى من حيث قيمة نسبة الاسترجاع والدقة وسهولة الطريقة.

ABSTRACT

A plastic membrane electrodes for the determination of cephalexin monohydrate were fabricated based on the use of cephaiexin-phosphotungstate as an active substance, and with different plasticizers, di-butyl phosphate (DBP), di-butyl phthalate (DBPH), di-octyl phthalate (DOP), tri-butyl phosphate (TBP) and o-nitro phenyl octyl ether (NPOE) in PVC matrix membranes. The study was carried out to investigate the electrode parameters, effect of pH and selectivity. Internal filling solution of 10^{-3} M Ceph + 10^{-3} M NaCl was used to fill the electrodes. The best electrodes were based on DBPH and DOP plasticizer which gave a slope 59.5 and 58.5 mV/decade, linear concentration range 10^{-4} – 10^{-2} M and detection limit of 5 x 10^{-5} and 3 x 10^{-5} M, respectively displayed good stability and reproducibility. The electrodes were used to determine the cephalexin monohydrate in oral suspension and capsules, the average recovery and standard deviation were (100.116 ± 0.393) and (100.344 ± 0.120), respectively. The results were compared with UV-derivative spectrophotometry (DS) and the results for the drugs obtained by first derivative are quite comparable with the recovery obtained by cephalexin selective electrodes.

Keywords; cephaiexin electrodes, phosphotungstic acid ionophore, Derivative spectrophotometry, cephaiexin monohydrate determination

INTRODUCTION

The name of cephalexin monohydrate was changed to cefalexin in British pharmacopeia's 1999. It prepared as; capsules, oral suspension and tablets. Cephalexin monohydrate is an oral antibiotic which has a wide spectrum of antibacterial activity, which has the empirical formula ($C_{16}H_{17}N_3O_4$ S, H_2O), and the molecular weight 365.4g/mol(1). cephalexin monohydrate contains not less than 95.0% and not more than 100.1% with reference to the anhydrous substances(1). Cephalexin occurs as white, or almost white, crystalline monohydrate powder. It is soluble in water, practically insoluble in alcohol and in

ether, resistant to acid and well absorbed orally. Ion selective electrodes (ISEs) are used today in a wide range of applications and new uses constantly being reported in the literature. Characterization of bulk drugs becomes increasingly important in the pharmaceutical industry. Analytical techniques are commonly employed for this purpose by using drug-selective electrodes(2-7).Several different methods have been used for determination of cephalexin monohydrate including; High-performance liquid chromatographic(8-12), A capillary zone electrophoresis method(12), Parallux, a solid-phase fluorescence(13). Spectropho -tometry and derivative spectrophotometry (DS) technique were utilized in determination of cephalexin (15,16). The methods for the determination of organic substances by the derivative spectrophotometry (DS) technique have been developed mainly for application in the analysis of pharmaceuticals and biochemical interesting systems.The general aspects of UV derivative spectropho -tometry and its advantages and limitations with respect to normal spectrophotometry are reviewed.(17,18) and it's used in chemical and in pharmaceutical analysis.In this work several cephalexin selective electrodes were prepared using phosphotungstic acid ionophore with different plasticizers in PVC membranes. The electrodes based on DBPH and DOP used for determination cephalexin in oral suspension and capsules and to compare the results with UV derivative spectrophotometry.

MATERIALS AND METHODS

Apparatus

1-Double-beam UV-Visible spectrophotometer model (UV-1650 PC) SHIMADZU (Japan), interfaced with computer via a SHIMADZU UV probe data system program (Version 1.10).
2- Infrared spectrophotometer SHIMADZU, FTIR-8000 (Japan).
3- Expandable ion analyzer, ORION, model EA 940, (U. S. A.).
4- Reference electrode single junction, ORION, model 90-01
5- Combined glass electrode Orion No.91-02, (Swiss made).

