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Short communication

Determination of the antimalaria drug artemether in pharmaceutical preparations by differential pulse polarography

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Abstract

A differential pulse polarographic method has been developed for the determination of artemether in its pharmaceutical formulations. The polarographic behaviour of artemether was examined in various buffer systems over the pH range 3.0–10.0. In phosphate buffer pH 5.5/methanol solution (7:3, v/v) the differential pulse polarograms displayed reproducible peaks at E_p – 0.01 V versus Ag/AgCl. Under these conditions strict linearity between artemether concentration and peak height was observed in 3.4×10^{-7}–3.0×10^{-5} mol/L concentration range ($R = 0.9998$). The detection limit was calculated to be 32 ng/mL. The polarographic method was applied to the determination of the content of artemether in tablets and capsules by using the standard addition method. The analysis of tablets containing 20 mg artemether showed a mean value of 19.73 mg with a relative standard deviation (R.S.D.) of ±1.01%. A content of 39.74 mg artemether was found in 40 mg capsules with a relative standard deviation of ±0.53%. The polarographic method is characterised to be cheap, precise and not time-consuming and can therefore be used for routine analysis of artemether in its pharmaceutical preparations.

Keywords: Artemether; Differential pulse polarography; Artemos® capsules; Riamet® tablets

1. Introduction

Malaria is one of the most widespread infectious diseases in the world. Every year about 500 million people are infected and over 2.7 million people die, most of them are children [1]. Because of the rapidly developing resistance of the malaria parasite *Plasmodium falciparum* to currently used alkaloidal drugs such as quinine and chloroquine, new non alkaloidal artemisinin type antimalarial drugs (artemisinin and its derivatives) have become increasingly important. Artemisinin is a sesquiterpene endoperoxide (Fig. 1) which is isolated from the herb of the Chinese medicinal plant *Artemisia annua* [2]. Artemisinin is a potent antimalarial drug against the resistant strains of *P. falciparum* [3,4]. Though the mechanism of action of the artemisinin type antimalarial drugs is not completely understood, there is growing evidence supporting the idea that the initial key step is the reductive cleavage of O–O bond of the endoperoxide group. This reaction presumably works by hemin, leading to oxygen and then carbon-centred radicals that subsequently lead to the biologically relevant damage to the malarial parasite [5–7].

Since artemisinin shows low solubility and poor oral bioavailability [8,9], derivatizations of artemisinin were carried out and yielded different semisynthetic antimalarial drugs such as artemether and sodium artesunate. Artemether (decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12H-pyrano [4.3-j]-1,2-benzodioxepin) (Fig. 1) is more active than the parent compound artemisinin [10]. Artemether is practically insoluble in water, very soluble in dichloromethane and acetone, freely soluble in ethyl acetate and dehydrated ethanol and shows a higher stability when dissolved in oils. The antimalarial action of artemether appears like artemisinin to be mediated by the generation of free radicals from the endoperoxy bridge of the drug. This endoperoxy bridge is essential for antimalarial activity because experiments with compounds having only one oxygen instead of two showed no activity [11].

The combination of artemether with lumefantrin is a well-tolerated, fast acting and effective blood schizontocidal drug. It is useful mainly in the treatment of uncomplicated *P. falciparum* malaria that is resistant to other antimalarial drugs [12].

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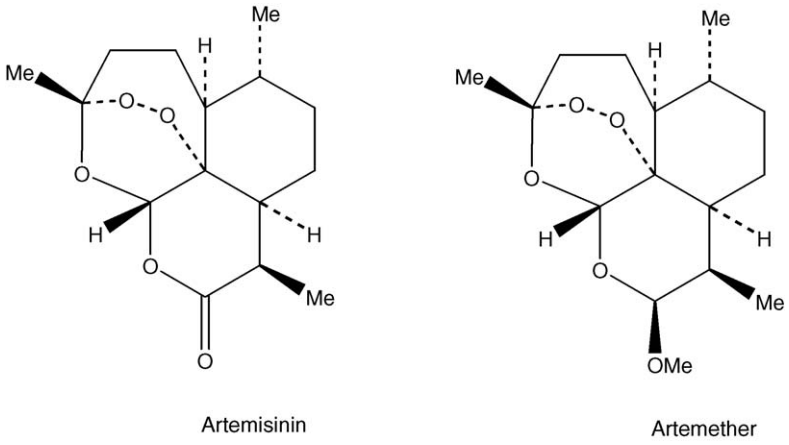

Fig. 1. Chemical structures of artemisinin and artemether.

