行政院國家科學委員會補助專題研究計畫成果報告

除臭製品中二氯苯氧氯酚與三氯二苯脲及化妝品與口腔衛生製品中雙氯苯雙胍己烷測定方法之研究

Determination of triclosan and triclocarbon in deodorants and chlorhexidine in cosmetic and oral hygine products

計畫類別：V☐個別型計畫　　☐整合型計畫

計畫編號：NSC　89－2113－M－041－007

執行期間：89 年 8 月 1 日　至　90 年 7 月 31 日

計畫主持人：王來好 副教授

共同主持人：

計畫參與人員：

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二氯苯氧氯酚(Triclosan，商品名 Irgason DP-300，2,4,4'-trichloro-2'-hydroxydiphenyl ether)與三氯二苯脲(Triclocarban，3,4,4'-trichlorocarbanilide)爲廣效的殺菌抗黴菌劑，對於感染於表皮及黏膜的微生物是強力的殺菌作用。所以兩者常被合併使用於治面皰抗痘的洗面乳、除臭體香製品、牙膏、藥皂及洗手乳等。本篇利用快速方便的高效率液相層析法，同時測定市售十八種化妝品與藥品中 triclosan 與 triclocarban 含量。樣品中的殺菌劑以甲醇微溫萃取，並於冰浴中離心而取得。液相層析分離係利用 Nucleosil C_{18} (4.6×250 mm)管柱，acetonitrile/water(7:3, v/v)爲移動相。由於 triclosan 與 triclocarban 的最大紫外線吸收波長分別爲 280 nm 與 260 nm，因此使用折射儀(Differential Refractometer)檢出器同時測定 triclosan 與 triclocarban 含量。Triclosan 與 triclocarban 的線性範圍分別爲 0.1 %~1.2 %和 0.02 %~0.16 %。該法用於測定樣品中 triclosan 與 triclocarban 之含量，回收率在 101 %~109 %之間。每個樣品分析只需要 7 分鐘，能滿足快速、準確、方便地分析各類樣品中 triclosan 與 triclocarban 的要求。

A rapid convenient high-performance liquid chromatography method for the simultaneous determination of some bacteriostats such as triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether) and triclocarban (3,4,4'-trichlorocarbanilide) is described. The liquid chromatography system consisted of a Nucleosil C_{18} column and 7:3 (v/v) acetonitrile : water as the mobile phase with a refractive index detector. The method has been applied in assaying commercial toothpastes, deodorant sticks, anti-acne washing cleaners and healthcare personal handwashes. Comparison with results obtained from differential pulse voltammetry show good agreement.

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Voltammetric behavior of chlorhexidine at a film mercury electrodes and its determination in cosmetics and oral hygiene products

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Abstract

The voltammetric behaviors of chlorhexidine on glassy carbon and electrodeposited mercury electrodes were investigated in an aqueous medium containing various supporting electrolyte. The various factors such as deposition material, time, concentration of mercury(II) and of coexisting inorganic, organic and surfactant interferences on the precision of the analysis have been explored. The possible reaction mechanisms were discussed by the relations of scan rate and peak potentials and currents. A comparison is made between the detection limit of glassy carbon, thin-film modified on glassy carbon and gold electrode. The electroreduction process is applied for the simultaneous quantitative determination of antiplaque agent and anticaries in oral hygiene products. The antiplaque agent (chlorhexidine) and anticaries (aluminum fluoride and sodium fluoride) of toothpaste in 1 + 1 mixture of 0.1 M lithium chloride and lithium hydroxide have a stripping peak at −1.88, −1.69 and −1.30 V for chlorhexidine, aluminum fluoride and sodium fluoride, respectively. Comparison with results obtained from high-performance liquid chromatography shows 98% good agreement.

Keywords: Chlorhexidine; Antiplaque agent; Anticaries; Film mercury electrode; High-performance liquid chromatography

1. Introduction

Chlorhexidine (CH), hexamethylenebis[5-(4-chlorophenyl)biguanide], and its salts, frequently chlorhexidine diglyconate (CHXG), are effective antibacterial agents. The use of chlorhexidine in the oral cavity has been the subject of numerous investigations [1–3]. Reviews on plaque control have concluded that to date chlorhexidine, used in mouthrinse, toothpastes, is the most effective chemical antiplaque agent. Chlorhexidine diglyconate is generally used as a skin disinfectant in various surgical hand scrubs, patient preoperatives skin preparation products, healthcare personal hand washing products and wound cleansing products [4]. Chlorhexidine and its diacetate and digluconate salts are used in cosmetics as preservatives. Chlorhexidine diglyconate was slightly toxic in oral and inhalation studies [5]. The paper reported DNA-damaging capacity of chlorhexidine diglyconate by the liquid *rec*-assay [6]. The chlorhexidine molecule will slowly degrade to form degradation products. This degradation of chlorhexidine is promoted by light, heat and ionizing radiation. *p*-Chloroaniline is the greatest concern product because of its toxicity. The FDA has approved the drug use of a mouthrinse containing 0.12% chlorhexidine diglyconate.