Chemicals and reagents

1- Standard antibiotic drugs: cephalexin monohydrate, Amoxicillin trihydrate, cloxacillin sodium, and ampicillin trihydrate were gift from the State Company of Drug Industries and Medical Appliances (IRAQ-SDI- Samara).
2- Commercial drugs: cephalexin capsule 250 (ACFLEX)* and oral suspension of cephalexin (ACFLEX)* 125 were marketed by (The Arab Co. for Antibiotics Industries), all drugs were purchased from local pharmacies
3-The plasticizers are di-butyl phosphate, di-butyl phthalate, di-octyl phthalate, tri-butyl phosphate, o-nitro phenyl octyl ether and phosphotungstic acid were obtained from Fluka and BHD companies
4-Tetrahydrofuran (E.Merck).

5-Polyvinyl chloride (PVC) of relatively high molecular weight (Breon S 110/10 B.P Chemical U. K. Ltd).

All chemicals and solvents were of an analytical reagent grade obtained from BDH, Fluka and Aldrich companies. Deionized distilled water was used throughout the study.

Procedure

The stock standard solutions of 0.01M cephalexin monohydrate was prepared by dissolving 0.1827g standard drug, into 50 ml water by using ultrasonicator to dissolve the drug. Other standards were prepared by serial dilutions of the stock solution. Stock solution of phosphotungstic acid (0.01 M) was prepared by dissolving 1.44 g of the acid in 50 ml of water.. 0.1M stock solutions of each interfering salts. NaCL, NH_4Cl, KCl, $CaCl_2$, $MgCl_2$, $CuSO_4$ and $Fe(NO_3)_3.3H_2O$ were prepared by dissolving 0.2922, 0.2672, 0.3729, 0.5550, 0.4761, 0.7980 and 2.0201 gm in 50 ml of water respectively. 0.01M solutions were prepared for amoxicillin trihydrate, cloxacillin sodium, ampicillin trihydrate, sucrose and gelatin.The pharmaceutical drugs oral suspension cephalexin 125 BP (ACFLEX) solution was prepared by dissolving all content to 1L with vigorous shaking followed by filtration the first part was rejected, the resultant concentration of drug is 125 µg/ml ($0.342x10^{-3}$ M). 0.01 M cephalexin capsules (AEFLEX) capsules 250 BP was prepared similar to standard the content of ten capsules were mixed and homogenized and weighted accurately, the weight of one capsule was diluted to 1L of water.

Preparation of ion-pair compound

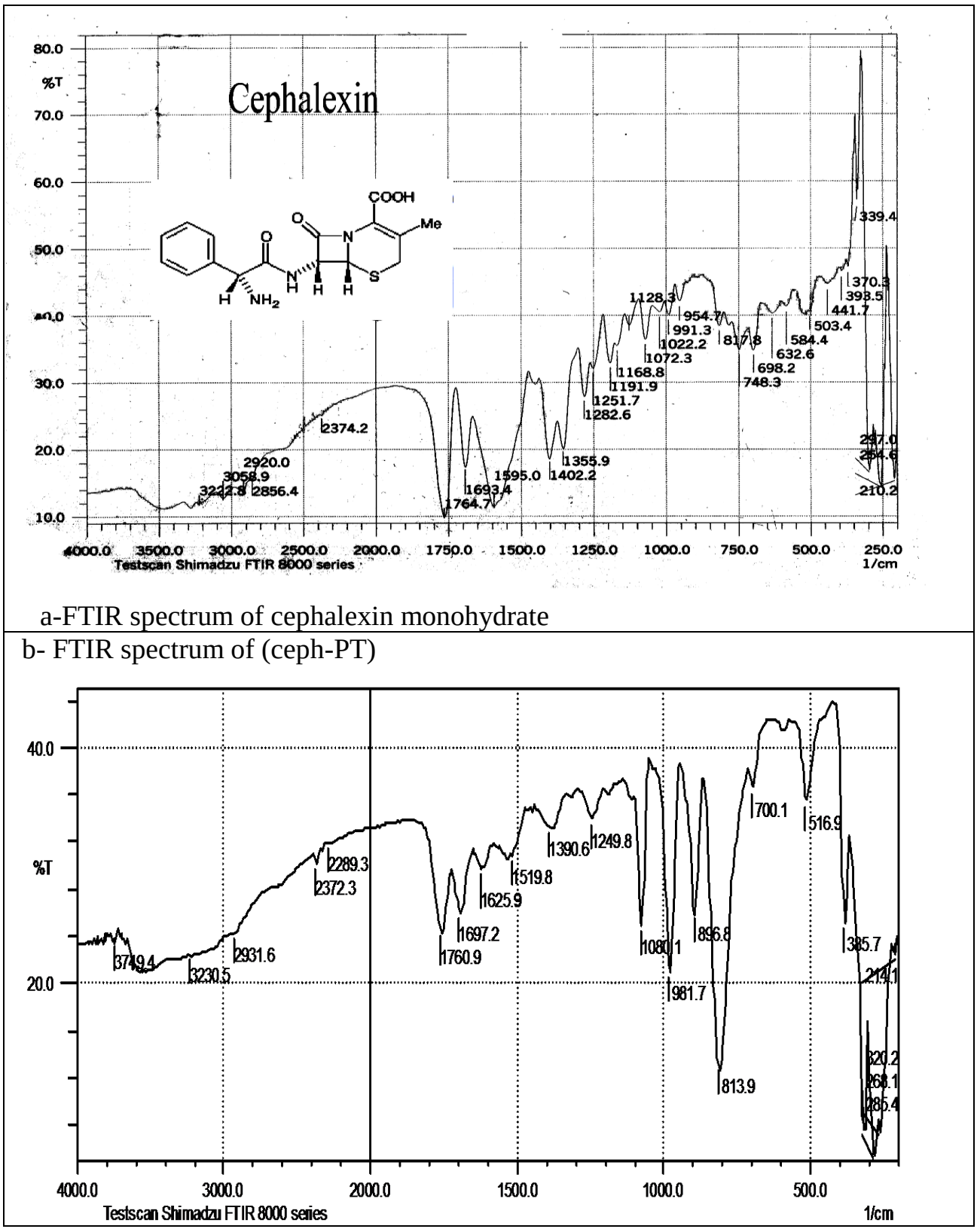
The ion-pair of cephalexin -phosphotungstate (ceph-PT) was prepared by mixing equal amounts of 0.01 M acidified solution of the drug with 0.01 M phosphotungstic acid with stirring. The resulting a yellow precipitate obtained was sediment by centrifugation and extensively washed with de-ionized water and dried for 2 days in evacuated desiccator's.

