ISSN : 0974 - 7540 Volume 4 Issue 5

Research & Reviews On Electrochemistry

Full Paper

RREC, 4(5), 2013 [161-167]

New potentiometric determination of clindamycin hydrochloride in pharmaceuticals

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ABSTRACT

A potentiometric membrane sensor responsive with satisfactory selectivity to clindamycin hydrochloride was prepared for simple and fast determination of this drug in pure form and pharmaceutical dosage forms without prior extraction process or separation from different dosage form excipients. The sensor was based on the formation of an ion association complex between clindamycin hydrochloride as a cationic drug and sodium phosphotungestate as anionic electroactive material. The produced ion association complex was incorporated in plasticized polyvinyl chloride membrane. The performance characteristics of this sensors were evaluated according to IUPAC recommendations-reveal fast, stable and near Nernestian response for 3.16×10^{-4} - 1×10^{-1} M for clindamycin hydrochloride. Statistical comparison between the results obtained by applying the proposed potentiometric method for the determination of the clindamycin hydrochloride and those obtained by applying the official method was done and no significant difference was found at $p = 0.05$. Validation of the method according to ICH guidelines shows the suitability of the sensor for quality control analysis of the cited drugs in pharmaceutical formulations. The proposed sensors can also be used as a detector for HPLC method.

KEYWORDS

Clindamycin hydrochloride;
Membrane sensor;
Potentiometry;
Sodium phosphotungestate.

INTRODUCTION

Clindamycin hydrochloride (CLD) is a lincosamide antibacterial drug with a primary bacteriostatic action against gram-positive aerobes and a wide range of anaerobic bacteria. It gives its action by binding to bacterial ribosome and inhibits the early stages of protein synthesis[1]. Different techniques were used for the determination of CLD, including spectrophotometric determinations based on color formation[2-5], HPLC determinations[6-27], capillary electrophoresis[28, 29], chemiluminescence methods[30], voltammetry[31-33] and conductometric methods[5].

The aim of the present work is to develop a potentiometric membrane electrode for the determination of CLD in pure form and pharmaceutical dosage forms

without prior extraction process or separation from different dosage forms excipients.

EXPERIMENTAL

Apparatus

All potentiometric measurements were made at 25 °C. A Jenway digital ion analyzer model 3503 (Essex, UK) with Ag/AgCl double junction reference electrode No. Z113107-1EAPW (Steinhum, Germany) was used. The influence of pH on the response of the electrodes was studied using a pH glass electrode Jenway (Jenway, UK) No. 924005-BO3-Q11C. The determination of the samples occurred using a magnetic stirrer, Bandelin Sonorox, Rx510S (Budapest, Hungaria). Silver wire of 1.0 mm diameter was purchased from Fluka (Steinheim, Germany) and used as internal reference electrode in the constructed electrodes.

Pure samples

Clindamycin hydrochloride: kindly supplied by Memphis Company, Cairo, Egypt. The purity of CLD was found to be 100.2 ± 0.90 according to the official method[7].

Market samples

- Clindam® 150 mg capsules, each capsule is labeled to contain 150 mg clindamycin HCl, batch number 92146, manufactured by SIGMA Pharmaceuticals Industries (Egypt).
- Clindam® 300 mg capsules, each capsule is labeled to contain 300 mg clindamycin HCl, batch number 90778, manufactured by SIGMA Pharmaceuticals Industries (Egypt).
- Dalacin-C® 150 mg capsules, each capsule is labeled to contain 150 mg clindamycin HCl, batch number 04P09D150, manufactured by Pfizer Pharmaceutical Company (Cairo, Egypt).
- Dalacin-C® 300 mg capsules, each capsule is labeled to contain 300 mg clindamycin HCl, batch number 9004, manufactured by Pfizer Pharmaceutical Company (Cairo, Egypt).

All of them were purchased from local market.

