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Quantitative Determination of Chloramphenicol in Milk by Differential Pulse Polarography

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Abstract: *The residual chloramphenical determination in milk by the voltammetric method(DPP) is the basis for sensitive measurement schemes for this compound. The DPP response is evaluated with respect to various experimental conditions. A negative shift in the peak potential, from -0.20 to - 0.70 V is observed upon increasing the pH from 2 to 9 for phosphate buffer. The detection limit for chloramphenicol is 200 ppb.*

Keywords : *Chloramphenicol, Differential Pulse Polarography, Milk.*

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Sütte Kloramfenikolün Diferensiyal Puls Polarografisi ile Miktar Tayini

Özet: *Sütteki kalıntı kloramfenikolün voltametrik(Diferensiyel Puls Polarografisi) yöntem ile tayini, bu bileşiğin duyarlı bir şekilde ölçülmesine olanak sağlamış ve DPP yanıtı çeşitli deneysel koşullarda incelenmiştir. Fosfat tamponunun pH'sı 2'den 9'a çıkarıldığında pik potansiyelinde -0.2 V'dan -0.7 V'a negatif bir kayma gözlenmiştir. Kloramfenikolün tayin sınırı 200 ppb olarak bulunmuştur.*

Anahtar sözcükler : *Kloramfenikol, Diferansiyel Puls Polarografisi, Süt*

Introduction

Chloramphenicol is a widely used antibiotic both in human and veterinary medicine, because of its broad spectrum of antimicrobial activity. But toxic side effects such as blood dyscrasias, gastrointestinal disturbances, neurotoxic effects and allergic hypersensitivity reactions limit its uses. Gray baby syndrome and a plastic anemia are two potentially adverse reactions which may be fatal.

Detection of chloramphenicol residues in the human food supplies such as meat products, milk and eggs is of public health concern because of the exposure to potantially toxic levels of drug residues without knowledge. Gray baby syndrome, which is a dose-related potentially adverse reaction, usually occurs in infants less than 30 days of age, who have received very high doses of chloramphenicol. Because of the presence of chloramphenical resudes in breast milk of chloramphenicol-treated mothers and animals, neonates fed by contaminated milk have a high incidence of gray baby syndrome[1,2,3,4].

Regarding the effects on consumers of chloramphenicol residues, various health-related organizations such as FDA, FAO and WHO proclaimed that the residues of chloramphenicol in the human food supply were unacceptable and recommended that chloramphenicol should be carefully controlled and used only in very special circumtances in both human and veterinary medicine[1,3,4]. Although the intensive attempts to enforce the ban on the use of any chloramphenicol products in food-producing animals, chloramphenicol is still being used in human and veterinary medicine because of its effectiveness in treating many diseases. Therefore, appropriate methods to monitor food-products for residues of chloramphenicol and its metabolites are needed.

The methods which were developed to detect and quantitate residues of chloramphenicol in food-

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products are radiommunoassay[5], enzyme-linked immunoassay[1], and various forms of chromatography, such as gas chromatography(GC), thin-layer chromatography, and high-pressure liquid chromatograpy(HPLC)[1,4,5,6,7]

In present study, it is aimed to detect chloramphenicol residues in milk by a rapid and simple method. For this purpose, a voltammetric (Differential Pulse Polarography) method is used and the lowest limit of detection by this method has been investigated using phosphate-buffer solutions, as well as the behaviour of chloramphenicol in different pH values.

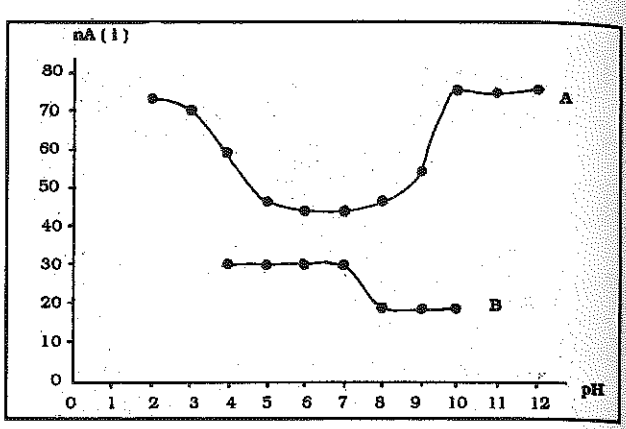

Figure 1. pH dependence of the diffusion-limited current of $2.3x10^{-6}$ M Chloramphenicol in (A) phosphate buffer, (B) phosphate buffer and milk which contains chloramphenicol.

