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Novel PVC-free All-solid-state Ion-selective Electrode for Determination of Chlorhexidine in Pharmaceutical Formulations

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Abstract- The potentiometric response characteristics and analytical applications of a poly(vinyl chloride) (PVC)-free all-solid-state ion-selective electrode for chlorhexidine (CHX) are examined and compared to those of PVC-based solid-state electrode. The PVC-free electrode is prepared by direct incorporation of chlorhexidine-tetraphenyl borate (CHX-TPB) as a sensing element into a commercial nail varnish (NV) containing cellulose acetate propionate. The composite was applied onto a 3 mm diameter graphite disk-electrode. The electrode exhibited a Nernestian slope of 29 mV/decade in the concentration range from 10^{-5} to $\leq 10^{-3}$ mol/L with a detection limit of 4×10^{-6} mol/L. The electrode is independent of pH in the range from 3.6 to 7.9. Cationic inorganic ions showed negligible interference, whereas, a strong electrode response was observed for cetrimide and benzalkonium chloride with selectivity coefficients of 8.62 and 7.59 respectively. Comparable pH working range and selectivity pattern were observed with the PVC-based electrode. The electrode was used for determination of CHX in mouthwash and gargle, without extraction, with high precision (%RSD≤2) and accuracy (±2%). The new electrode is simple, economical and rapid when compared to the PVC-based electrode.

Keywords- Solid-state ion-selective electrode, PVC-free, Chlorhexidine, Potentiometry, Pharmaceutical preparations

1. INTRODUCTION

Conventional ion-selective electrodes (ISEs) have been extensively used for the analysis of pharmaceutical [1,2] and environmental samples [3]. They have the capability of performing direct measurements in turbid and colored samples. In conventional ISEs, a sensing membrane, usually a PVC membrane is sandwiched between two solutions, the sample and the inner filling solutions. Such configuration requires a good deal of practice and patience and, requires large volumes of samples for analysis. In contrast, in solid-state ISEs the inner filling solution is eliminated and the sensing membrane is casted directly onto a solid contact (e.g, Pt, gold, graphite) [4]. Polymers other than PVC were employed in the development of ion-selective electrodes including, substituted PVC [5], polyurethane [6] and silicon rubber [7-9]. The advantage of these materials over PVC is that they are biocompatible and can be used for in vivo measurements.

Commercial nail varnish is inexpensive, made of biocompatible materials and is available everywhere. Typically, it is composed of film forming agents, resins and plasticizers (water resistant) and some other ingredients. The nail varnish is made with optimum composition that renders it safe, water resistant, stable and hardens quickly in a few minutes in traditional UV light or lamp.
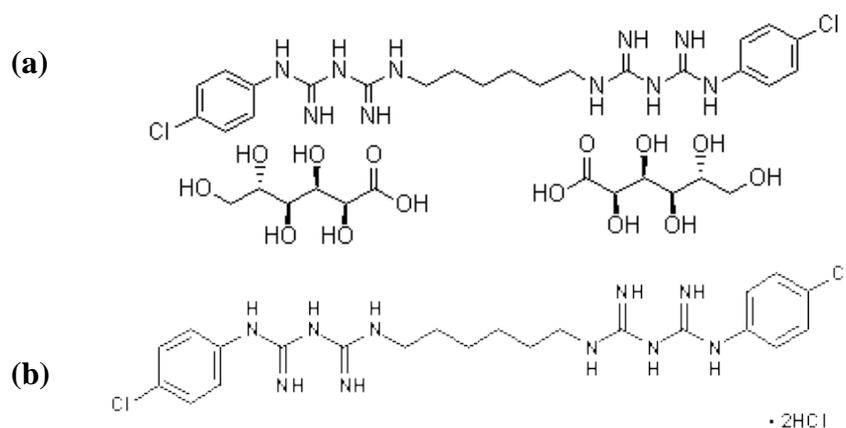
Scheme 1. The chemical structure of **(a)** chlorhexidine digluconate and **(b)** chlorhexidine hydrochloride