Reagents and chemicals

All chemicals and solvents used were of analytical grade

Polyvinyl chloride (PVC) high molecular weight, Dibutyle sebathate (DBS) and Sodium phosphotungestate (PTA) were purchased from Fluka (Steinheim, Germany), while Terahydrofuran (THF), 99% was obtained from BDH (Poole, England). KCl, HCl and NaOH were obtained from Prolabo (Pennsylvania, USA) and deionized water was used

Standard solution for linearity

Stock solutions of CLD (1×10^{-1} M) and working solutions of CLD ($1 \times 10^{-1} - 3.16 \times 10^{-4}$ M) were prepared in deionized water.

Procedures

Preparation of clindamycin HCl ion exchangers:

The ion-pair complex of CLD-phosphotungestate was prepared by slow addition of 10 mL 1×10^{-2} M aqueous solution of CLD to 10 mL 1×10^{-2} M Na-phosphotungestate solution with stirring. The resulting precipitate was filtered using Whatmann No 42 filter paper, washed with cold water, dried at room temperature (about 25 °C) and grinded to fine powder. The resultant ion association complex was confirmed by elemental analysis.

In a Petri dish (5 cm diameter), 0.01 g of ion pair complex was mixed thoroughly with 0.19 g of PVC, 0.35 g DBS and 5 mL THF. The dish was covered with a filter paper and left to stand overnight to allow slow evaporation of the solvent forming the master membrane with about 0.1 mm thickness[34].

Electrode assembly

A disk of an appropriate (about 8 mm) diameter was cut from the previous prepared master membrane and cemented to flat end of an adhesive of PVC tubing by dissolved in THF. The other end of the PVC tubing was then connected to an appropriate glass outer casting.

A mixture of equal volumes of 10^{-2}M CLD and 10^{-2}M KCl solutions was used as an internal reference solution and Ag wire was dipped inside the electrode after being plated in concentrated HCl to be Ag/AgCl internal reference electrode. The membrane was conditioned by soaking in 10^{-2}M aqueous drug solution over night and stored in the same solution when not in use.

Sensor calibration

The prepared sensor was immersed in conjunction with the double junction Ag/AgCl reference electrode in working solutions of CLD in the range of 3.16×10^{-4} - 1×10^{-1} M, after pH checking, allowed to equilibrate whilst stirring and the e.m.f. readings were recorded within ±1 mV. The membrane sensor was washed between measurements with double distilled water. The e.m.fs were recorded as a function of the drug concentrations, then the calibration graph of the recorded potentials vs. -log drug concentrations were plotted. The computed regression equation for the linear part of the curve was used for the subsequent determination of unknown concentrations of CLD.

Application to pharmaceutical formulations

The contents of 10 capsules (from each dosage form) were emptied as completely as possible, accurately weighed and mixed. Transfer an accurately weighed portion of the powder, equivalent to 0.2308 g CLD to a 50-mL volumetric flask add about 35 mL deionized water and sonicate for 5 min then completed to volume with deionized water to prepare 10^{-2} M solution of CLD, then serial dilution to 3.16×10^{-4} M was done.

RESULTS AND DISCUSSION

ISEs play an important role in pharmaceutical analysis due to their simplicity and rapidity over some other analytical methods like spectrophotometry and HPLC[35]. Selective membrane in ISEs have shown both ion exchange and perm-selectivity of the sensor ions and signal is generated by charge separation at the interface between the ion selective membrane and the solution due to selective partitioning of ionic species between these two phases[36, 37]. Furthermore, they may be used for the measurement over a wide concentration range. ISEs are generally tolerant to small changes of pH. A further advantage is that they are relatively simple and not expensive to develop, set up and run[38].

In the present work, CLD is an antibacterial agent; its structure does not have any conjugation therefore exhibiting a very poor UV absorption, Figure (1). Determination of CLD using spectrophotometric method requires previous derivatization and adjusting the condition of experiment which increase both difficulty and time of the method. By reviewing the literatures, we found that there is no ion selective electrode method was reported for the analysis of CLD and other reported electrochemical methods were complex and need expensive apparatus. This acquired our attention to develop simple, cheap, rapid, selective, precise and accurate method for determination of CLD in pure bulk and in pharmaceutical dosage forms without previous pretreatment steps (derivatization or extraction, ….etc).