Various spectrophotometric methods for the determination of chlorhexidine have been reported [7–11].

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Materials used include *o*-hydroxyhydroquinonephthalein–manganese, copper complex and sorbents. Since most of these methods require solvent extraction, they have such disadvantages as complexity of procedure and lack of reproducibility. High-performance liquid chromatography is widely applied to the analysis of chlorhexidine pharmaceutical creams [12–19]; the methods usually involve time-consuming and laborious sample clean up steps, such as liquid–liquid extraction and excipient precipitation by cooling of an appropriate sample solution. The polarographic behavior of chlorhexidine has previously been described [20–23]. It based on its adsorptive accumulation at a hanging mercury drop electrode. However, the effects of interferences by other surface-active substances were not explored. These interferences might affect the accuracy in determination of chlorhexidine. A thin-film mercury electrode (TFME), coated on glassy carbon electrode (GCE) or gold (Au), can be useful for the determination of chlorhexidine in cosmetics and pharmaceuticals by differential pules voltammetry (DPV). TFME is more sensitive than DME [24]. Various factors influencing the determination of chlorhexidine are discussed.

2. Experimental

2.1. Apparatus

All electrochemical experiments were performed using an EG&G Princeton Research (Princeton, NJ, USA) Model 253 Versatat connected to a EG&G Model 616 Rotating Electrode system. A three-electrode system was employed, consisting a working electrode (Hg^{2+}/GCE and Hg^{2+}/Au), a platinum counter and a saturated calomel electrode (SCE) reference electrode.

The HPLC system consisted of a Model 576 pump (Gasukuro Kogyo, Japan), a Model 7125 injector equipped with a 20 μl sample loop and a Model 502 U spectrodetector. Chromatograms and peak area were obtained with a SISC Chromatogram Data Integrator.

2.2. Reagents and materials

Chlorhexidine, chlorhexidine digluconate and 4-chloroaniline were obtained from Aldrich, Sigma, and TCT Chem. Co., respectively. All other chemicals were of analytical-reagent grade. The following surfactants were used: Anion, sodium dodecylsulfate (SDS) and sodium dodecylbenzenesulfate (SDBS); nonionic, Span 60, Tween 60, Brij 35; cationic, benzalkonium chloride (BKC), cetylpyridinun chloride (CPC) and cetyltrimethylammonim bromide (CTMAB).

Anionic, cationic and nonionic surfactants were purchased from Sigma and E. Merck, respectively.

Flavors: menthol and methyl salicylate were purchased from Lancaster and Acors, respectively.

The supporting electrolyte was a 1 + 1 mixture of 0.1 M lithium chloride and 0.1 M lithium hydroxide (E. Merck). Sample of cosmetic, mouthrinse, toothpaste and antiseptic liquid were bought from a number of outlets in south Taiwan.

3. Procedure

3.1. Determination of chlorhexidine by DPV

The thin-film metal electrode was produced by the following method, prior to analysis, the glassy carbon and gold electrodes (4 mm diameter) were mirror polished sequentially with aqueous suspension of 1.0, 0.5 and 0.05 μm alumina, respectively. The glassy carbon or gold electrode was rinsed with deionized water and electrolytically plated with mercury from 25 ml of acetate buffer (pH 4.23) that was 1.0×10^{-4} to 4.0×10^{-3} M mercury(II). Plating times were 4 and 8 min, with a potential scan from −0.8 to 0.0 V at 1500 rpm.

Comparative tests of various pH and supporting electrolytes were taken for chlorhexidine in a phosphate buffer (pH 2.14, 6.08 and 11.68) and ammonium buffer (pH 7.27 and 10.38) methanol or water containing various salts such as sodium perchlorate, lithium perchlorate, tetraethylammonium tetrafluoroborate, tetrabutylammonium hydroxide and 1 + 1 mixture of lithium chloride and lithium hydroxide solution.