Both for quality assurance and consumer safety the quantification of artemether in its commercial pharmaceutical products is particularly important. Suggested methods of determining the quantity of artemether are complex chromatographic (HPLC, TLC scanning) and NMR methods [13–15]. The analyses of artemether in tablets and/or capsules is till now carried out by using TLC, HPLC, TLC scanning techniques and one spectrophotometric method [14,16–18].

The purpose of the present study was to develop and validate an analytical method for the determination of artemether. The method ought to be not time-consuming and simple and therefore suitable in routine work. Since artemether contains the electrochemically active peroxide (–O–O–) group it can be reduced easily at various electrodes [19–24]. On the basis of these considerations, the electrochemical behaviour of artemether at a mercury electrode was studied in order to develop a differential pulse polarographic method. Then as a proof of principle the estimated method was tested in the mono-preparation Artemos® and in the compound preparation Riamet®.

2. Experimental

2.1. Reagents and compounds

Artemether (pure substance, 99.7%) was kindly provided by Beijing Novartis Pharma Ltd. (Beijing, China). Riamet® tablets (containing 20 mg artemether and 120 mg lumefantrin per tablet) were manufactured by Novartis Pharma AG (Basle, Switzerland) and Artemos® softgel capsules (containing 40 mg artemether per capsule) were from ETDZS Industry (Chongoing, China). All reagents were of Suprapur and/or Proanalysis grade (Merck, Darmstadt, Germany). Distilled water was purified with a Milli-Q Nanopure® (Millipore, Bedford, MA, USA) system and was stored in Nalgene® containers. The nitrogen used was 99.9995% pure, whilst the mercury was 99.999% pure (Oegussa, Graz, Austria).

$(NH_4)_2SO_4$ (0.05 M) pH 4–8, KH_2PO_4 (0.1 M) pH 4–7, acetic acid/sodium acetate buffer (0.1 M) pH 3.5–5.5 and Britton Robinson buffer solutions (0.1 M) pH 2–10 were used as supporting electrolytes for fundamental polarographic tests. The proper pH value was obtained by adding 0.5 M NaOH to the buffer components mentioned above. The construction of the calibration curve and the analysis of tablets and capsules were performed in a phosphate buffer pH 5.5 mixed with methanol (7:3, v/v). This methanolic buffer solution has now a pH value of 6.4. The aqueous buffer solution can be used for two weeks but the mixture with methanol was prepared freshly just before determination.

Stock solution of artemether was prepared by transferring 15.0 mg artemether to a 50 mL volumetric flask, dissolving in ethanol and bringing to volume. This solution contains 0.30 mg/mL and is stable for 2 weeks. Further standard artemether solutions were prepared freshly by diluting the stock solution with ethanol.

2.2. Apparatus and polarographic conditions

The plarographic investigations were carried out with a polarographic analyzer/stripping voltammeter model 264 A (EG&G, PARC, New Jersey, USA) in combination with a polarographic stand model 303 A SMDE (EG&G) and plotter model RE0150 (EG&G). This electrode stand consists of a dropping mercury electrode (DME) as working electrode, an Ag/AgCl reference electrode (3 M KCl), and a platinum wire as an auxiliary electrode.

For preparation of the calibration curve and analysis of the tablets and capsules, respectively, the analyser was operated under following parameters: drop size, *M*; drop time, 1 s; potential range, +0.15 to −0.45 V; scan rate, 5 mV/s; pulse amplitude, −50 mV; current sensitivity, 1–2 μA. The following apparatus parameter were set for cyclic voltammetric analysis: drop size, *M*; potential range +0.15 to −0.35 V; scan rate, 10–500 mV/s; current sensitivity, 1 μA; equilibration time, 15 s.