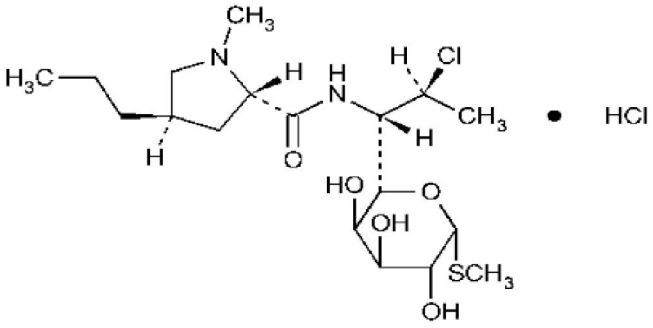

Figure 1 : Chemical structure of clindamycin hydrochloride

Optimization of experimental conditions

First we try to prepare different sensors for determination of CLD the first 2 sensors were based on using 2-hydroxypropyl-β-cyclodextrin (2-HP-βCD) as ionophore with PVC-COOH which has two recommended properties, (partial dissociation and high adhesion)[39]. The first sensor was plasticized by dioctyl phthalate while the second was plasticized using o-nitophenyl octyl ether. Poor Nernestian responses were obtained (30.1 and 45.2) upon using dioctyl phthalate and o-nitophenyl octyl ether, respectively. So we oriented to the fact that CLD behaves as a cation in acidic medium due to the protonation of amino group (Figure 1), this fact suggests the use of an anionic exchanger.

Choice of appropriate ion exchanger

The type of the ion exchanger affects the response of the sensor[36], therefore the two anionic exchanger namely, ammonium-reineckate and PTA were tried. CLD-reineckate membrane gave very poor Nernestian response, about 40 mv, while phosphotungestate was found to be the optimum anion exchanger for the studied drug. The resulting precipitates have low solubility products and suitable grain size. CLD reacted with PTA ($Na_3O_{40}PW_{12}$) to form stable 3:1 water insoluble ion association complex.

This ratio was confirmed by elemental analysis data, TABLE (1), and by Nernestian response of the suggested electrode which was about 60 mV, the typical value of monovalent substance, indicating that CLD behaves as monovalent cation.

TABLE 1 : Elemental analysis of CLD-PTA complex.

Parameters	Analysis %[a]		
	C	H	N
Calculated %	15.6	2.4	2.0
Found %	14.4	2.6	1.8

[a] Calculated on 3:1 basis

Effect of plasticizers

The effect of plasticizers was investigated using DBS (a non polar plasticizer) and o-nitrophenyl octyl ether (polar plasticizer) for the preparation of the proposed electrode. According to the result obtained, DBS was found to be the more effective. This indicates that DBS adjusts both membrane permeability and ion exchange sites mobility to give the highest possible selectivity and sensitivity.

Effect of pH

The effect of pH on the potential- of the electrode system was studied over pH range 2-10 at 1 pH interval by immersing the electrode in 10^{-3} M and 10^{-2}M aqueous solutions of CLD. Gradual increase or decrease in pH was obtained by adding aliquots of 1M sodium hydroxide or hydrochloric acid solutions, respectively with constant stirring. The potential obtained at each pH value was recorded. It was found that the investigated electrode response was stable over pH range 4-6, Figure (2).

Effect of some additives and related drug

The performance of the electrode in the presence of some pharmaceutical additives that are commonly used in drug formulation e.g. sodium chloride, potassium chloride, purified talc, magnesium stearate, lactose, urea and lincomycin HCl was studied.