A 1.0 g amount of cosmetic and oral hygiene sample was accurately weighed, dissolved in 10 ml of 1:1 (v/v) mixture of methanol and water and mixed with vertex treatment for 20 min. After centrifuging, the supernatant was transferred into a 10 ml calibrated flask and make up to a volume with methanol and water (1:1, v/v). In order to obtain calibration graph for the chlorhexidine, 10 ml of supporting electrolyte was

pipetted into a voltammetric cell and de-aerated with nitrogen for 4 min before voltammetric measurement. By micropipette, aliquots of 1000 ppm chlorhexidine solution were added. After each addition voltammograms were obtained; the solution de-aerated for 1 min after each addition before obtaining the voltammogram. Quantitative analyses were performed in the differential pulse mode. The potential was set at −1.0 to −2.0 V versus saturated calomel electrode (SCE) for reduction. The pulse height was 50 mV, and the scan rate 10 mV s^{-1} with a film electrode. For sample solution analysis, 1 ml of the solution was pipetted to volume with 1 + 1 mixture of lithium chloride and lithium hydroxide solution. This solution was analyzed by DPV using the same condition as for calibration graph.

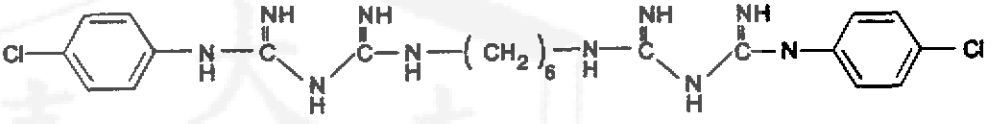
3.2. Determination of chlorhexidine by HPLC

Since chlorhexidine and *p*-chloroaniline each contain amine functionalities, HPLC analyses have used ion-pairing reagents and/or amine modifiers to minimize peak tailing. HPLC methods are either based on separation by cation exchange (Alltech, RP-H IC Cartridge) or on a combination of ionpair and reversed phase chromatography using acetate, phosphate or sodium 1-heptane sulfonate as the counterion and C_{18} material as the stationary phase. Stock solution of standard was prepared by dissolving the appropriate amount of chlorhexidine in methanol. A set of standard solutions were produced by diluting aliquots of the stock solutions with methanol to 10 ml in calibrated flasks. Taking into account about the chlorhexidine content of the antiperspirant, toothpaste, gargle mouthwash and antiseptic liquid, samples (approximately 0.5–2.0 g) of the latter were weighed accurately in a 15 ml beaker, diluted to about 1 ml with dichloromethane and 9 ml methanol, dissolved and centrifuged. The supernatant was transferred into a 10 ml calibrated flasks. An aliquot of the solution load a conditioned cation exchange column (condition with 1 ml water) and filtered through a 0.45 μm membrane filter prior to HPLC analysis. A μBondapack C_{18} column (particle size 5 μm, 3.9 mm i.d.) was used for reverse-phase HPLC. The mobile phase were 80:20 and 90:10 methanol–water (containing phosphate or acetate buffer) at a flow rate of 1.0 ml min^{-1}, the UV detector was operated at 240 nm. By means of the injection value, 25 μl of the prepared sample solution and standard solution was chromatographed under the operating conditions described above. Quantitation was based on the peak area of the sample.

4. Results and discussion

4.1. Choice of analytical method

Chlorhexidine is a bisbiguanide which presents a hexametilen–(CH_2•) radical between two biguanide groups.



The flexibility and length of a hexametilen radical, permit the molecule to fold and unfold with relative facility, changing its structure when the pH, temperature and composition of the medium in which it is dissolved are modified [21]. For the various electrolytes such as phosphate (pH 2.45–11.87) and ammonia (pH 7–10) buffer, tetraethylammonium tetrafluoroborate lithium perchlorate, sodium perchloride, tetrabutylammonium hydroxide (Bu_4NOH, pH 12.47), (1 + 1) lithium chloride and lithium hydroxide (LiCl/LiOH, pH 12.36) were examined. Preliminary experiments showed that cyclic voltammograms of chlorhexidine in acidic solutions exhibit only a poorly-defined wave, with peak potential close to the reduction wave of the supporting electrolyte, which is not useful for a voltammetric determination of the cosmetics and pharmaceuticals. On the other hand, voltammograms recorded for alkaline medium (Bu_4NOH and LiCl/LiOH) exhibit a single well-defined wave. The effect of pH on the reduction wave of chlorhexidine was investigated by Jacobsen and Glyseth [22]. The limiting current is independent of pH in the range 6.5–8.3 but decreases of higher pH values. In accordance with the behavior of chlorhexidine a one-electrode wave was observed in acid solutions. This wave corresponds to the reduction of the protonized form of chlorhexidine. The steepest wave was obtained at pH values above 9. At higher