Herein, a commercial nail varnish containing cellulose acetate propionate is exploited for the preparation of a PVC-free solid-state ion selective electrode for the determination of CHX salts (digluconate and hydrochloride) in pharmaceutical products. Scheme 1 shows the chemical structure of chlorhexidine digluconate and chlorhexidine hydrochloride. The response characteristics of the new electrode were evaluated by comparison to that of a CHX-selective PVC-based solid-state electrode. Chlorhexidine (CHX) is a cationic antiseptic [10]

that is widely used in human to prevent and treat redness, swelling, and bleeding gums associated with gingivitis [11]. The electrode is based on the incorporation of CHX-TPB ion pair as a sensing element into the nail varnish. The proposed electrode has been used for the assay of chlorhexidine salts directly in mouth and gargle without extraction or separation. The selectivity of the electrode towards common interfering compound was assessed using the separate solution method (SSM) [12]. The proposed solid-state ion selective electrode is an inexpensive and reliable tool for rapid analysis.

2. MATERIALS AND METHOD

2.1. Chemicals and reagents

Chlorohexidine digluconate (CHXD) (20% w/v), Hexitol mouthwash (1.25% w/v chlorhexidine hydrochloride) and Antiseptol gargle (0.1% w/v chlorhexidine digluconate) were obtained from El Kahira Pharma. & Chem Ind. Co. (Amirya, Egypt). Dioctylphthalate (DOP) was purchased from Merck and Poly(vinyl chloride) (PVC) of high molecular weight was purchased from Aldrich. A commercial nail varnish was obtained from a local pharmacy, Cairo, Egypt. Tetrahydrofurane (THF) was obtained from Lab-Scan Analytical Science. All reagents were of chemically pure grades and bi-distilled water was used throughout.

2.2. Preparation of solid-state CHX-selective electrodes

CHX-TPB ion-pair was prepared by adding 100 mL of 0.01 mol/L Na-TPB to 50 mL 0.01 mol/L chlorhexidine digluconate (CHXD). The solution was stirred for 10 min. The white precipitate was filtered, washed with bi-distilled water and left to dry over night at room temperature.

The CHX-selective nail varnish-based solid-state electrode was prepared by mixing 42 mg of CHX-TPB with 340 mg of a commercial nail varnish. About 0.5 mL of acetone was added to insure good mixing. A 25 µL of the mixture was applied onto the top of 3 mm diameter graphite disk electrode. The electrode was left to dry at room temperature for 30 min.

The CHX-selective PVC-based solid-state electrode was prepared by dissolving 120 mg of CHX-TPB ion pair, 740 mg DOP and 340 mg PVC in 3 mL THF. 25 µL of the mixture was applied onto the top of 3 mm diameter graphite disk electrode. The THF was allowed to evaporate and then the electrode was dipped two times in the mixture solution. The electrode was allowed to dry over night at room temperature.

2.3. Electrochemical system

Potentiometric measurements were carried out with HI 9321 microprocessor pH meter. A saturated Ag/AgCl electrode was used as an external reference electrode. The internal reference electrode was a coated wire Ag/AgCl electrode

The electrochemical system is represented as follows:

Graphite disk-electrode/sensing matrix (nail varnish or PVC)/test solution//KCl salt brige/3M KCl/Ag/AgCl.

2.4. Construction of calibration graph

The calibration graphs were constructed using solutions of different concentrations of chlorhexidine digluconate (CHXD) covering the concentration range from 10^{-7} to 10^{-2} mol/L. The cell potential was recorded for each solution at constant stirring at room temperature and plotted against log [CHXD]. The slope of the calibration graph was calculated using Nernestain equation (Eq. 1).

$$E = E_{ISE}^{\circ} + 2.303 \frac{RT}{zF} \log[CHXD] \qquad\qquad (Eq.\ 1)$$

Where: R is the gas constant, F is the Faraday equivalent and z is the charge of the analyte. The term E_{ISE}° is a constant which is the sum of all invariants in the system.