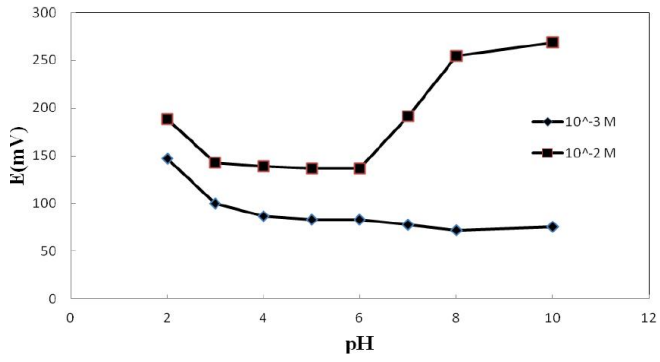

Figure 2 : Effect of pH on the response of CLD-PTA electrode.

Selectivity coefficient values ($K^{pot}_{primary\ ion,\ interferent}$) were measured using separated solution method[40] in which concentration of the measured interferent was 10^{-3}M. The small values of selectivity coefficient showed no significant interference from these additives, indicating selectivity for CLD, TABLE (2).

TABLE 2 : Potentiometric selectivity coefficient ($K^{pot}_{primary\ ion,\ interferent}$) for the investigated CLD electrode.

Interferent 10^{-3}M	Selectivity coefficient[a]
K^+	12.4×10^{-2}
Na^+	9.4×10^{-2}
Mg^{++}	3.06×10^{-2}
Talc	3.3×10^{-2}
Lactose	4.64×10^{-2}
Urea	4.98×10^{-2}
Lincomycin HCl	13.2×10^{-2}

[a] Average of three determinations.

The coefficients were calculated from the equation:

$$\text{Log } K^{pot}_{primary\ ion,\ interferent} = \frac{EB - EA}{S}$$

Where $K^{pot}_{primary\ ion,\ interferent}$ is the potentiometric selectivity coefficient, S the slope of the calibration plot, EA and EB are the potential for CLD and the interfering ion, respectively.

Identification of the slope, response time and operative life of studied electrode

The electrochemical performance of the studied electrode was evaluated, CLD phosphotungestate, according to IUPAC recommendation[41].

The slope of the calibration curve was typically 54.36 mV/concentration decade. Deviation from the ideal Nernestian slope (60 mV), stems from the fact that the electrode responds to the activities of the drug cation rather than its concentrations. TABLE (3) showed the response characteristics of the electrode.

The response time of the electrode was tested for concentrations 10^{-3} M and 10^{-2} M of the drug. The measurements were characterized by a fast stable response within 20 seconds. The electrode displayed constant and stable potential readings within ±2 mV from day to day. Calibration slopes did not change by

TABLE 3 : Electrochemical response and validation of the investigated CLD electrode.

Parameter	CLD-PTA
Slope (mV/decade)	54.36
Intercept (mV)	248.48
Correlation coefficient (r)	0.9999
Response time (seconds)	20
Working pH	4.0-6.0
Concentration range (M)	$3.16x10^{-4}$-10^{-1}
Stability (weeks)	2
Accuracy (mean ±SD)[a]	100.0 ± 0.45
Error (% RSD/$\sqrt{n}$)	0.314
Repeatability[b] ± S.D.	100.2 ± 1.320
Intermediate precision[b] ± S.D.	100.0 ± 1.114

All values measured at room temperature (≅25°C); [a] Average of five determinations; [b] = 3x3.(Concentration 10^{-3},$3.16x10^{-3}$, 10^{-2} M of CLD were measured three times).

more than 2.0 mV/decade over a period of 2 weeks.

Method validation

The validity of the proposed potentiometric method was assessed by studying the following parameters: linearity, range, accuracy, precision and selectivity according to the ICH guidlines[42].

Linearity

The potentiometric response of the electrode at the optimum pH and at room temperature was linear with constant slope over a drug concentration range $3.16x10^{-4}$ - 10^{-1} M. The regression equation was computed and found to be:

y = 54.36 x + 248.48 r = 0.9999

Where y is the potential in mV, x is –log CLD molar concentration, and r is the correlation coefficient.

