Determination of Aminophylline by Cathodic Stripping Voltammetry

التقدير الكمي للمركب الدوائي أمينوفلين بطريقة الانتزاع المهبطي الفولتمتري على قطرة الزئبق المعلقة

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Abstract

Aminophylline is determined by cathodic stripping voltammetry (CSV) in BR buffer, pH 7.5 at a hanging mercury drop electrode. The detection limit was $3x10^{-8}$ M after 60 s accumulation at -0.60 V versus Ag/AgCl reference electrode. The linear range demonstrated up to $5x10^{-7}$ M using CSV. The interference caused by some purine derivatives, anions and some metal cations on the peak current was studied. The peak current is enhanced by theophylline, some methylguanines and copper (II) while it decreased or disappeared by citrate, chloride and Triton X-100 surfactant. The method has good sensitivity and its application to pharmaceutical samples is possible.

Keywords: Determination of aminophylline, stripping voltammetry, cathodic stripping voltammetry, adsorptive voltammetry, dosage forms

ملخص

يعتبر دواء الأمينوفلين aminophylline مناسبا لعلاج الأزمة الصدرية وهو يشبه في تركيبه المركب الدوائي theophylline وقد تم تعيين هذا الدواء في عينات المادة الخام وفي أحد المستحضرات الصيدلانية بطريقة الانتزاع المهبطي hodic stripping voltammetry cat وهي طريقة كهروكيميائية حساسة تعين بواسطتها كثير من المركبات المستخدمة في صناعة الأدوية. وقد كان الحد الأدنى من تركيز الدواء الذي يمكن قياسه هو $3x10^{-8}$ مول لكل لتر بعد فترة تجميع قدرها ستون ثانية على جهد تجميعي يساوي 0.60- فولت.وكان القطب المرجعي هو Ag/AgCl مقابل قطرة الزئبق المعلقة hanging mercury drop electrode كقطب عامل في محلول منظم درجة حموضته pH 7.5 وكانت العلاقة خطية مستقيمة حتى تركيز $5x10^{-7}$ مول لكل لتر. وهناك مواد متداخلة في التعيين تزيد من قيمة التيار الكهربائي (القراءة) مثل أيون النحاس الثنائي، theophylline ، guanine ، كما أن هناك أيونات تزيل القراءة كليا أو تنقصها مثل أيون الكلوريد وأيون السترات. وتعتبر الطريقة ناجحة في تعيين مركب الأمينوفلين عند تراكيز منخفضة جدا مع احتمال اللجوء الى طريقة فصل(مثل الكروماتوغرافي السائلة) اذا وجد شيء من المواد المتداخلة بتراكيز مؤثرة.

Introduction

Aminophylline is a 2:1 mixture of theophylline and ethylenediamine which is widely used as a bronchorelaxant for the treatment of asthma. More recently, it has been claimed that formulations of aminophylline in topical creams are useful for the reduction of lower body fat [1]. High Performance Liquid Chromatography, HPLC has been utilized [1-10] for the determination of aminophylline. A selective HPLC determination of aminophylline in cream preparations has been published [1]. The determination was applied to capsule formulations [2-3] in presence of chlopheneramine maleate and noscapine. In hyoscine tablets, the selective determination of aminophylline [4] was based on separation on ODS column. Coupled with UV detection at 275 nm, an RP-C_{18} column with a mobile phase consisting of water and methanol, (29:71)v/v % mixture gave high resolution. The tablets were dissolved in dilute NaOH solution and the recovery was 98% with RSD of< 1%.

In another work [5], HPLC was applied to the analysis of a mixture of cefuroxime, theophylline and aminophylline, using UV detector at an analytical wavelength of 254 nm. Orcinol (900 μg/ml) was used as an internal standard. Both theophylline and ethylenediamine were separated and determined simultaneously in aminophylline dosage forms (tablets, injections and oral solutions) [6]. Aminophylline and promethazine were determined in suppositories [8] using HPLC. Titrimetric methods using HCl or NH_4SCN titrants were used for the determination of aminophylline [11] in addition to TLC methods [12].