The pH values of the solutions were adjusted employing a Metrohm pH meter Model 632 and a glass electrode model 6.0202.000 (Metrohm AG, Herisau, Switzerland). All measurements were carried out at room temperature.

2.3. Validation of the method

The method was validated according to ICH Guidelines Q2A and Q2B.

2.3.1. Working procedure for calibration graph including linearity, range, LOQ, LOD

About 10 mL of the mixture of phosphate buffer pH 5.5 and methanol (7:3, v/v) were transferred to a polarographic cell and deoxygenazed by purging 8 min with nitrogen. After determining the blank value, six aliquots (each 50 μL) of artemether stock solution were added successively and the cell was purged after each addition with nitrogen for another 30 s. The polarogram was then recorded using the instrumental parameters described above. The peak height was evaluated by applying the tangents method, considering the increase in volume. Using suitable standard solutions, it is possible to determine artemether in the concentration range of 0.10–9.0 μg/mL.

The detection limit (LOD) and the limit of quantitation (LOQ) were calculated according to the Analytical Methods Committee [25]. LOD is defined as the mean value of the intercepts (blank mean y_B) of the calibration curves plus three times of the standard deviation of the intercepts (blank S_B). LOQ is estimated similarly to the LOD value, but: $y_B + 10\ S_B$.

2.3.2. Specificity and recovery experiments

The specificity of the proposed method was investigated by testing the excipients of Riamet® tablets (polysorbate 80, hypromellose, cellulose, silicon dioxide, croscarmellose – sodium, magnesium stearate, magnesium oleate, magnesium palmitate) and of Artemos® capsules (soybean oil and gelatine). These excipients were tested in the concentration of 20 μg/mL corresponding to a 10-fold excess of artemether. Furthermore experiments of artemether under "stress conditions " (0.1N NaOH, 3N HCl, and heat 150 °C) were carried out. The polarographic parameters as well as the conditions of measurements were achieved as described in Section 2.3.1.

The recovery experiments in tablets were carried out by adding a known amount of artemether (10, 15, and 20 mg) to the homogenised powder of Riamet® tablets (200 mg). This mixture was placed in to a 50 mL volumetric flask using the same procedure as described for analysis of Riamet® tablets. For Artemos® soft gel capsules 50, 100, and 150 mg of pure artemether was added to five dissolved capsules and placed in to a 500 mL volumetric flask following the same procedure as outlined under analysis of Artemos® capsules.

2.4. Working procedures for the determination of artemether

2.4.1. Analysis of Riamet® tablets

Ten tablets were weighed and finely ground in an analysis mill. The average weight of one tablet was 241.9 mg. An aliquot of 200 mg of the homogenised powder was placed in a 50 mL volumetric flask, brought to volume with a mixture of ethanol and phosphate buffer pH 5.5 (1:1, v/v) and allowed to extract for 3 min in an ultrasonic bath. After extraction it was centrifuged for 5 min to get a clear solution of the tablet extract for subsequent polarographic analysis. Then 10 mL of a mixture of phosphate buffer pH 5.5 and methanol (7:3, v/v) were transferred to the polarographic cell and purged with nitrogen for 8 min. After determination of the blank value, 50 μL of the tablet extract (mean linearity range) was added and purged with nitrogen for another 30 s. The polarogram was then recorded using the instrumental parameters described above. The content of artemether was determined applying the standard addition by adding 50 μL stock solution two times (15 μg/50 μL). The peak height was evaluated using the tangents method.

2.4.2. Analysis of Artemos® capsules

To avoid inconstancy of weight five Artemos® soft gel capsules were placed in a 500 mL volumetric flask. Then 30 mL of 1% HCl were added to dissolve the capsules using an ultrasonic bath (5 min). After adding 400 mL ethanol, the solution was allowed to extract for further 2 min in the ultrasonic bath. The volume was adjusted with ethanol up to 500 mL. The polarographic analysis was subsequently carried out following the procedure described under tablets analysis.