Material and Method

Reagents

Potassium dihydrogen phosphate, dipotassium hydrogen phosphate, sodium hydroxide, and phosphoric acid were purchased from Merck. Chloramphenicol was obtained from Carlo-Erba. 1/2 kg of Tetrapak commercial forms of Türkiye Süt Endüstrisi Kurumu(SEK) were used as milk samples.

All solutions were prepared from double distilled water and analytical-reagent grade chemicals.

Apparatus and techniques

Polarographic assays were performed using a Metrohm(E 505: 626 Polarecord) instrument with a 25 mL of electrochemical cell. A dropping mercury electrode was used as the working electrode and a saturated calomel electrode as reference electrode and a platinum coil as the counter electrode. A Nelmod. 821 pH-meter with Ingold electrode was used for pH determinations.

The supporting electrolyte solution was prepared by the dilution of phosphate-buffer solution. The mercury flow rate, pulse amplitude, screening rate and dropping size were choosen as 0.5 s. 10mV. 100 mV/s and medium, respectively.

For the determination of chloramphenicol residues in milk by DPP, the modified methods of Zuman[8] and Alfonso et.al.[9,10] were used. The optimum amount of milk in mixture with phosphate-buffer solution which provides the most sensitive measurement of chloramphenicol residues and the optimum pH value were investigated. For this purpose the pH values of certain amounts of phosphate-buffer solution (pH 7.0, 0.05 M) were adjusted to 2.0, 3.0, 4.0, 5.0 and 6.0 by phosphoric acid, and to values above 7.0 by 4M NaOH solution.

The pH values of the mixtures prepared by adding 10 mL phosphate-buffer solutions with different pH values to 10 mL aliqouts of milk samples were measured and the peak heights obtained by the addition of $2.3x10^{-6}$ M chloramphenicol at those pH values were evaluated. The optimum pH value for determination of the drug residues was chosen as 6.0 on the basis of preliminary assay results. The DPP curves of the mixtures of 10 mL aliquots of phosphate buffer solution with the same volume of milk samples at this pH value were recorded after adding different amounts of chloramphenicol from stock solution to the mixtures.

Oxygen was removed by passing a stream of nitrogen through the solutions for 10 minutes and the measurements were performed at room temperature (25±1°C) during the study.

Results and Discussion

Different trend for chloramphenicol is observed for both buffer(A) and milk containing buffer(B) (Fig. 1.)