Colorimetric determinations of aminophylline and theophylline were made at 410 and 440 nm, respectively after reaction with diazotized 4-nitroaniline [13]. A very selective determination of aminophylline was carried out by a fluoroimmunosensor with an antibody covalently immobilised on a solid support (protein A)[14]. No interference was observed even from theophylline and caffeine which have similar structures to aminophylline. The interference of both caffeine in UV-spectrometric assay [15] and of aminophylline in voltammetric assay [16] is observed in the determination of theophylline. This work aims at developing a sensitive stripping voltammetric method for the

determination of aminophylline in aqueous pharmaceutical samples. Voltammetric determinations need no extractions or pretreatment of samples, and this provides a cheap and rapid analysis.

Experimental

All the voltammograms were obtained using EG&G polarographic analyzer / stripping voltammeter (USA), Model 264B equipped with an electrode stand, 303 A, automatic stirrer, 305 and RE 0150 x-y recorder. The three-electrode system was completed using an auxiliary platinum electrode with Ag/AgCl reference electrode (3M KCl). The pH measurements were carried out using HANNA pH meter, model HI 8424. Adsorptive voltammetric measurements were carried out using a Hanging Mercury Drop Electrode (HMDE). Aminophylline, with a purity of 99.3% was obtained locally from Berzeit-Palestine Pharmaceutical Company in Palestine. All chemicals were analytical grade(Aldrich), doubly distilled water was used for preparing all stock and working solutions. Aminophylline stock solution ($1x10^{-3}$M) was kept refrigerated for one month.

General procedure: voltammograms were obtained as follows:

Britton Robinson (BR) buffer (10 ml, pH 7.5) was pipetted into a previously cleaned and dried electrochemical cell, the solution was stirred while purging with oxygen free nitrogen for 6-8 minutes. An optimum accumulation potential (-0.60V) was applied for a duration of 60 s. A new drop was formed while stirring continued and at the end of accumulation and equilibration, a cathodic scan was initiated at a sweep rate of 10 mVs^{-1} from -0.20 to -1.0 V. The pulse amplitude was 50 mV.

Purging with nitrogen for 1-2 minutes was carried out between successive measurements depending on the length of the preceding accumulation period. All experiments were carried out at room temperature. A calibration curve of concentration against peak current was used for quantitative determinations. When complexation with copper (II) was used to enhance the peak current, the accumulation was made at 0.00 V and the scan was started from there to -0.80V. The

complexation effect of Cu(II) will be discussed later in the "Conclusion" part.

Effect of pH and Buffer Constituents

Three different buffers (phosphate, borate, BR), all at the same pH of 8.5, were tried with $2x10^{-6}$ M of aminophylline after accumulation at -0.60 V for 60 s. BR and phosphate buffers were stable for about two hours, while borate buffer showed a decrease in the signal of about 50% after 10 minutes. The maximum peak current was obtained with Britton Robinson(BR) buffer; at -0.31 V. The phosphate buffer gave lower currents and the borate one showed less reproducibility.

The effect of pH of the selected buffer was tested. Only neutral and slightly alkaline solutions of aminophylline gave significant peak heights. The values of peak current and peak potential at different pH values of BR buffer are shown in Table 1 below.

The shift in peak potential with pH change is not constant, and this may indicate a complicated mechanism of adsorption at the electrode surface.