2.5. Selectivity

Potentiometric selectivity coefficients $\mathbf{K_{i,j}^{Pot}}$ for different inorganic and organic cations were evaluated using the separate solution method (SSM) [12]. In this method the EMF value (E_i and E_j) of the electrode in pure solution of each of the primary (a_i) and the interfering ion (a_j), of equal concentration ($a_i=a_j=10^{-3}$ mol/L), are used for calculating the selectivity coefficient. The selectivity coefficient $\mathbf{\log K_{i,j}^{Pot}}$ is calculated using Nickolsky-Eisenman (Eq. 2).

$$\log K_{i,j}^{Pot} = \frac{(E_j - E_i)}{2.303 RT/z_i F} + \left(1 - \frac{z_i}{z_j}\right) \log a_i \qquad a_i = a_j \qquad (Eq.\ 2)$$

3. RESULTS AND DISCUSSION

3.1. Response characteristics of the electrodes

A commercial nail varnish containing cellulose acetate propionate is exploited for the preparation of CHX-selective all-solid-state electrode. A nail varnish free from CHX-TPB showed no response to CHX even though after soaking the electrode in 10^{-3} mol/L chlorhexidine solution for 1 hour (Fig. 1a). On the other hand, a nail varnish containing 5%

CHX-TPB (w/w) showed a non-Nernestian slope of 19 mV/decade over a narrow concentration range from 8×10^{-5} to $\leq10^{-3}$ mol/L. The slope and the linear range were improved by increasing the amount of CHX-TPB. A nail varnish containing 9-13% (w/w) CHX-TPB showed a Nernestian response with a slope of 29 ± 1 mV/decade over the concentration range from 10^{-5} to $\leq10^{-3}$ mol/L (r=0.9997) with a detection limit of 4×10^{-6} mol/L (Fig. 1b). Further increase of CHX-TPB to 16% led to a sluggish response with a non-Nernestian slope of 22 mV/decade.

Therefore, a nail varnish containing 11% (w/w) CXH-TPB was used as an optimum composition for preparation of CHX-selective solid-state electrode. The average slope of ten electrodes was 28.95 mV/decade with RSD of 1.9%, which means that the method of preparation is reproducible. The calibration graph showed a deviation from the Nernestian response at CHX concentration $\geq10^{-3}$ mol/L. This deviation would be due to the decrease in chlorhexidine digluconate activity as a result of dimer formation [13]. The measurement of increasing and decreasing the concentration of CHX (ascending and descending calibration) showed slightly different e.m.f. values (Fig. 1b and c).

Comparable results were obtained with a PVC-based solid-state electrode containing 10 or 13% (w/w) CHX-TPB. The PVC-based electrode exhibited a near Nernestian slope of 27 ± 1 mV/decade over the concentration range from 10^{-5} to $\leq10^{-3}$ (r=0.9993) and a detection limit of 5×10^{-6} mol/L. A calibration curve for CHX using the PVC solid-state electrode is shown in Fig. 1d. The composition and potentiometric response characteristics of CHX-selective nail varnish- and PVC-based solid-state electrodes are shown in Table 1.
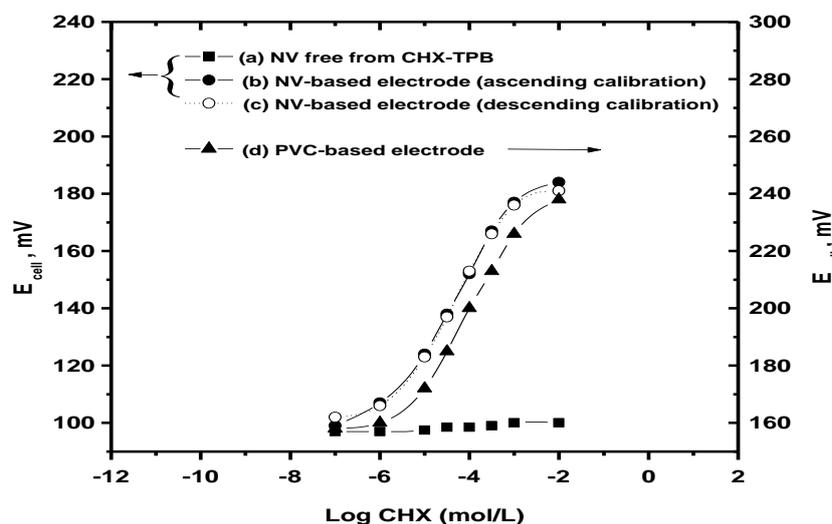
Fig. 1. Calibration curves for the CHX-selective solid-state electrodes: (a) nail varnish free from CHX-TPB, (b) nail varnish modified with 11% w/w CHX-TPB (ascending calibration), (c) nail varnish modified with 11% w/w CHX-TPB (descending calibration) and (d) plasticized PVC modified with 10% w/w CHX-TPB