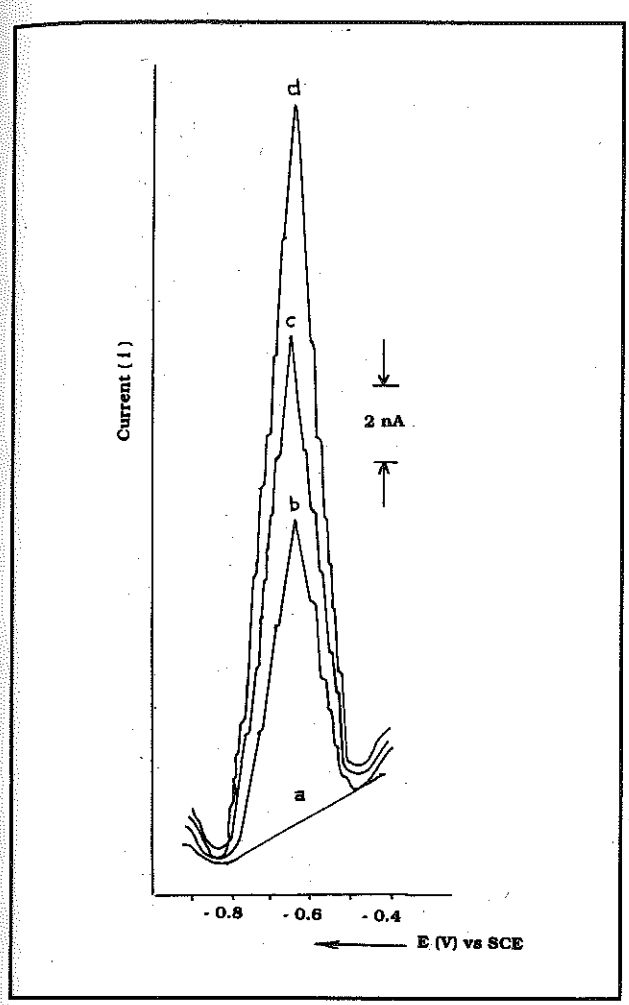

Figure 2. Differential-pulse voltammograms for chloramphenicol in milk sample (a) 0: (b) 1.3; (c) 1.5 and (d) 1.7 ppm. Supporting electrolyte. 0.05 M phosphate buffer and milk sample (1:1) (pH 6.0), differential-pulse waveworm; scan rate 5mVs^{-1} and amplitude 10mV.

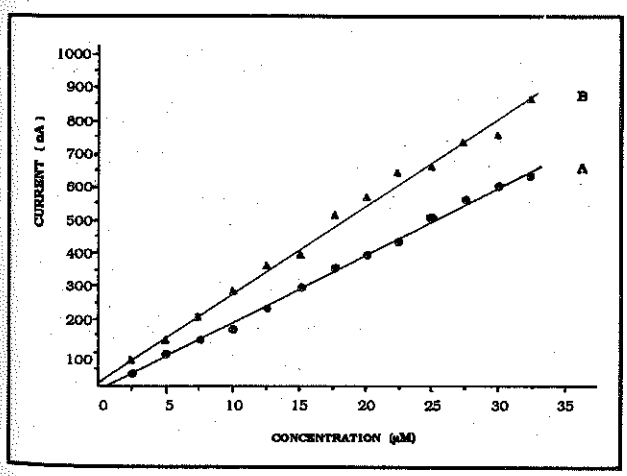

Figure 3. Peak current versus concentration for buffer containing chloramphenicol at (A) pH 6 and (B) pH 10. Other conditions are as in Fig. 2.

Response in phosphate buffer is higher than the response in milk containing buffer. Milk proteins cause the peak current to be depressed in milk samples. There is no peak under pH 4 for milk containing buffer. The optimum response is obtained at pH 4-7. pH 6 is used for all subsequent work.

Figure 2 shows well defined peaks for chloramphenicol in milk sample. Ep(peak potantial) = -0.68 V vs SCE with half peak height width of 194 mV. The differential pulse waveform offered improved response, and waveform parameters of 5 mV/s scan rate and 10 mV amplitude yields the best compromise between peak height and broadening.

Resulting plots of peak current versus concentration exhibit a linear relation up to 32.2 x 10^{-6} M (r = 0.99) (Figure 3 and 4) for buffer and milk samples.

Figure 5 shows the effect of the pH upon the peak potantial for 2.3x10^{-6} M chloramphenicol. A negative shift in the peak potential from -0.20 to -0.70 V is observed upon increasing the pH from 2 to 9 and it levels off after pH 9. There is a similar trend for both buffer(A) and milk sample(B). A negative shift in the peak potential from -0.70 to - 0.80 V is also observed, changing the solution from buffer(A) to milk sample(B).

Detection limit of 200 ppb(μg/mL) chloramphenicol can be estimated on the basis of signal to noise characteristics(S/N = 3). Milk samples are investigated at pH 6 due to high peak response as shown in Figure 1.

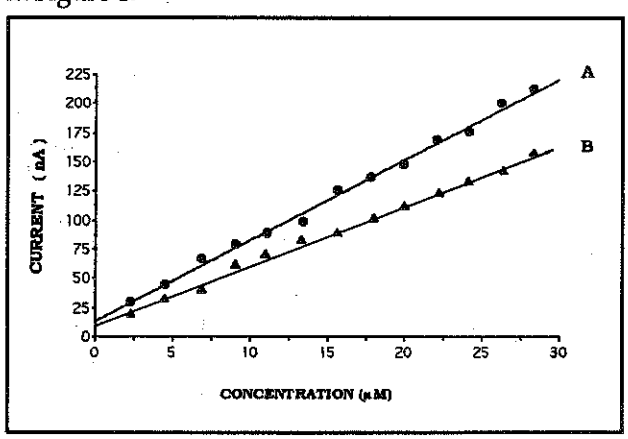

Figure 4. Peak current versus concentration for milk sample with buffer containing chloramphenicol at (A) pH 6 and (B) pH 10. Other conditions are as in Fig. 2.