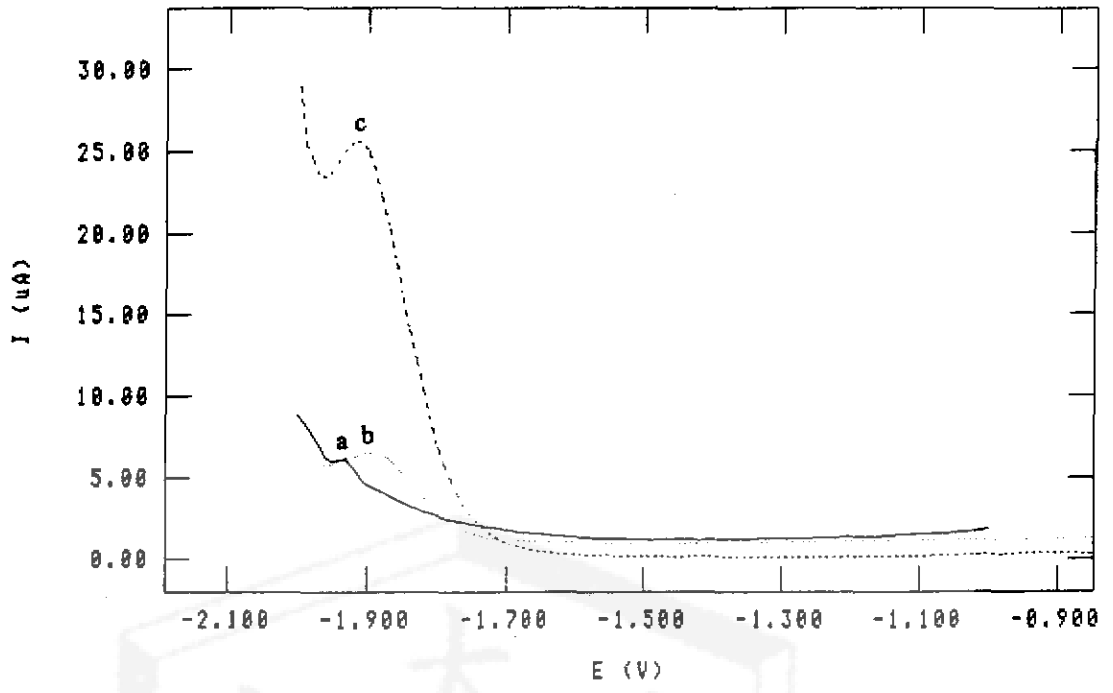


Fig. 1. Differential pulse voltammograms of chlorhexidine (20 ppm) lithium chloride and lithium hydroxide: (a) at GCE, (b) thin-film mercury modified GCE, (c) at thin-film mercury modified Au electrode, scan rate 10 mV s^{-1}, pulse height 0.05 V.

pH values only one two-electrode wave was observed for chlorhexidine. This change is probably due to the protonization reaction for the chlorhexidine transferred to the reaction layer by diffusion. Moreover, a better separation of chlorhexidine wave from that of the supporting electrolyte was obtained at higher pH values. The peak height of chlorhexidine in solutions of (1 + 1) LiCl/LiOH was found to be higher than Bu_4NOH. Consequently, 0.1 M LiCl/LiOH was chosen as supporting electrolyte in the following experiments.

Chlorhexidine have four azomethine bond and may undergo reduction at a dropping or hanging mercury electrode by a similar mechanism to other imine group double bond systems [20,23]. The cathodic wave obtained −1.47 V for chlorhexidine on a dropping mercury electrode in pH 7.05 Britton–Robinson buffer aqueous dimethylformamide media [20]. The peak potential of chlorhexidine was −1.53 V on a hanging mercury drop electrode in pH 9.70 ammonia–ammonium acetate buffer [23]. The reduction of the bearing substituent R_1, R_2 and R_3 imine group double bond was influenced of the exchange of R_1, R_2 and R_3 [25]. The effect of the reduction of the azomethine bonds to amino groups, the chlorophenyl (R_1 or R_2 = C_6H_5Cl) portion of the chlorhexidine is more instructive than that of the remaining part. This difference in the course of the electrode process may be caused by the resonance and inductive effect of the chlorophenyl grouping. The reduction of chlorhexidine in 1 + 1 mixture LiCl/LiOH was studied on GCE, thin-film mercury deposited on GCE (TFM/GCE) and thin-film mercury deposited on gold electrode (TMF/Au). It can be seen in Fig. 1 that TFM/Au give a better performance than TFM/GCE. In this reduction, three kinds of working electrodes were investigated and detection limits were 20.0, 2.23 and 0.742 mg l^{-1} for GCE, TFM/GCE and TFM/Au, respectively. Therefore, the TFM/Au was chosen for use in the determination of chlorhexidine in antiperspirant, toothpaste, gargle mouthwash and antiseptic liquid samples.