Table 1. Response characteristics of CHX-selective nail varnish- and PVC-based solid-state electrodes

Electode	Composition % (w/w)				Slope (mV/decade)	Linear range (mol/L)	Detection limit (mol/L)
	CHX-TPB	NV	PVC	DOP			
NV- solid state	0	100	-	-	-	-	
	5	95	-	-	19	8×10^{-5} to $\leq10^{-3}$	
	7	93	-	-	21	6×10^{-5} to $\leq10^{-3}$	
	9	91	-	-	29	110^{-5} to $\leq10^{-3}$	4×10^{-6}
	11	89	-	-	29	10^{-5} to $\leq10^{-3}$	3×10^{-6}
	13	87	-	-	28.5	10^{-5} to $\leq10^{-3}$	4×10^{-6}
	16	84			22	6×10^{-5} to $\leq10^{-3}$	
PVC-solid state	5	-	30	65	26	4×10^{-5} to $\leq10^{-3}$	
	10	-	28.3	61.7	27	10^{-5} to $\leq10^{-4}$	5×10^{-6}
	13	-	27	60	27	10^{-5} to $\leq10^{-4}$	

The nail varnish-based solid-state electrode was used directly for CHX measurements. In contrast, soaking the PVC-based electrode in bidistilled water for at least 30 min was necessary for a Nernestian response. This difference would be attributed to the difference in hydrophilicity/lipophilicity of cellulose acetate propionate and PVC. The effect of soaking time on the electrode response to CHX was studied by calibrating the electrodes at different time intervals and recording the slope and the detection limit. The slope of the nail varnish-based electrode was constant at 29 ± 1 mV/decade with a linear dynamic range from 10^{-5} to $\leq10^{-3}$ mol/L and a detection limit between 4×10^{-6} and 6×10^{-6} mol/L for 3 days. The slope decreased after five days to 25 mV/decade with a narrower linear range from 5×10^{-5} to $\leq10^{-3}$ mol/L and a detection limit of 3×10^{-5} mol/L. Longer life time has been observed when the electrode was stored in air, the electrode has retained its slope at 29 ± 1 mV/decade with a linear range from 10^{-5} to $\leq10^{-3}$ mol/L and a detection limit between 4×10^{-6} and 7×10^{-6} mol/L for seven month.

On the other hand, the slope of the PVC-based electrode was constant for 13 days of continuous soaking in bidisttilled water. The electrode exhibited a Nernestian slope of 27 ± 1 mV/decade with linear range from 10^{-5} to $\leq10^{-3}$ mol/L and detection between 5×10^{-6} and 8×10^{-6} mol/L. The slope decreased after 15 days to 23 mV/decade with a linear range from 5×10^{-5} to $\leq10^{-3}$ mol/L and a detection limit of 2×10^{-5} mol/L.

The dynamic response time of the proposed electrodes was studied by measuring the time required to achieve a steady state potential (within ± 1 mV) after successive immersion of the electrode in a series of stirred CHXD solutions (1.0×10^{-6} to 1.0×10^{-3} mol/L), each having a 10-fold increase in concentration. A small potential drift (0.8 mV/min) was observed for the NV-based electrode at lower concentrations of CHXD (10^{-6} mol/L). The cell potential remained constant (within ± 1 mV) for 5 minutes at 10^{-5} and 10^{-4} mol/L. A drift in the cell potential of 1 mV/min was observed again when the electrode was immersed in 10^{-3} mol/L solution. Similar response time was observed for the PVC-based electrode. The electrode showed a small potential drift (0.5 mV/min) in 10^{-6} mol/L solution. The cell potential was steady within ± 1 mV at 10^{-5} mol/L and up to 10^{-4} mol/L CHXD solution. The potential time response of the proposed electrodes is shown in Fig. 2.
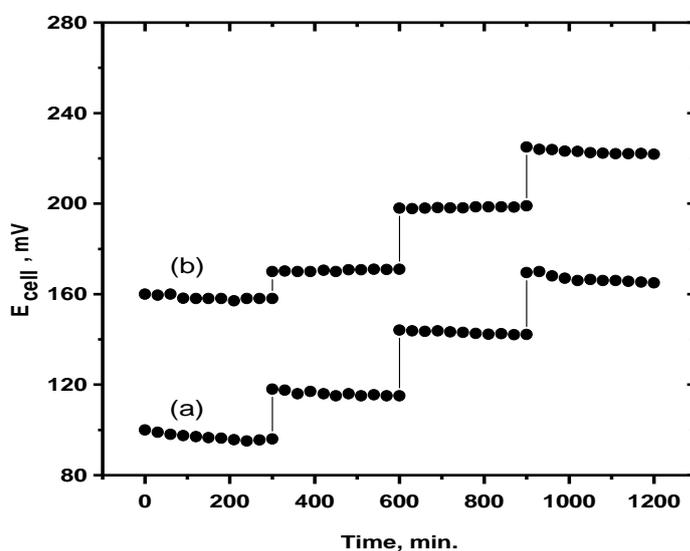