Fabrications of the membrane and electrode

The method of immobilization the ion-pair compound into the PVC matrix membrane was made as described by Craggs et al. (18). A 0.04 g of cephalexin - phosphotungstate mixed with 0.36 g plasticizer and 0.17 g PVC dissolved in 6 ml THF with stirring until a clear viscous solution was obtained. The resultant solution was poured into a glass casting ring about 35 mm in diameter, the solution was left for 2 days to allow slow evaporation of the solvent and formation a sensing membrane. Laboratory-made electrode body was used, which consisted of glass tube containing silver wire coated with silver chloride and internal filling solution. The electrode was made according to the procedure given in reference(20).

RESULTS AND DISCUSSION

Cephalexin -phosphotungstate as an electro active compound was used to prepare new sensors. The electroactive compound was confirmed by using FTIR, the coordination sites of cephalexin monohydrate involve in the bonding with phosphotungstic acid had been determined by comparision of the IR spectrum of the complex with that of the parent cephalexin monohydrate as in Figure 1. The potentiometric response characteristics based on (ceph-PT) and five plasticizers, DBP, DBPH, DOP, TBP and NPOE in PVC matrix were examined. The effect of the plasticizers was studied with respect to the slope, concentration range, detection limit, response time, life time and pH effect. All the membranes were soaked in 10^{-3} M cephalexin monohydrate solution for one hour in order to conditioning the membrane before use. Two different internal filling solutions were used; the first one was 10^{-3} M cephalexin + 10^{-3} M HCl to calibrate the electrodes from 10^{-2} M to 10^{-7} M cephalexin.. The second was 10^{-3} M + 10^{-3} M NaCl. The best conditions for determination cephalexin monohydrate were with internal filling solution of 10^{-3} M + 10^{-3} M NaCl. The slopes were near Nernistain slope with correlation coefficients around one for the electrodes based on DBPH, DOP, and NPOE. Therefore, this internal solution was fixed for all measurements. The results of electrode parameters measurements for cephalexin monohydrate selective electrodes are listed in Table 1. Electrode based on DBPH, DOP, and NPOE gave a very good Nernistain slopes equal to 59.5, 58.5 and 62.0 mV/decade and detection limit of 5.0 x 10^{-5} M, 3 x 10^{-5} M and 6 x 10^{-6} M respectively displayed good stability and reproducibility during the measurements. Also good electrode parameters were obtained for electrodes DBPH and DOP with life times around 20 and 30 days but the life time for NPOE around 7 day. This short life time can be attributed to the behavior of the plasticizer with (ceph-PT) complex or may be the low viscosity of the plasticizers or incompatibility of the plasticizer with PVC matrix. A typical plot for calibration curves of electrodes based five plasticizers are shown in Figure 2.