Three cyclic voltammograms (CV) dependence of peak current i_p on scan rate of chlorhexidine were shown in Fig. 2. The cathodic current ($i_{p,c}$) depends on square root of scan rate ($v^{1/2}$) and anodic current ($i_{p,a}$) is zero; thus that ($i_{p,c}$)/($i_{p,a}$) is zero for all scan rate. The fact that no peaks were observed in the

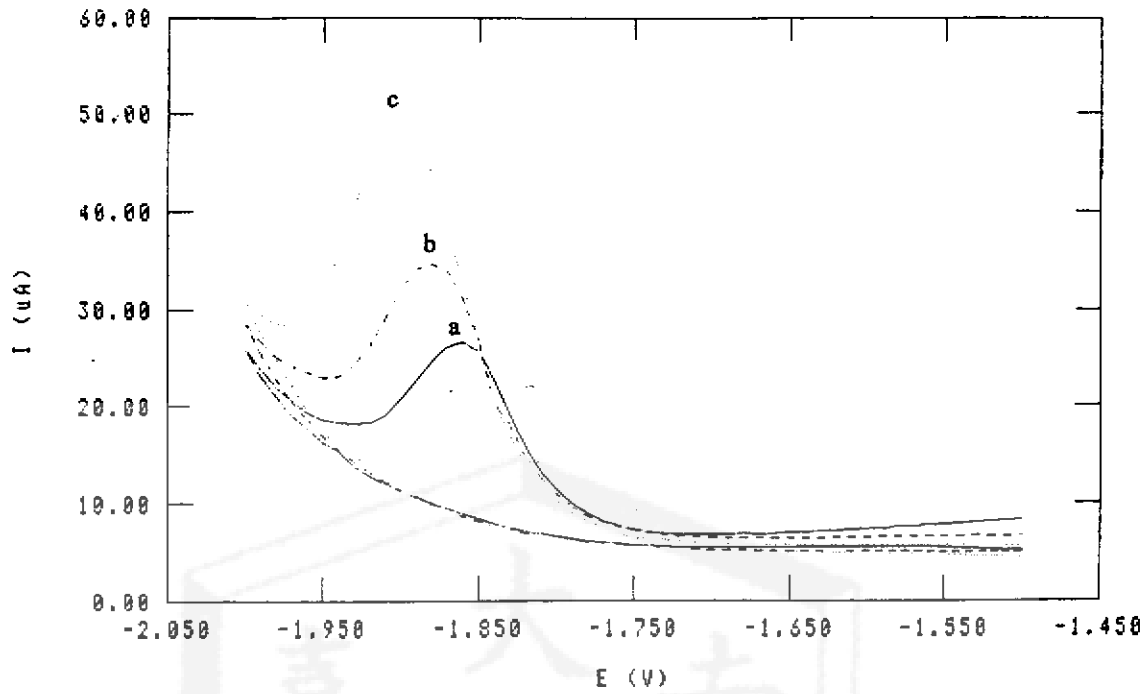
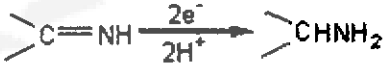


Fig. 2. Cyclic voltammograms of chlorhexidine (5×10^{-5} M) in 0.1 M lithium chloride and lithium hydroxide (pH 12.20) at a thin-film mercury modified Au electrode scan rate: (a) 25 mV s^{-1}, (b) 50 mV s^{-1}, (c) 100 mV s^{-1}.

anodic direction suggests that the process is an irreversible one [26]. The $i_{p,c}$ of linear sweep voltammetry (LSV) depends on scan rate ($v^{1/2}$) and of peak potential (E_p) becomes more negative with increasing scan rate. Fig. 3 shows the peak current of chlorhexidine increasing linearly with the concentration. Hence, the class of electrode process of chlorhexidine in CV and LSV is irreversible $A + ne \rightarrow B$. Proposed scheme for the mechanism of reduction of chlorhexidine may be the following: the reaction may be schematically pictured as a two-electron reduction to form an amino group.



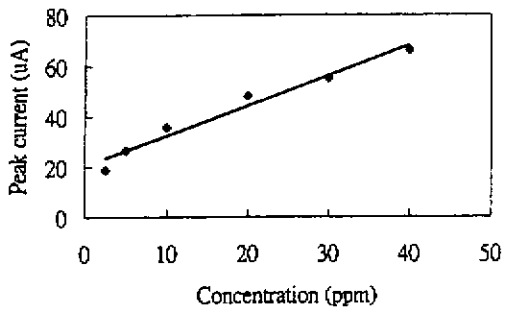


Fig. 3. The peak current of linear sweep voltammetry dependence on concentration of chlorhexidine. Scan rate 50 mV s^{-1}.