Fig. 2. Typical potential-time plot for the response of a) NV-based and b) PVC-based solid-state CHX-selective electrodes

The effect of pH on the potential of electrodes was investigated by recording the variation in the cell potential when small volumes of hydrochloric acid and sodium hydroxide (0.1-1.0 mol/L of each) was added to 10^{-4} mol/L chlorhexidine digluconate solution (Fig. 3). It is obvious that the electrodes do not respond to pH changes in the range from 3.6 to 9.7. The decrease in cell potential at pH less than 4.6 may be due to interference from H^+, while the decrease that takes place at pH values higher than 9.7 is most probably due to the formation of free chlorhexidine base in the test solution.

3.2. Selectivity of the electrodes

Assessment of the selectivity of ISEs has been so far a controversial issue [14]. For simplicity, here, the selectivity of the proposed electrodes to CHX in presence of common ions is assessed using the SSM [12]. Besides the numerical values of $\log K_{i,j}^{Pot}$, the calibration curves of the electrodes using the interfering ions is presented. The logarithmic $K_{i,j}^{Pot}$ values for divalent inorganic cations Ca^{2+} and Mg^{2+} showed no interference; this is consistent with the calibration curves which showed a negligible response of the electrodes to these ions (Fig. 4). In contrast, the logarithmic $K_{i,j}^{Pot}$ for monovalent inorganic cations (Li^+, Na^+ and K^+) showed a slight interference while the constructed calibration curves showed a negligible inference that is similar to that for divalent cations. Such contradiction would be due to difference in charge number. High interference was observed for the lipohilic compounds cetrimide and benzalkonium chloride (Fig. 4). The selectivity coefficient $K_{i,j}^{Pot}$ of the NV-based electrode was 8.62, and 7.59 for cetrimide and benzalkonium chloride, respectively. The interference can be explained by the diffusion of these compounds into the sensing part of the electrode at the electrode solution interface. The selectivity coefficient values of the NV-based and PVC-based solid state electrodes are summarized in Table 2.
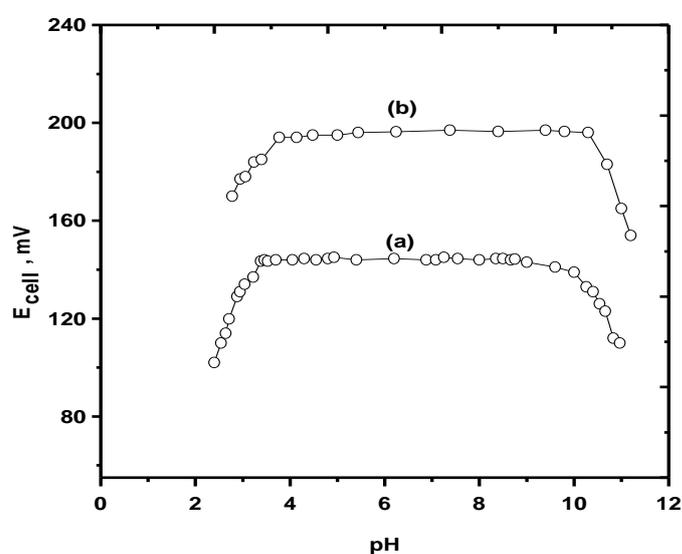
Fig. 3. Effect of pH of a test solution of 10^{-4} mol/L chlorhexidine digluconate on the potential response of CHX-selective solid-state electrodes: (a) nail varnish-based electrode and (b) PVC-based electrode
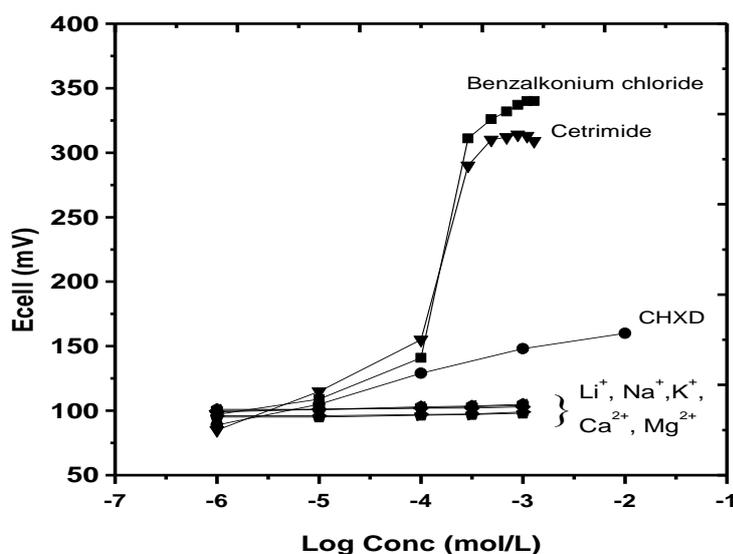
Fig. 4. Calibration curves for chlorhexidine digluconate (CHXD) and different interfering ions using the NV-based solid-state electrode

