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Electrochemical analysis of cephalosporin antibiotics

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Abstract: A review is presented on electroanalysis studies and methods for the determination of cephalosporin antibiotics in either simple solutions or biological fluids. Some general data about the electroactivity of cephalosporins are given and the analytical utilization of those data together with the corresponding analytical parameters, compiled from different literature sources.

Keywords: *Review; electroanalysis; voltammetry; polarography; cephalosporins.*

Introduction

Cephalosporins are semi-synthetic antibiotics of the β-lactam family and are thus closely related in structure to penicillins. Because of their antibacterial activity, β-lactamase resistance and pharmacokinetic properties, the cephalosporin antibiotics have gained importance in pharmaceutical research in the last two decades and are now widely used in clinical practice for the treatment of severe infections. This interest is manifested in the literature with many papers and monographs on the physicochemical, pharmacological, medical and other aspects of cephalosporins [1–6].

Cephalosporins comprise the cephem nucleus (Fig. 1) and side-chains at positions 3 and 7 that determine their properties and bioactivity [5]. They act bactericidally by selectively inhibiting the synthesis of mucopeptide in the bacterial cell wall because of their structural similarity to the corresponding natural components; thus these antibiotics inhibit the growth and multiplication of bacteria [3, 4]. The compound depicted in Fig. 1 with $R_1 = R_3 = H$ and $R_2 = OCOCH_3$, i.e. 7-amino-cephalosporanic acid (7-ACA), is the starting compound for the synthesis of all cephalosporins. Because of their excellent solubility in water, cephalosporins are usually formulated as the sodium salts. Details of their structures can be found in the literature [1, 3–5].

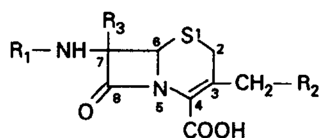

Figure 1
The basic cephem nucleus.

There are several methods for the determination of cephalosporins in simple solution or in body fluids. Some reviews are available with collected data on the most frequently employed methods [7, 8]. Among these are microbiological, colorimetric, chromatographic (especially high-performance liquid chromatography, HPLC) [9, 10], spectroscopic [11], fluorimetric, enzymatic and electrometric methods.

For a long time electroanalytical techniques, particularly differential pulse (DPP) and direct current (DC) polarography, square wave voltammetry (SWV), cyclic voltammetry (CV) and linear sweep voltammetry (LSV), have been well recognized and successfully employed for the determination of compounds of biological importance because of their sensitivity, accuracy, simplicity and low cost [12–14]. Basic reviews on the application of polarography to the determination of antibiotics, including some cephalosporins, were presented by Siegerman [15, 16]. A brief review of the polarography and voltammetry of cephalosporins was included by Bersier and Bersier within a long review of the application of these techniques in industrial laboratories [17].

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The scope and aim of this work was to review all accessible articles on the electrochemical analysis of cephalosporin antibiotics and to present data on their determination, compiled from different literature sources. Its purpose is to provide a basis and reference source for all concerned in research on the electrochemical analysis of cephalosporins.

Classification of Electroanalytical Methods for Cephalosporins

The first article on the electrochemical analysis of a cephalosporin antibiotic was published in 1967 by Dušinský and Antolík and dealt with the oscillopolarographic determination of cephaloridine [18]. This article was overlooked (probably because of language and information difficulties) by many later authors who listed a paper by Jones *et al.* [19], published in 1968, as being the first article on the subject. Since then, many reports have been published on the polarographic and voltammetric determination of cephalosporins; a substantial increase in the number of publications has been noted in the last few years. Not many workers, however, have investigated the electrode reaction mechanisms; the others have been concerned more with the electrochemical responses and consequent analytical applications.

Literature on the electroactivity of cephalosporin antibiotics and the resulting analytical applications can be divided into two main groups: papers on the direct polarographic or voltammetric activity of cephalosporins [18–45, 54–56]; and papers on the polarography, voltammetry or amperometry of the degradation products of cephalosporins after acidic, neutral or alkaline hydrolysis or UV photolysis [42, 46–51, 58, 59]. Only a few articles on the electroanalysis of cephalosporins describe other techniques; this shows the predominant rôle of polarography and voltammetry (including amperometry) in the electrochemical analysis of cephalosporins.

Most cephalosporins are electroactive and give a faradaic response on an electrode (mercury or solid) immersed in their solution. The majority have been reported to be electroreducible and the common electrode reaction has been shown to be the two-electron reduction of the Δ^3 double bond of the cephem nucleus. It is well known that a C=C bond is generally very difficult to reduce. Although the reduction of the Δ^3 double bond in the cephalosporin thiazine ring is facilitated through its conjugation with the carbonyl moiety of the cephem carboxylic group, its polarographic signal occurs at relatively high negative potentials (at about -1.0 V vs Ag/AgCl or SCE reference electrode, in acidic media pH 2–4), and is dependent upon the presence and nature of the substituent at position 3 (R_2 in Fig. 1). Details of this reduction will be given later in the text.