4.2. Reproducibility and accuracy

Fig. 4 shows the results obtained by standard addition of chlorhexidine solution and the peak height of the wave at −1.85 V. The analytical curves show good linearity over the range of 5.0–40.0 ppm. For chlorhexidine, the regression equation being $y = 2.68 + 1.284x$ (correlation coefficients, $r = 0.9999$). The relative standard deviation value was 2.0%. Fig. 5 shows the DPV of chlorhexidine for commercial antiseptic sample spiked with standard solutions. Recovery tests were carried out on cosmetic and pharmaceutical products were spiked with the amounts reported in Table 1 and subjected to the whole procedure. As shown in Table 1, excellent recoveries and precision were observed (recoveries ranging form 99.4 ± 3.2 to $101 \pm 5.0\%$).

Table 1
Recovery of chlorhexidine and chlorhexidine digluconate added to commercial antiperspirant, toothpaste and antiseptic liquid by DPV at a thin-film mercury electrode[a]

	Chlorhexidine					Chlorhexidine digluconate			
	Added (ppm)	Found (ppm)	Mean	Recovery (%)	R.S.D. (%)[b]	Added (ppm)	Found (ppm)	Recovery (%)	R.S.D. (%)
Antiperspirant	10.00	9.92 ± 0.01, 10.20 ± 0.02, 9.8 ± 0.01	9.97 ± 0.24	99.7	0.5	–	–	–	–
Toothpaste	20.00	20.49 ± 0.25, 20.15 ± 0.20, 22.3 ± 0.3	20.98 ± 0.42	105	2.0	–	–	–	–
Antiseptic liquid.	–	–	8.01 ± 0.38	101	4.7	8.00	8.13 ± 0.15, 7.77 ± 0.20, 8.14 ± 0.28	99.4	3.2

[a] Number of determination ($N = 3$).
[b] Relative standard deviation.

4.3. Interferences

It is well known that chlorhexidine is a cationic compound, and that it forms salts of low solubility with anions such as phosphate, sulfate and chloride. Aluminum fluoride, sodium fluoride, sodium benzoate and sodium monofluorophosphate are therapeutic agent added to toothpastes and gargle mouthwashes. A unique group of inorganic salts of aluminum(III) ion are the basis for all commercial antiperspirant metal salts utilized today. In general, surface-active agents are through to lower the surface tension penetrate and loosen surface deposits, and emulsify or suspend the debris which the abrasives in dentifrice remove from the tooth surface. Sodium dodecyl sulfate (SDS) is one of the most widely used synthetic detergents in dentifrices. The concentration of SDS in dentifrices usually range from 0.5 to 2.0%. A cationic quaternary ammonium compounds such as benzalkonium chloride, cetylpyridium chloride, cetyltrimethyl ammonium bromide and chlorhexidine digluconate, which are widely used in clear ophthalmic, gargle mouthwash and antiseptic solution. The effect of the ingredients in cosmetic and pharmaceutical products on the determination of chlorhexidine was investigated. As shown in Table 2, on interference effect

Table 2
Effect of ingredients of cosmetic and pharmaceuticals on the determination of chlorhexidine

Ingredient	Chlorhexidine				
	Peak potential (V)	Added ($mg\,l^{-1}$)	Present ($mg\,l^{-1}$)	Found ($mg\,l^{-1}$)	Recovery (%)
Aluminium fluoride	1.69	200.0	10.0	10.1	101
Sodium fluoride	1.31	200.0	10.0	9.72	97.2
Sodium dodecyl sulfate	–[a]	80.0	10.0	10.4	104
Sodium dodecyl benzene sulfate	–	80.0	10.0	10.1	101
Benzalkonium chloride	1.66	160.0	10.0	9.54	95.4
Cetylpyridinium chloride	0.95	40.0	10.0	9.86	98.6
Cetyltrimethylammonium bromide	1.24	40.0	10.0	10.3	103
Tween 60	–	80.0	10.0	10.5	105
Span 60	–	80.0	10.0	10.4	105
Brij 35	–	80.0	10.0	9.56	95.6
Menthol	–	80.0	10.0	9.90	99
Methyl salicylate	1.57	80.0	10.0	10.3	103

[a] Not detected by the DPV method.

were observed. The chlorhexidine wave appears in the voltage range between −1.84 and −1.88 V and can be used for the determination of chlorhexidine in the presence of reasonable amounts of aluminium(III) and sodium(I) ions. The TFM/Au could perform the simultaneous determination of these compounds in a mixture as can be seen in Fig. 5.