a-FTIR spectrum of cephalexin monohydrate

b- FTIR spectrum of (ceph-PT)

Fig.-1: a- FTIR spectrum of (ceph), b- FTIR spectrum of (ceph-PT).

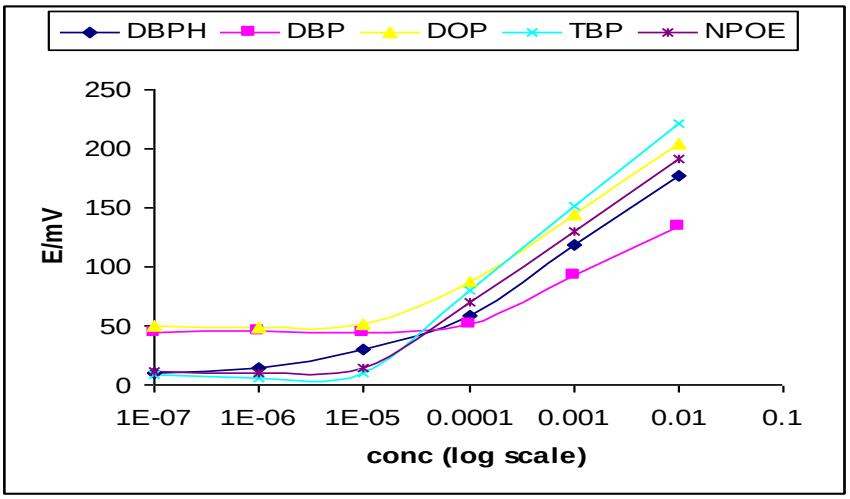


Fig. -2: The calibration curves for the five membranes: DBP, DBPH, DOP, TBP and NPOE.

Table -1: The characteristics parameters of cephalexin electrodes.

Membrane composition	Slope mV/decade	Concentration Range M	PH	Detection limit	Response /second		Life time/ day
					10^{-3} M	10^{-4} M	
Ceph-PT +DBP	42.0	$10^{-4} – 10^{-2}$	3.9- 5.1	$5x10^{-5}$	40	60	40
Ceph-PT+DBPH	59.5	$10^{-4} – 10^{-2}$	3.9- 5.1	$5x10^{-5}$	15	30	20
Ceph-PT +DOP	58.5	$10^{-4} – 10^{-2}$	3.9- 5.1	$3x10^{-5}$	25	45	30
Ceph-PT +TBP	71.5	$10^{-5} – 10^{-2}$	3.9- 5.1	$8x10^{-6}$	10	35	4
Ceph-PT +NPOE	62.0	$10^{-5} – 10^{-2}$	3.9- 5.1	$6x10^{-6}$	20	35	7

Effect of pH

The effect of pH on the response was examined by measuring the pH from 1.0 to 11.0 for the two concentrations of cephalexin monohydrate 10^{-2} and 10^{-3} M respectively. The pH range are listed in Table 2, At the strongly acid solution pH< 3 the drug undergoes a complex series of reactions leading to variety of inactive degradation products and protonated of the lacton nitrogen. At higher pH>6 the cephalexin monohydrate like other penicillin group drugs was hygroscopic and effected by pH of solutions. This may be attributed to the reactivity of the strained ß-lactam ring, particularly to hydrolysis.

Table-2: Working pH ranges for cephalexin electrodes.