Many cephalosporins contain additional or possibly other reducible groups, the electrode response of which can be utilized for analytical purposes. These groups are incorporated into side-chains at positions 3 or 7 and usually give rise to a less cathodic reduction signal compared with that of the Δ^3 double bond reduction. Their response is, therefore, in most cases preferred for analytical applications. As an illustration, sampled DC and DP polarograms of cefotaxime are presented in Fig. 2. The more cathodic peak (wave) is due to the Δ^3 double bond reduction; the other peak is due to the reduction of the methoxyimino group in the side-chain at position 7. Also, the analytical advantage of DPP (peak shape and sensitivity) over DC polarography is evident here; hence the DC mode is mostly used only for electrode process characterization owing to its simpler electrical background and more elaborated theoretical basis.

Few papers have dealt with the oxidation of cephalosporins at solid electrodes and on the utilization of the anodic response for their determination [28, 30, 54–56]. Nevertheless, oxidation of the aminothiazole group, the functional group contained in the 7 side-chain of some cephalosporins (e.g. ceftizoxime, cefmenoxime, ceftazidime and ceftriaxone) was reported to enable a promising amperometric detection mode to be developed for liquid chromatography or possibly other flow analytical procedures [54–56]. Some papers have also described polarographic catalytic [52, 53], indirect amperometric [56, 57] and potentiometric [60–63] methods.

Over 30 different cephalosporin antibiotics have been examined electrochemically, the most frequently investigated being cephalexin, cephalothin, cefotaxime, cephaloridine, ceftriaxone and cefazolin. These studies will be discussed in the following two sections. Reports on the electroanalysis of cephalosporins in a minor capacity, e.g. those using

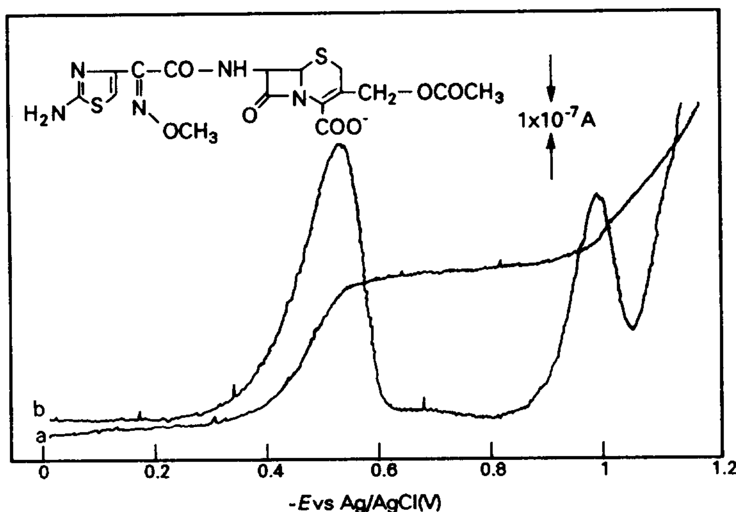

Figure 2
(a) Sampled DC and (b) DP polarograms of 1×10^{-4} M cefotaxime in Britton–Robinson buffer (pH 3). Instrumental settings: drop time 2 s (a), 1 s (b); scan rate 2 mV s^{-1} (a, b); pulse amplitude 100 mV (b). Structural formula of cefotaxime anion in upper left corner.

cephalosporins as test compounds, are not considered in this review.

Polarographic and voltammetric studies on electroactive cephalosporins

As mentioned earlier, Dušinský and Antolík [18] described the oscillopolarographic behaviour of cephaloridine in neutral, alkaline and acidic media, and discussed the possibility of using the technique for the determination of the drug and its degradation products. Jones *et al.* [19] reported a DC polarographic investigation of cephalosporin *C*, cephalothin and cephaloridine; only cephaloridine was quantitatively evaluated. It was found that all three cephalosporins gave rise to a single cathodic wave. On the basis of diffusion current data for cephaloridine, a two-electron reduction was assumed, but no attempt was made to determine the reduction pathway or functional group involved.

Although the reaction was assumed by previous authors to take place, Ochiai *et al.* [20] were the first to present evidence for electroreduction of the cephem double bond. In their report describing a macroscale electrosynthesis of cephalexin ($R_2 = R_3 = H$) starting from 7-ACA or cephalosporin *C*, via intermediate 3-methylenecepham derivatives to 3-methylcephem derivatives (e.g. cephalexin) after a final isomerization procedure, cleavage of the substituent at position 3 (R_2 on Fig. 1) was proved. In later work, Ochiai *et al.* [21] studied the mechanism of this reduction using deuteration and DC polarography. They found that all investigated derivatives with a substituent R_2 different from H (e.g. R_2 = O—,N—, or S—substituent) gave 3-methylenecepham derivatives as a result of cathodic reduction. 3-Methylenecepham or 3-methylcephem derivatives did not give an appreciable polarographic response, indicating the inability of the cephem ring to undergo cathodic reduction if R_2 = H. The authors proposed a reduction mechanism involving a double one-electron transfer step via a very unstable intermediate radical anion and splitting off the R_2 group to obtain an intermediate anion with final solvent protonation of its C_4 carbon to form the 3-methylenecepham derivative.