Various anionic, cationic and nonionic surfactants such as SDS, SDBS, BKC, CPC, CTMABC, Span 60, Tween 60 and Brij 35 were examined with respect to their interferences with the determination of chlorhexidine. The results showed that 10 mg l^{-1} of chlorhexidine can be determined in the presence of anionic and nonionic surfactants, since no wave for them is observed below −1.84 V. The peak heights of the waves at −1.66, −1.40, −1.24 V for BZC, CPC and CTMAB, respectively. The chlorhexidine peak height has been found to be practically unaffected by addition of cationic surfactants below 40 ppm. Surfactants changes the differential capacity and there force the charging current. The change in charging current would interfere with many determinations. Chlorhexidine reduction is not affected in the concentration of surfactants below 80 ppm. However, the background current will increase with the concentration of surfactants. If the background changes in an unknown and unpredicatable fashion, the use of a calibration curve for the determination of a species would of course be dubious. The calibration curve for chlorhexidine is $y = 5.89 + 0.884x$ in the presence of surfactant and sensitivity is lesser than without surfactant. Hence in this study, determination of the concentration of the commercial cosmetic and pharmaceutical products was accomplished by means of a standard additions procedure.

4.4. Application to cosmetic and pharmaceutical products

The proposed DPV method was applied to the determination of chlorhexidine in antiperspirant, toothpaste, gargle mouthwash and antiseptic liquid products. *p*-Chloroaniline is more oxidation than reduction, and not cause interference in the range of 1.0–2.0 V. Analytical results are given in Table 3. A separation of chlorhexidine and *p*-chloroaniline chromatogram is shown in Fig. 6. These results agreed with those obtained by a performance liquid chromatographic method. DPV is a specific and selective method for chlorhexidine as an alternative to that of

Table 3
Analytical results for the determination of chlorhexidine and chlorhexidine diglyconate in commercial antiperspirant, toothpastes, gargle mouthwashes and shower bath liquid

Samples	Concentration (w/w, %)[a]			
	Chlorhexidine		Chlorhexidine diglyconate	
	DPV	HPLC	DPV	HPLC
Antiperspirant	0.036 (5.0)[b]	0.024 (5.5)	–	–
Toothpaste				
1	0.045 (4.5)	0.040 (2.7)	–	–
2	0.037 (3.9)	0.033 (0.5)	–	–
Gargle mouthwash				
1	–	–	0.066 (2.6)	0.064 (2.5)
2	–	–	0.116 (1.6)	0.119 (5.0)
3	–	–	0.199 (5.0)	0.210 (2.7)
Antiseptic liquid				
1	–	–	3.382 (5.0)	3.372 (4.5)
2	–	–	0.164 (4.7)	0.216 (1.2)
3	–	–	0.041 (2.1)	0.048 (1.4)
4	–	–	0.257 (4.1)	0.244 (1.4)

[a] Number of determination ($N = 3$).
[b] Relative standard deviation.

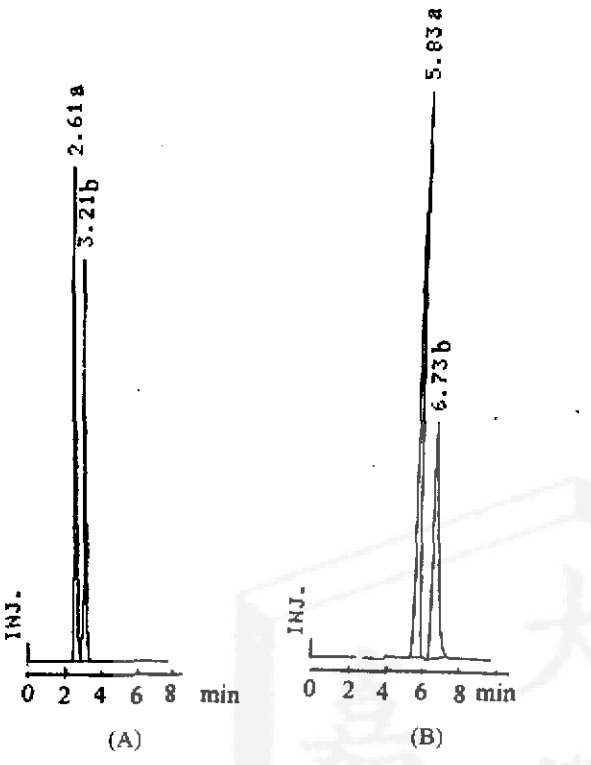


Fig. 6. (A) Separation of a = chlorhexidine, b = 4-chloroaniline. Stationary phase, μBondapack C_{18} (3.9 nm × 300 nm); mobile phase, methanol–water (90:10, v/v) containing 0.1 M phosphate buffer (pH 2.15); flow rate 1.0 ml min^{-1}, detection 240 nm. (B) Separation of a = chlorhexidine, b = 4-chloroaniline. Stationary phase, μBondapack C_{18} (3.9 nm × 300 nm); mobile phase, acetonitrile–methanol–water (2:1:2, v/v) containing 5 M sodium 1-heptane sulfonate and acetate buffer (pH 4.77); flow rate 1.0 ml min^{-1}, detection 240 nm.