Table 1: Effect of pH (BR buffers) on the peak current, peak potential of $2.5x10^{-6}$M aminophylline. Accumulation potential, E_{ac}= -0.60V; accumulation time, t_{acc} = 60s; sweep rate = $10mVs^{-1}$; pulse amplitude= 50mV; the scanning being from 0.00 to -0.80V.

pH	Peak current, nA	Peak potential, V
4.00	1.8	-0.04
5.00	3.7	-0.10
6.00	5.1	-0.23
7.00	6.4	-0.27
7.50	**7.7**	-0.31
9.00	5.8	-0.30
10.0	2.1	-0.32

Effect of accumulation potential and accumulation time

Accumulation of aminophylline at potentials equal to or more negative than -0.60 V gives enhancement in the peak current. This may be explained due to stronger adsorption of the reduced form of this drug at such potentials. The peak shape is improved if the accumulation is made at -0.60 V compared with -0.20 V, while a tailing effect is observed at more negative potentials. The optimum accumulation potential of aminophylline is thus -0.60 V.

The effect of accumulation time is illustrated in Fig 1. A linear increase in peak current was observed with increasing accumulation time up to 60 seconds. A desorption phenomenon is supposed at longer accumulation times, and this is more pronounced at low concentrations of aminophylline(less than $1x10^{-6}$ M). A better linearity is obtained at shorter accumulation times, but the sensitivity is sacrificed. The selected accumulation time is 60 seconds.

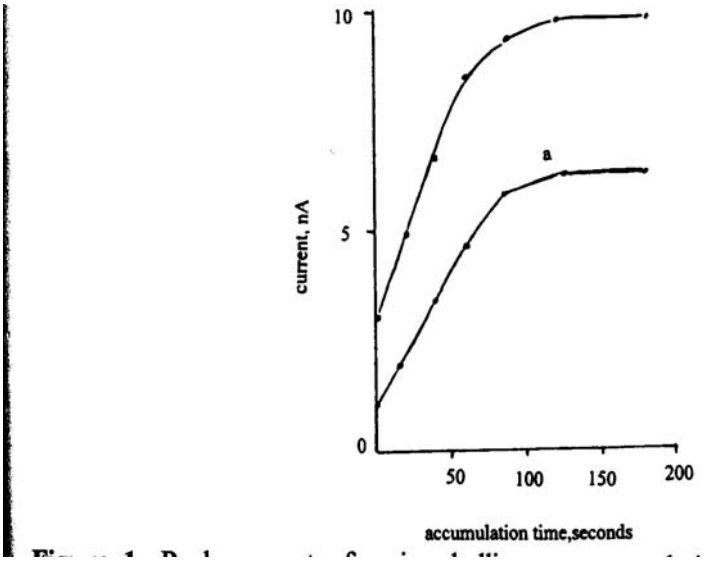

Figure 1: Peak current of aminophylline vs accumulation times in BR buffer, pH 7.5. Accumulation at -0.60 V for 60 s, scanning from 0.00 to -0.80 V at 10 mVs^{-1}. a: $1x10^{-6}$ M, b: $2.5x10^{-6}$ M aminophylline.

Determination of aminophylline by Cathodic Stripping Voltammetry (CSV)

A calibration curve of CSV peak current against concentration of aminophylline is shown in Fig 2. The detection limit under conditions of the calibration curve is equal to $3x10^{-8}$ M (signal to noise ratio=3). The detection limit was brought down to $1x10^{-9}$ M when $5x10^{-7}$ M Cu (II) was added to the voltammetric cell, accumulation time increased to 5 minutes, and the pulse amplitude increased to 100 mV. The RSD was about 3% for six determinations at 2.5 x 10^{-7} M level.

The addition of copper ions enhances the peak currents, but the reproducibility of the results becomes unsatisfactory. The lack of good reproducibility is also observed for long accumulation times, perhaps due to an adsorption/desorption equilibrium [17] as time exceeds about one minute. CSV peaks are important for trace analysis, which is usually required in pharmaceutical and biological samples. The analysis of aminophylline injections, manufactured by TEVA in Israel gave an average recovery of 97 % for six determinations at $2.5x10^{-7}$ M level. The RSD was about 4% for these determinations. The average recovery was equal to 96% for six determinations at $4x10^{-7}$ M level and the RSD was about 4% also.