Membrane Composition.	pH range	
	10^{-2} M	10^{-3} M
Ceph-PT+DBPH	3.0-6.5	2.5-6.0
Ceph-PT+DOP	3.0-6.5	3.0-6.0
Ceph-PT+NPOE	3.5-5.5	3.0-6.5

Selectivity

The influence of some inorganic cations and antibiotic of penicillin group on the response characteristics of the electrodes were investigated. Potentiometric selectivity coefficients were performed by separate solution method using 10^{-3} M concentration for both cephalexin and interfering species ($a_A = a_B = 10^{-3}$ M). The following equation was used to calculate the selectivity coefficients according to references (21) and (22).

$$\text{Log } K^{pot}{}_{ceph} = [\ (E_B - E_A\ ) / (\ 2.303\ RT/zF)\ ] + (\ 1- z_A / z_B\ ) \log a_A$$

E_A, E_B; z_A, z_B; and a_A, a_B are the potentials, charge numbers and activities for the primary A and interfering B ions, respectively, the values were calculated at $a_A = a_B$ .The results for selectivity coefficients were summarized in Table 3 using electrodes based on DBPH, DOP, and NPOE plasticizers and the potential of cephalexin monohydrate (a_A) at 10^{-3} M equal to 98, 102 and 95 mV respectively. The results in Table 3 show that the selectivity coefficients of monovalent cations are higher than divalent and trivalent cations. This may be attributed to the differences in ionic size, charge density, mobility and permeability. The values of selectivity coefficients for mono-valent (NH_4^+ and K^+) ranged from 0.227 to 0.685 for NH_4^+, 1.264 to 2.934 for K^+. Bad selectivity for Na^+ with K_{AB} ranged from 5.150 to 6.128 may be due to filling solution of the electrodes (10^{-3} M of NaCl). There is also an interference of amoxicillin, ampicillin and cloxacillin on responses of cephalexin selective electrodes. Other neutral species, sucrose and gelatin can not interfere with electrode response due to low values of selectivity coefficients, 0.092 and 0.272, respectively. None of the investigated species interfere seriously except monovalent ions.

Table-3: Selectivity coefficient values for the cephalexin electrodes.

Interfering Ion	Ceph-PT+DBPH		Ceph-PT+DOP		Ceph-PT+NPOE	
	LogKAB	K_{AB}	$LogK_{AB}$	K_{AB}	$LogK_{AB}$	K_{AB}
amoxicillin	-0.305	0.274	-0.207	0.169	-0.113	0.771
Ampicillin	-0.169	0.677	-0.121	0.739	-0.274	0.532
Cloxacilln	0.372	2.360	0.138	1.374	0.548	3.535
Na^+	0.712	5.150	0.827	6.128	0.726	5.319
NH_4^+	-0.644	0.227	-0.621	0.685	-0.441	0.363
K^+	0.102	1.264	0.466	2.924	0.306	2.025
Cu^{2+}	-0.856	0.139	-0.845	0.046	-0.613	0.244
Ca^{2+}	-0.754	0.176	-0.586	0.073	-0.774	0.168
Mg^{2+}	-1.449	0.036	-1.552	0.019	-1.613	0.024
Fe^{3+}	-0.373	0.424	-0.569	0.326	-0.532	0.294
Sucrose	-0.983	0.103	-0.810	0.092	-0.563	0.272

Sample analysis

Quantitative determination of cephalexin monohydrate in solutions was used using potentiometric techniques, direct method and increment method which include single standard addition (SSA), multi standard addition (MSA) and Gran's plot. In increment method a 0.5 ml of cephalexin monohydrate standard solution 10^{-2} M was added to 20 ml of the sample. The results of quantitative measurements for the electrodes are listed in Table 4. The (MSA) for electrodes based on DBPH and DOP and a typical plot is shown in Figure 2 and 3 The Gran's plot was constructed by using Orion Gran's plot paper with 10% correction for electrodes based on DBPH and DOP and a typical plot is shown in Figure 4 for cephalexin concentration at 2.6 x 10^{-4} M and 2.0x10^{-4} M respectively.. Direct method was used for determination of cephalexin in cephalexin oral suspension and cephalexin capsules. From the results in Table 5, the average recovery and standard deviation of cephalexin oral suspension and cephalexin capsule were (100.116 ± 0.393) and (100.344 ± 0.120), respectively These recoveries are quite comparable with that given in the certificate of British pharmacopeia's 2000 (1). Due to the interference of cephalexin monohydrate with the response of amoxicillin electrode or may be other drugs can be interfere. Therefore, UV-derivative spectrophotometry (DS), first, second, third and fourth derivatives were shown in Figure 5, used in this study for determination of cephalexin drug and to compare with cephalexin selective electrodes. The values of the wavelengths for normal spectrum and derivative spectra (^{1}D, ^{2}D, ^{3}D and ^{4}D) for cephalexin in the range 2 to 150 µg/ml were determined and the results of applying the UV-derivatives for 40 µg/ml cephalexin solution are listed in Table 6. As we noticed the accurate results were obtained by using all derivatives spectrophotometry. The accuracy of the method depending on the wavelength chose not just the order of derivative.