3. Results and discussion

3.1. Electrochemical investigations of artemether

The differential pulse polarographic (DPP) analyses have shown that artemether can be easily reduced at the mercury electrode with one well defined peak using various buffer systems (acetate buffer, Britton Robinson buffer, phosphate buffer and ammonium sulfate) mixed with methanol (7:3; v/v). The potential of the peak occurs at −0.01 V versus Ag/AgCl and is independent upon the pH in the range of 3.0–10.0 (within the error of measurement ±10 mV).

The cyclic voltammetric behaviour of artemether was investigated using the above described buffer/methanol solutions (7:3; v/v) in the pH range 3.0–10.0 with the result that the cyclic voltammograms were quite similar. Fig. 2 illustrates a typical cyclic voltammogram of artemether in phosphate buffer 5.5/methanol solution. This figure shows a very distinct cathodic

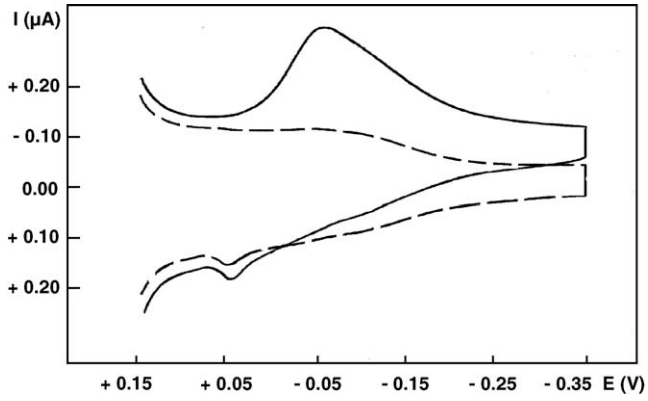

Fig. 2. Cyclic voltammogram of 2×10^{-5} M artemether in a phosphate buffer pH 5.5/methanol mixture (7:3, v/v). Scan rate 200 mV/s. The dashed line showed the supporting electrolyte.

peak at −0.04 V versus Ag/AgCl. In anodic direction, however, no corresponding peak can be registered, which indicates that the electrode reaction is irreversible. The small anodic signal which appears in the reverse scan at about +0.05 V is due to the supporting electrolyte (Fig. 2). The above described behaviour (E_p approx. 0.0 V, pH independent, irreversible process) is typical for the endoperoxide moiety [21,26,27] and is also in accordance with the electrochemical data given for artemisinin [28–30]. The reduction of the O–O bond in this type of compounds is characterised to be a dissociative process where electron uptake and bond fragmentation act together [31–33]. The characteristic voltammetric behaviour of a dissociative reduction was also observed as expected for artemether: The reduction appears as single, broad, irreversible peak at all scan rates (50–500 mV/s). The peak widths, $\Delta E_{p/2}$, increase with increasing scan rate (e.g. 127 mV at 100 mV/s and 148 mV at 500 mV/s, respectively). The cathodic peak potential E_p shifts to more negative values as a function of scan rate (ν) by an average of 66 mV/ln ν.

So the reaction is in accordance with a two electron reduction, probably by starting with dissociative O–O bond fragmentation followed by the actual reduction of the compound [28].

3.2. Determination of artemether by means of DPP

With a view to develop an analysing method for the determination of artemether, differential pulse polarography (DPP) was selected as one of the most sensitive among electrochemical procedures. Exhaustive studies were carried out with acetate buffer pH 3.5–5.5, Britton Robinson buffer pH 3–10, phosphate buffer pH 4–7 and ammonium sulfate pH 4–8. The intensity of the peak current (I_p) of artemether was influenced both by the type of buffer systems and the pH value. It was necessary to add a solvent as solubilizer to the buffer solution. Best results regarding peak form was obtained using methanol as solvent. It was found that the peak current was reproducible, with optimal sensitivity, in phosphate buffer pH 5.5 mixed with methanol solution (7:3, v/v) (Fig. 3).