HPLC. However, due to the surfactants interaction of electrode surface, leading to competitive adsorption and significant suppression of signal. HPLC data for chlorhexidine, the preliminary sample clean-up can eliminate interfering substances but lose the recovery rate. Recovery normally ranged between 89 and 108%. Although the reversed-phase LC is a sensitive assay for chlorhexidine but due to the potential interaction of chlorhexidine with the stationary phase, these LC quantitations may not be accurate unless the solution media is of working standard. Of the 10 samples of Table 3, toothpaste and gargle mouthwash have similar concentrations by DPV and HPLC. Samples less than 0.1% of concentration showed not so good agreement. UV active compounds were also quantified at 240 nm and the results were more than those obtained by DPV.

5. Conclusions

This paper presents a new DPV method for the analysis of aqueous solutions of chlorhexidine. The method can be performed without any time-consuming separation from the fatty constitutents and simple extraction procedure with good recovery of chlorhexidine.

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References

[1] S. Jenkins, M. Addy, W. Wade, J. Clin. Periodontal. 15 (1988) 415.
[2] A. Kalaga, M. Addy, B. Hunter, J. Periodontol. 60 (1989) 127.
[3] R.G. Smith, J. Moran, M. Addy, F. Doherty, J. Clin. Periodontol. 22 (1995) 613.
[4] C.S. Xue, T.K. Lin, Central English–Chinese Medical Dictionary, National Central Book Co., Tapei, 1995, p. 410.
[5] L. willis, J. Am. Coll. Toxicol. 12 (1993) 201.
[6] Y. Sakogami, H. Yamazaki, N. Ogasawara, H. Yokoyama, Y. Ose, T. Sato, Mutat. Res. 209 (1988) 155.
[7] J.M. Calatayud, J.V. Garciamateo, J. Pharmaceut. Biomed. Anal. 7 (1989) 1441.
[8] Y. Fujita, I. Moricand, M. Toyoda, Anal. Sci. 6 (1990) 807.
[9] E. Dinc, F. Onur, J. Fac. Pharm. Gazi. 11 (1994) 135.
[10] D. Bonazzi, V. Andrisano, R. Gatti, V. Cavrini, J. Pharmaceut. Biomed. Anal. 13 (1995) 1321.
[11] R. Borissova, S. Mandjukova, Fr. J. Anal. Chem. 357 (1997) 977.
[12] O.Y.P. Hu, S.Y. Wang, Y.J. Fang, Y.H. Chen, M.L. King, J. Chromatogr. 523 (1990) 321.
[13] M. Hoang, J.F. Moellmer, M.A. Khan, J. Liquid Chromatogr. 13 (1990) 2677.
[14] A.P. Cheung, R. Mavar, C. Carlson, W.K. Chiang, J. Pharmaceut. Biomed. Anal. 9 (1991) 41.
[15] W.K. Gavlick, J. Chromatogr. 623 (1992) 375.
[16] H. Ogase, I. Nagai, K. Kameda, S. Kume, S. Ono, J. Appl. Bacteriol. 73 (1992) 71.
[17] Y.W.F. Lam, D.C.N. Chan, S.Y. Rodriguez, J.H. Lintakoon, T.H. Lam, J. Chromatogr. 612 (1993) 166.
[18] A.M.D. Pietra, V. Andrisano, R. Gotti, V. Cavrini, J. Pharmaceut. Biomed. Anal. 14 (1996) 1191.
[19] Y. Ha, A.P. Cheung, J. Pharmaceut. Biomed. Anal. 14 (1996) 1327.

[20] F.T. Vert, F.V. Pedros, J.M. Calatayud, V.P. Martinez, Talanta 30 (1983) 977.
[21] M.A. Nuhez-Flores, C. Monleon, F. Vicente, Port. Electrochim. Acta. 7 (1989) 541.
[22] E. Jacobsen, B. Glyseth, Talanta 22 (1975) 1001.
[23] P. Gratteri, S. Pinzauti, E. Lapolta, G. Papeschi, V. Cavrini, G. Santoni, Analyst 116 (1991) 723.
[24] H. Bloom, B.N. Noller, in: Proceedings of the Aust. Electrochem. Conf. on Trends Electrochem. Plenary Invited Contrib., 4th Edition, 1976, pp. 241–252.
[25] I.S. Longmuir, Advances in Polarograply, Vol. 3, Pergamon Press, Oxford, 1960, p. 805.
[26] A.M. Bond, Modern Polarographic Methods in Analytical Chemistry, Marcel Dekker, New York, 1980, p. 195.