ceph-PT +DBPH
Slope=59.5

vml	Antilog E/s
0	22.4
0.5	42.7
1	60.5
1.5	73.4
2	89
2.5	116.7

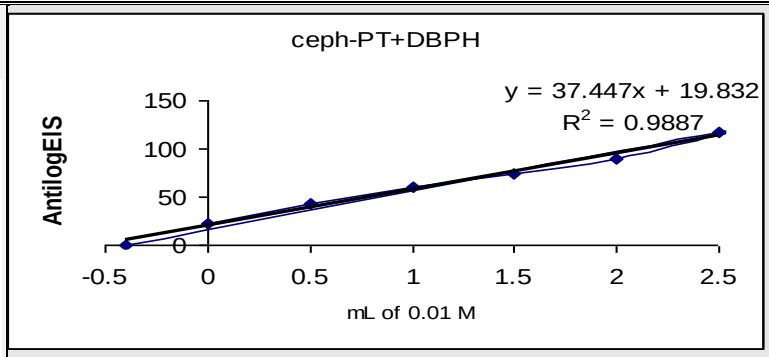


Fig.-3: Antilog (E/S) versus the volume of the added standard for the determination of cephalexin solution (2.6x10^{-4}M) MSM using electrode ceph-PT+DBPH.

Ceph-PT+DOP
Slope=58.5

Volume	Antilog E/s
0	53.27
0.5	1.17E+02
1	175.75
1.5	237.7
2	301
2.5	366.5

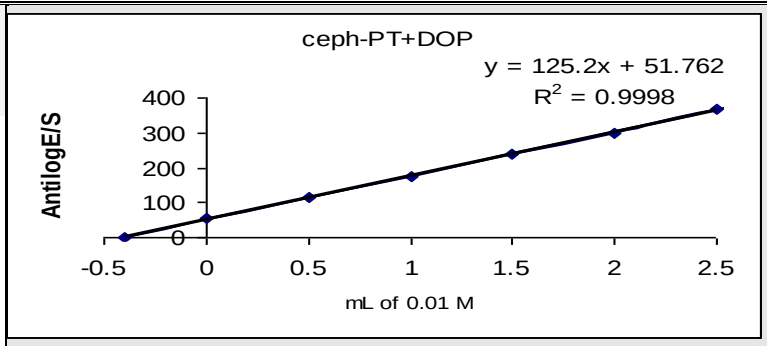
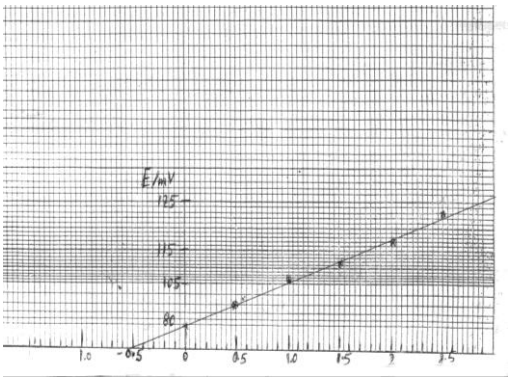
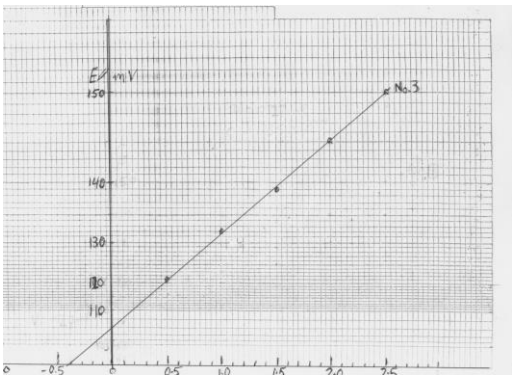


Fig.-4: Antilog (E/S) versus the volume of the added standard for the determination of cephalexin solution ($2x10^{-4}$M) by MSM using ceph-PT+DOP electrode.