Under these conditions strict linearity between peak height and concentration of artemether in a range of 0.10–9.00 μg/mL

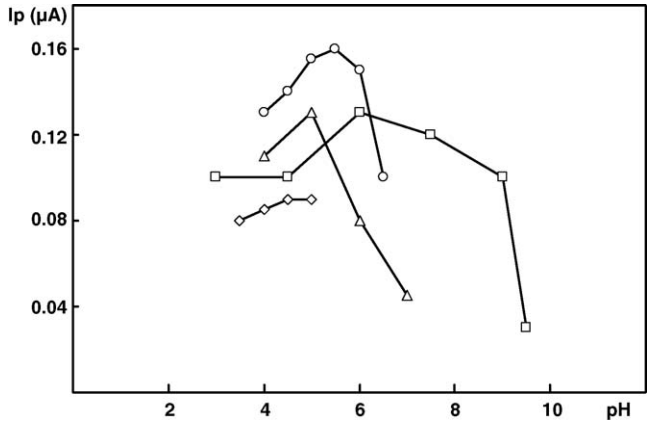

Fig. 3. Influence of pH on I_p of artemether (4×10^{-6} M) using various buffer/methanol solutions (7:3; v/v); acetate buffer (◊), Britton Robinson buffer (□), phosphate buffer (◯), ammonium sulfate (△).

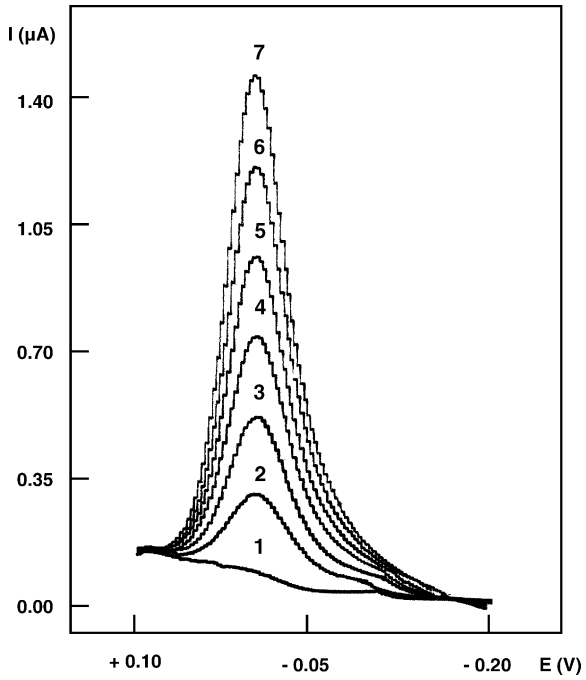

Fig. 4. Differential pulse polarograms of artemether in phosphate buffer pH 5.5/methanol solution (7:3, v/v). The concentration of artemether employed were: (1) blank, (2) 1.5, (3) 3.0, (4) 4.5, (5) 6.0, (6) 7.5, and (7) 9.0 μg/mL.

(3.35×10^{-7}–3.02×10^{-5} M) was observed. Typical differential pulse polarograms of artemether are shown in Fig. 4. Intra day determination of the calibration line (measurment of five calibration curves on 1 day; six measuring points per curve) resulted in the following linear equation: I_p (μA) = 0.136 × C (μg/mL) + 0.0004 (μA) with a correlation coefficient (R) of 0.9998 and a relative standard deviation of the slope of ±2.3%.

The precision of the polarographic method for the determination of artemether was tested on solutions with a concentration of 2.0 μg/mL ($n = 6$) corresponding to the concentration in the tablets and/or capsules by means of the standard addition method. During one day this analysis revealed a mean value of 2.00 ± 0.016 μg/mL corresponding to a 0.80% relative standard deviation (R.S.D.). In addition, inter day precision was analysed by measuring three solutions of artemether (2.0 μg/mL) on three different days. This analysis resulted in 1.98 ± 0.022 μg/mL (R.S.D. = 1.11%).