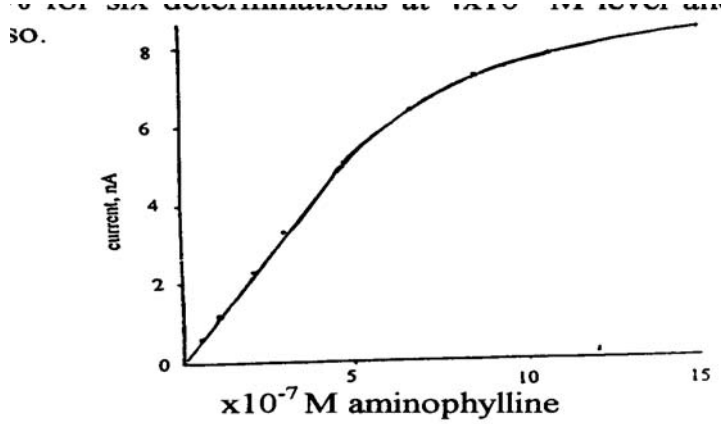

Figure 2: Calibration curve of aminophylline: DP-CSV peak currents versus its concentration. Conditions: BR buffer, pH 7.5; accumulation at -0.60 V for 60s; scanning from-0.20 to- 0.80 V at sweep rate of $10mVs^{-1}$. Pulse amplitude = 50 mV.

Interferences

The addition of $5x10^{-7}$ M Cu(II) to $1x10^{-6}$ M aminophylline caused a reasonable DP-CSV peak current enhancement(more than 500 %). Pb(II), Zn(II), Co(II) and Cd(II) caused no interference at $1x10^{-6}$ M level. Nitrate, sulphate, perchlorate and EDTA(at 0.02 to 0.10 M level) have no significant effect on the peak current and peak potential of $5x10^{-7}$ M aminophylline (calibration curve conditions). Citrate, chloride and Triton x-100 cause the peak current to decrease from 20 to 60% or totally disappears at similar conditions. No significant interference was observed for xanthines, dimenhydrinate,uric acid, theobromine, guaiphenisin and 6-chloropurine at $1x10^{-6}$ M level of each. Guanine, 1-methylguanine, 3-methylguanine and theophylline interferred by increasing the peak current from 50 to 90% at $1x10^{-6}$ M level of each, added to a similar concentration of aminophylline. The effect on peak potential is limited and does not exceed 20mV, except for Cu(II),-40mV; citrate, $_+$ 60mV and Triton x-100, $_+$70 mV.

Disadvantages of CSV method as applied to aminophylline

The main disadvantage of this method is its high sensitivity to the constituents of the solution in the voltammetric cell. The peak currents are only in nanoampere order, and so the relative error is high unless many replicate measurements are carried out. The calibration curve should be constructed again when necessary or at least checked for stability of measurements. Other disadvantages are the interference of particularly chloride and citrate ions, a problem that can be solved by a separation technique.

Comparison with other methods

CSV is a fast and cheap technique and after a calibration curve is established, it has the following advantages over the other methods:

1. No internal standard or other chemicals are used except the BR buffer and the analyte solution itself.
2. No separation is needed except in the case of the few interferents mentioned above.

3. Low detection limit is obtained ($3x10^{-8}$ M) with short accumulation times (60 sec).

Conclusion

Upon addition of Cu (II), a complex is expected to be formed, having the structure of Cu(I)-(aminophylline)$_2$. Practical evidence of complex structure obtained with theophylline (a similar structure) was given [16 - 17]. The peaks obtained for aminophylline alone are not expected to be due to reduction or oxidation of the adsorbed species, but rather due to an adsorption/desorption phenomenon, ie tensammetric peaks. The method is useful in routine analysis for aminophylline assay in pharmaceutical forms. The interference of theophylline is not important here because it is not usually mixed with aminophylline in pharmaceutical preparations.

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