Ceph-PT+DBPH
Slope= 59.5
v=0.53 mL c=$2.65x10^{-4}$ M
RE%=1.88



ceph-PT+DOP
Slope=58.5
v=0.41mL c=$2.05x10^{-4}$ M



RE%=2.5

Fig.-5: Gran's plot for ceph-PT +DBPH and ceph-PT +DOP electrodes

Table-4: Determination of cephalexin sample using cephalexin electrodes.

Membrane composition	Ceph. Conc./M sample	Measured concentration (M) Using potentiometric method					
		Direct~	gran's plot	SAM*	MSM	OPC~	TM~
Ceph-PT+DBPH	$2.600x10^{-4}$	$2.570x10^{-4}$	$2.650x10^{-4}$	$2.620 X 10^{-4}$	$2.650 X 10^{-4}$	$2.630x10^{-4}$	$2.645x10^{-4}$
	RSD%	1.24		5.95		1.34	3.74
	RE%	-1.15	1.88	0.769	1.92	1.15	2.25
Ceph-PT+DOP	$2.0x10^{-4}$	$2.07x10^{-4}$	$2.05x10^{-4}$	$1.95 X 10^{-4}$	$2.06x10^{-4}$	$2.06x10^{-4}$	
	RSD%	2.21		1.687		1.87	
	RE%	3.5	2.5	-2.5	3.0	3.0	

*The result of five additions. ~Each concentration represents an average of at least three measurements.

Table-5: sample analyses of pharmaceutical cephalexin using ceph-PT+ DBPH electrode.

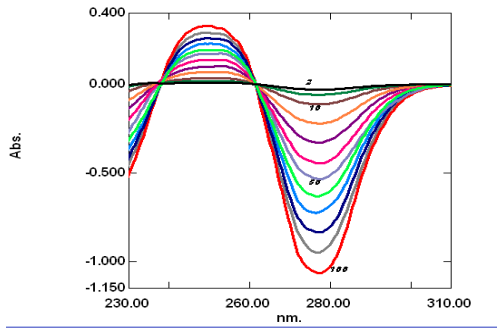
pharmaceutical	cephalexin oral suspension BP		cephalexin capsules BP	
	Direct method	OPC	Direct method	OPC
Concentration	$6.842x10^{-4}$	$6.842x10^{-4}$	$8x10^{-4}$	$8x10^{-4}$
Found*	$6.874x10^{-4}$	$6.852x10^{-4}$	$8.02x10^{-3}$	$8.03x10^{-3}$
Recovery %	100.468	100.146	100.25	100.375
RE %	0.468	0.854	0.25	0.375
Concentration	$1.368x10^{-3}$	$1.368x10^{-3}$	$2x10^{-3}$	$2x10^{-3}$
Found*	$1.372x10^{-3}$	$1.362x10^{-3}$	$2.01x10^{-3}$	$2.005x10^{-3}$
Recovery %	100.292	99.561	100.50	100.25
RE %	0.292	-0.439	0.50	0.25

*Each concentration represents an average of at least three measurements.
Relative Standard deviation ranged 0.232 to 1.156

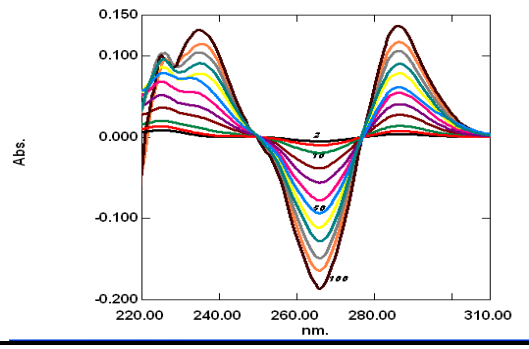
Comparison between ISE and DS

Cephalexin monohydrate was determined by First derivative using wavelength 249 nm. (n=7) and by ISE using ceph-PT+DBPH electrode (n=5). The values of F at 95% confidence level is 4.53, standard deviation (s) were 0.298 and 0.213 for the ISE and DS methods, respectively. Therefore, the resulting F is equal to 1.957. The results obtained by ISE were quite comparable with DS method and indicated no significant difference between the two methods. Other parameters for the methods are listed in Table 7, which show that two methods are equivalent in the accuracy and simplicity but differ in linear range and detection limit, which were not effected on determination of cephalexin in the pharmaceutical samples.