The influence of temperature was investigated (20–40 °C). As expected an increase of the peak height of about 1% per 1 °C appeared but this effect is completely compensated applying the standard addition method.

The limit of detection (LOD) was 32 ng/mL and the limit of quantitation LOQ 103 ng/mL.

In order to investigate the specificity of the analytical method in presence of all excipients used in tablets/capsules, a known amount of artemether was added to the relevant excipients. The corresponding peak height of artemether was evaluated and compared with the peak height of a solution containing only artemether. As a result of these experiments no influence of the tested excipients was observed. Stress conditions led to degradation products which showed no signal in the potential range

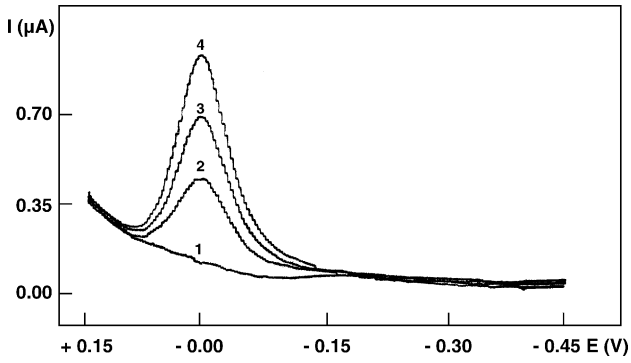

Fig. 5. Determination of artemether in Riamet® tablets using phosphate buffer pH 5.5/methanol solution (7:3; v/v). Differential pulse polarograms of (1) blank, (2) 50 μL tablet extract, and (3 and 4) addition of artemether stock solution, 50 μL each.

of +0.15 to −0.45 V, so there is no effect on the determination of artemether. The degradation is presumably associated with the break of the electrochemically active peroxide group [34–36].

To investigate the accuracy [37] of the polarographic method recovery experiments were performed by spiking tablets/capsules samples. These investigations resulted in a mean recovery rate of 99.3% for tablets and 99.8% for capsules, respectively.

These experiments show that the developed DPP method is suitable for the determination of artemether in tablets and capsules.

3.3. Analysis of artemether tablets and capsules

To evaluate the content of artemether in Riamet® tablets (a combined preparation with lumefantrin) and Artemos® soft gel capsules (a mono preparation) the sample preparation had to be optimised. Several solvent systems, different extraction volumes as well as the extraction time were examined.

For Riamet® tablets best results were obtained by dissolving a proper aliquot of the homogenised tablet powder in a mixture of ethanol and phosphate buffer pH 5.5 (1:1, v/v) at room temperature. Following the analysis of the tablets as described in the working procedure a mean value ($n = 10$) of 19.73 ± 0.20 mg artemether per Riamet® tablet was obtained (R.S.D. of ±1.01%). This result is in a quite good agreement with the manufacturer's declaration (20 mg).

The determination of Artemos® soft gel capsules using the described working procedure gave a mean value ($n = 10$) of 39.74 ± 0.21 mg artemether per capsule (R.S.D. ±0.53%) and corresponds closely to the producer's specification (40 mg).

The polarograms of artemether in tablets and capsules are quite similar. Such a typical polarogram is shown in Fig. 5.

4. Conclusions

In summary, a simple differential pulse polarographic method has been developed for the determination of artemether in pharmaceutical formulations. The significant advantage of this DPP method is that the analysis requires neither extensive separation nor extraction of artemether, with the result that the method is selective without being time consuming. In addition the established method is robust, not expensive and suitable for routine analysis which is reflected in the successful analysis of Riamet® tablets and Artemos® soft gel capsules. Furthermore the relative standard deviation of ±1.01% for Riamet® and ±0.53% for Artemos® indicates an excellent reproducibility. Since the proposed method is not very temperature sensitive it is applicable in all climate zones of the earth, thus making it suitable for quality assurance of malaria drugs in the known risk areas.

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