Accuracy

The proposed electrode can determine different concentrations of bulk powder with mean percentage recovery of 100.0 ± 0.45 for CLD. To prove the accuracy of the proposed method, the results of the assay of CLD in pure form by the proposed electrode were compared with those obtained using the official method [7].

Statistical comparison of the results obtained by the proposed electrode with those obtained by the official method using student *t*-test and variance ratio *F*-test revealed no significant differences between the perfor-

TABLE 4 : Statistical analysis of the results obtained by the proposed electrode and the official method[7] for the determination of CLD.

Value	CLD-PTA	Official method[a](7)
Mean	100.0	100.2
± SD	0.45	0.90
%RSD	0.45	0.90
n	6	5
Variance	0.198	0.810
Student's t (2.365)[b]	0.463	-----
F test (5.192)[b]	4.091	-----

[a] HPLC method based on using C_{18} (250 mm × 4.6 mm 5 µm) column and phosphate buffer solution: acetonitrile (55:45, *v/v*) as a mobile phase, the pH adjusted to 7.5, with UV detection at 210 nm; [b] The values between parenthesis are the corresponding theoretical values of t and F at (*p= 0.05*) (43).

TABLE 5 : Determination of CLD in its pharmaceutical preparations by the proposed electrode.

Conc. Taken (-log M)	Conc. Found (-log M) Clindam® capsules 150mg[a]	Found%[e] Clindam® capsules 150mg[a]	Conc. Found (-log M) Clindam® capsules 300mg[b]	Found%[e] Clindam® capsules 300mg[b]	Conc. Found (-log M) Dalacin-C® capsules 150mg[c]	Found%[e] Dalacin-C® capsules 150mg[c]	Conc. Found (-log M) Dalacin-C® capsules 300mg[d]	Found%[e] Dalacin-C® capsules 300mg[d]
3.50	3.48	99.4	3.47	99.1	3.47	99.1	3.46	98.9
3.00	3.02	100.7	2.97	99.0	3.02	100.7	3.01	100.3
2.50	2.52	100.8	2.51	100.4	2.52	100.8	2.52	100.8
Mean		100.3		99.5		100.2		100.0
± SD		0.781		0.781		0.954		0.985
%RSD		0.779		0.785		0.952		0.985

[a] B.No. 92146, Nominal content of each capsule equal 150.0 mg CLD; [b] B.No. 90778, Nominal content of each capsule equal 300.0 mg CLD; [c] B.No. 04P09D150, Nominal content of each capsule equal 150.0 mg CLD; [d] B.No. 9004, Nominal content of each capsule equal 300.0 mg CLD; [e] Average of four determinations.

mances of the two methods[43], TABLE (4).

Precision

Precision of the assay was determined in relation to repeatability (intra-assay) and intermediate precision (inter-assay). In order to evaluate the repeatability of the methods, nine samples were determined during the same day for three concentrations of CLD. Intermediate precision was studied by comparing the assays performed on three different days. The SD values were less than 2% demonstrating that the method was precise. Good recoveries were obtained for each concentration, conûrming that the method was accurate, TABLE (3).

Selectivity (Application to pharmaceutical formulation)

The selectivity of the proposed electrode was established by its ability to determine CLD in capsules (two different concentrations and two different manufacturers), in presence of common capsule excepients and without prior extraction procedures.

Satisfactory results were obtained as shown in TABLE (5).

CONCLUSION

The studied electrode is sufficiently simple and selective for the determination of CLD in the pure powder form, in its pharmaceutical formulation and in the presence of other lincosamide antibiotic.

The use of the proposed electrode offers the advantage of fast response, elimination of drug pretreatment or separation steps, wide pH range and direct determination of drug in turbid solutions. It also provides a good solution for the low UV absorption of CLD. It can therefore be used for routine analysis of drug in quality control laboratories and may be used as detector for CLD in HPLC method.

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