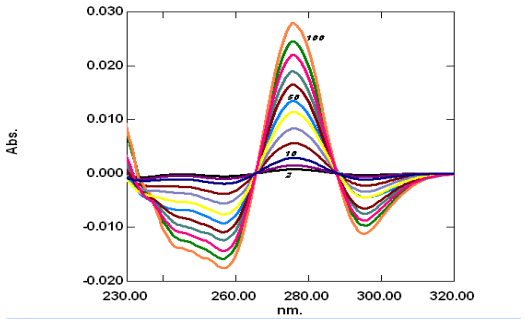
a-First derivative of ceph. S=10.Δλ=5



b-Second derivative of ceph. S=20.Δλ=10.



c-Third derivative of ceph. S=30.Δλ=10.



d-Fourth derivative of ceph. S=40.Δλ=10.

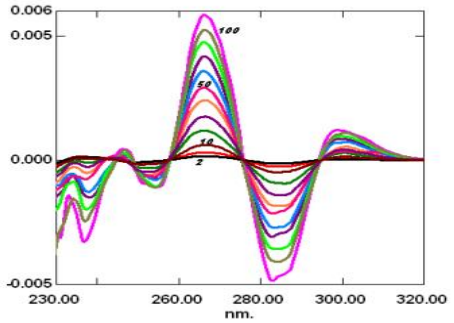


Fig.-6: The UV-derivatives: spectra first, second, third and fourth derivative for ceph.

Table-6: The equations and correlation coefficient of calibration curves for the suitable range of the cephalexin concentrations.

Method	Range mg/L	Wavelength nm	Linear equation	r
Normal	4-80	262	Y=0.02296x+0.00639	0.99974
^{1}D	12-100	P=249	Y=0.00211x-0.00042	0.99953
	8-100	V=277	Y=-0.00687x+0.00196	0.99977
^{2}D	10-50	P=286	Y=0.00178x+0.00320	0.98809
	10-50	P=236	Y=0.00164x+0.00340	0.99970
	8-80	V=268	Y=-0.00067x+0.00037	0.99971
^{3}D	8-60	P=270	Y=0.00027x-0.00025	0.99938
	8-100	V=297	Y=-0.00020x-0.00047	0.99938
^{4}D	8-100	P=268	Y=0.00151x-0.00152	0.99973
	8-60	P=297	Y=0.00071x-0.00058	0.99910
	8-100	V=286	Y=-0.00148x+0.00382	0.99951

Table-7: The parameters of ISE and DS methods to determine cephalexin monohydrate (60 mg/L).

parameter	ISE using ceph-PT+DBPH	DS using D1 at 249 nm
Linear range	$1x10^{-4}$- $1x10^{-2}$ M (36.5-365.4 mg/L)	$2x10^{-6}$-$2x10^{-2}$ M (8-100 mg/L)
Detection limit	$5x10^{-5}$M (14.6 mg/L)	$4x10^{-6}$ M (2 mg/L)
Working pH range	≈ 3.0 – 6.5	3.0 – 7.0
Standard deviation	0.298	0.213
RSD%	0.812 – 1.99	0.103 – 0.214
S^2	0.0888	0.0454
ceph .found mg/L	60.288	60.137
RE%	0.48	0.228
Recovery%	100.48	100.228

We can conclude:

Several cephalexin selective electrodes can be prepared based on phosphotungstic acid as an ionophor with different plasticizers in PVC matrix membranes. Electrodes based on DBPH and DOP plasticizers with longer life times can be used for determination of cephalexin in drugs. The recovery obtained by the electrodes was good comparable with the recovery obtained by first derivative spectrophotometer. The proposed electrodes were successfully applied to the determination of cephalexin in the pharmaceutical preparation. The analytical method proposed proved to be a simple rapid and accurate method.

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