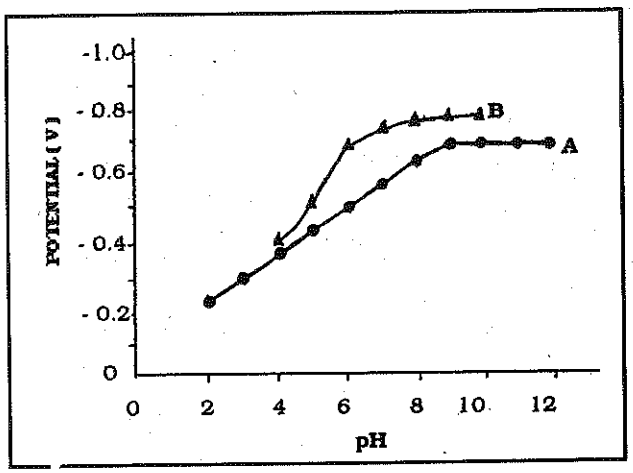

Figure 5. Dependence of peak potantial on the solution pH for 2.3×10^{-6} M chloramphenicol in buffer (A), in milk sample (B) Other conditions are same as Fig 2.

In conclusion, the determination of chloramphenicol in milk samples without sample preparation, enhance sensitivity of its voltammetric quantitation. These features of voltammetry, coupled with the simplicity of the method, make it extremely attractive for sensing other biologically important electroactive compounds.

References

1. Allen E.H., "Review of Chromatographic Methods for Chloramphenicol Residues in Milk, Eggs and Tissues from Food-Producing Animals", *J. Assoc. Off. Anal. Chem.*, 68 (5), 990-999, 1985.
2. Kayaalp S. O., "Amfenikoller", in Kayaalp S.O. (ed), *Tıbbi Farmakoloji*, 1. 5. Baskı, 706-716, 1989.
3. Abel R. S., Kleiman M. B., "Chloramphenicol", in Kacew S. (ed), *Drug Toxicity and Metabolism in Pediatrics*, Florida, C. R. C. Press Inc., 155-172, 1990.
4. Nelson J. R., Copeland K. F. T., Forster R. J., Campbell D. J., Black W. D., "Sensitive Gas-Liquid Chromatographic Method for Chloramphenicol in Animal Tissues Using Electron-Capture Detection", *Journal of Chromatography*, 276, 438-444, 1983.
5. Arnold D., Somogyi A., "Trace Analysis of Chloramphenicol Residues in Eggs, Milk and Meat: Comparison of Gas Chromatography and Radioimmunoassay", *J. Assoc. Off. Anal. Chem.*, 68 (5), 984-990, 1985.
6. Parker R.M., Shaw I. C., "Determination of Chloramphenicol in Tissues-Problems With in Vitro Metabolism", *Analyst*, 113, 1875-1876, 1988.
7. Arnold D., von Berg D., Boertz A. K., Mallick U., Somogyi A., "Radioimmunologische Bestimmung von Chloramphenicol Rückstanden in Muskulatur, Milch und Eiern", *Archiv für Lebensmittelhygiene*, 35, 121-148, 1984.
8. Zuman P., "Polarography in Attacking Practical and Theoretical Problems in Analytical Chemistry", *Proc. Analyt. Div. Chem. Soc.*, 12 (7), 199-208, 1975.
9. Morales A., Toral M. I., Richter P., "Polarographic Behavior and Determination of Nitrofurantoin, Chloramphenicol and Related Compounds", *Analyst*, 109, 633-636, 1984.
10. Morales A., Richter P., Toral K. L., "Voltammetric Behaviour of Nitrofurazone, Furazolidone and Other Nitro Derivatives of Biological Importance", *Analyst*, 112, 965-970, 1987.