Table 2. Selectivity coefficient values $\log K_{i,j}^{Pot}$ of the nail varnish-based and PVC-based solid-state electrodes

Inteferent	$\log K_{i,j}^{Pot}$	
	NV-solid electrode	PVC-solid state electrode
Li^+	0.52	0.45
Na^+	0.41	0.48
K^+	0.45	0.55
Ca^+	-2.52	-2.66
Mg^+	-2.52	-2.52
Cetrimide	8.62	8.03
Benzalkonium chloride	7.59	7.86

3.3. Analytical application

The proposed electrodes were successfully applied for the determination of CHX in pure solution and in pharmaceutical preparations. The samples were prepared by appropriate dilution from the pharmaceutical products using bidistilled water. The detection limit found for CHX with the nail varnish solid-state CHX-selective electrode was 0.22 µg/mL. This is better than the detection limits of spectrophotometric methods which range from 1 to 20

µg/mL; moreover, owing to the broad linear range (from 10^{-5} to $\leq 10^{-3}$ mol/L) of the electrode described here, it might be more flexible in applications where a series of samples with widely varying CHX concentrations have to be analysed. Potentiometric determination of CHX salts (digluconate and hydrochloride) in pure solution and in pharmaceutical products using the CHX selective nail varnish and the PVC-based solid-state electrodes by potentiometric titration are summarized in Table 3. The potentiometric titration curves of CHXD in the range from 0.45 to 44.89 mg/50 mL against Na-TPB are presented in Fig. 5. A well defined potential break at the titration end-point was obtained when the concentration of the titrant was 20 fold that of chlorhexidine salt solution. The end point was determined using the derivative method. The mean recovery data (n=3) for all concentrations was within ±2% with RSD of less than 2%. The LOQ of CHX using the nail varnish-based solid-state electrode was 9 µg/mL (0.45 mg/50 mL) with a RSD of 3.34% calculated from five replicate samples. The potentiometric titration curve of 9 µg/mL CHXD against 10^{-3} mol/L Na-TPB is presented in Fig. 5a.

Other methods were proposed for quantification of chlorhexidine in pharmaceutical products including chromatography [15-19], solid-phase extraction with UV spectrophotometry [20], capillary electrophoresis [21,22] and flow injection extraction-spectrophotometry [23]. Although good results were obtained with these methods, they require dilution and other time-consuming steps for sample preparation.
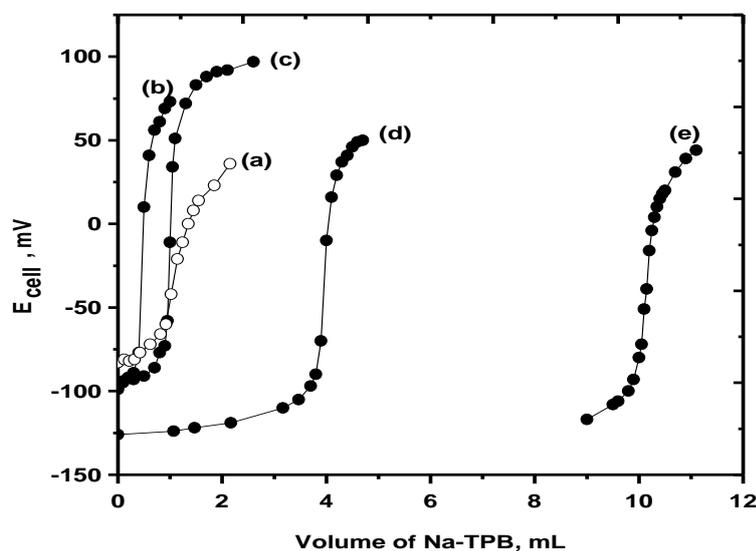
Fig. 5. Potentiometric titration curves of chlorhexidine digluconate against sodium tetraphenyl borate using the nail varnish-based solid-state electrode. Curve (a): 0.44 mg chlorhexidine digluconate against 10^{-3} mol/L sodium tetraphenylborate. The other curves (b), (c), (d) and (e) are for 2.24, 4.49, 17.96 and 44.89 mg of chlorhexidine digluconate against 10^{-2} mol/L sodium tetraphenylborate

Table 3. Accuracy and precision results of determination of chlorhexidine digluconate and chlorhexidine hydrochloride in pure solution pharmaceutical formulations (Antiseptol gargle and Hexitol mouthwash) using the nail varnish- and PVC-based solid-state CHX-selective electrodes

Taken	Nail varnish-based solid-state electrode		PVC-based solid-state electrode	
	Found	% Recovery	Found	% Recovery
(mg/50 mL)	(mg/50 mL)	average ± %RSD [a]	(mg/50 mL)	average ± %RSD [a]
Pure solution (20% chlorhexidine digluconate)				
2.24	2.17	96.7±1.49	2.15	95.8±1.51
4.49	4.42	98.5±1.34	4.42	96.8±1.66
17.96	17.66	98.3±0.73	17.58	97.9±0.74
44.89	44.36	98.83±0.29	44.22	98.5±0.51
Hexitol mouthwash (1.25% chlorhexidine hydrochloride)				
2.88	2.78	96.75±1.23	2.75	95.5±1.23
3.13	3.07	97.95±1.91	2.97	95.00±0.55
3.75	3.66	97.96±1.93	3.70	98.65±1.84
Antiseptol mouthwash and gargle (0.1% chlorhexidine digluconate)				
1.00	1.01	97.07±1.91	0.96	96.00±1.63
2.00	2.00	100.23±1.30	1.99	99.66±1.13
2.50	2.48	99.28±0.63	2.44	97.60 ±0.43

[a] Mean recovery and relative standard deviation of three determinations

4. CONCLUSION

Commercial nail varnish proves useful for the preparation of solid-state CHX-selective electrode. The potentiometric characteristics were comparable to that of a PVC-based CHX-selective electrode. The nail varnish–based electrode is reliable for the determination of CHX in the range from $5×10^{-5}$ to $≤10^{-3}$ mol/L and, therefore, can be alternative for the chromatographic techniques which are lengthy and solvent consuming. The interference from common inorganic ions was negligible. A strong response was observed to cetrimide and benzalkonium chloride. Under these circumstances, separation of CHX prior to analysis would be useful. The nail varnish would be used for the preparation of solid-state ion selective electrodes for other important drugs. The nail varnish-based electrode is easily

prepared and cheap when compared to the conventional and solid-state PVC-based electrodes.

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