In his first contribution, Hall [22] reported on the DC polarographic investigation of an unnamed cephalosporin derivative with a substituted methyl group at position 3. It was found that this compound was reducible in acidic medium giving two diffusion-controlled polarographic waves as a consequence of one two-electron electrode reaction (ascribed to reductive elimination of 3-substituent) and one six-electron electrode reaction (ascribed to known reduction of sydnone side-chain). Both waves could be analytically exploited, but only the first was used to follow acidic and alkaline hydrolysis as well as enzymatic degradation processes. In a later work by Hall *et al.* [23], the electrochemical behaviour and reaction pathway of cephalothin reduction was studied by DC and AC polarography, cyclic voltam-

metry and coulometry. A reduction mechanism that differed in part from that shown by Ochiai *et al.* was presented. According to this mechanism, the 4-carboxylate anion of the cephem ring was first protonated, followed by a slow one-electron transfer producing a transition state (not anionic, but closely related to 3-exomethylene) and a fast one-electron attack with cleavage of the acetoxy group and final protonation of cephem C_4 carbon, yielding predominantly a 3-methylene cephalothin (Fig. 3). The authors found that this reduction was totally irreversible and claimed that desacetoxy cephalothin (R_2 = H) might undergo further reduction at high negative potentials and low pH. For the first time, it was also reported that adsorption phenomena influenced the electrode behaviour of a cephalosporin molecule. After this thorough study, no new ideas about the Δ^3 double bond reduction pathway were presented; this implies overall agreement on such a mechanism.

Benner [24] elaborated a procedure for the polarographic determination of seven penicillins and three cephalosporins (cephalothin, cephaloridine and cephalexin) in an ultrafiltrate of a human serum sample. He found that cephalexin with an unsubstituted 3-methyl group gave a polarographic signal in either strong acidic or alkaline solution, but was not very stable. Rickard and Cooke [25] reported a polarographic study of cefamandole and its formyl ester (cefamandole nafate) using DC polarography and controlled potential coulometry. The two techniques were used in analytical control, stability and purity tests. Both compounds were found to undergo a two-electron process for which a reductive cleavage

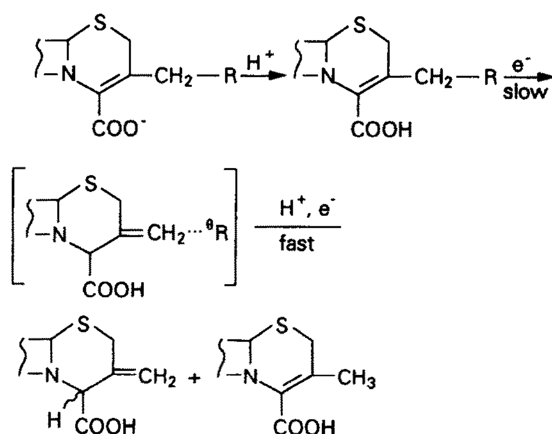

Figure 3
Scheme of cephalosporin Δ^3 double bond reduction.

of the thioether linkage at position 3 was assumed to be responsible. As reported by other authors dealing with the polarography of cephalosporins, they found the same pH and concentration dependence of the cefamandole polarographic wave (shift of half-wave potential to more negative potentials with increasing pH or concentration); similarly the limiting current was diffusion controlled. Comparison of polarographic with microbiological and iodometric analytical procedures in terms of precision, accuracy and selectivity showed the advantage of the polarographic method in all three aspects.

In a polarographic study carried out by Fogg *et al.* [26], several cephalosporins (cephalothin, cephalosporin *C*, cephaloridine, cephalonium, cefuroxime, cephoxazole, cephalexin, cephradine, desacetylcephalosporin *C*, 7-ACA and 7-aminodesacetoxycephalosporanic acid) and some of their degradation products were investigated using the DPP mode. These workers claimed that cephalosporins containing an unsubstituted 3-methyl group but no reducible group (i.e. cephalexin and cephradine) did not give rise to DPP peaks, whereas some of their degradation products did. In work by Fogg and Fayad [27], the same cephalosporins were examined by the DPP technique, with emphasis on the determination of cephalexin and cephradine with DPP detection of their degradation products after neutral or alkaline hydrolysis.

DPP and LSV methods for the determination of cefadroxil, cephalexin and cephapirin were described by Ivaska and Nordström [28]. Cefadroxil and cephalexin, each with an unsubstituted methyl group at position 3 (R_2 = H), were found to give a distinct DPP peak at about −1.3 V (vs Ag/AgCl) in a very acidic medium (pH 1–2), and this peak was stable for several hours. On the basis of DC polarographic and literature data, a two-electron hydrogen discharge, catalysed by one molecule of either compound, was assumed to be the relevant electrode reaction. Cefadroxil also gave an anodic oxidation wave at the glassy carbon electrode, when LSV was used; this wave, which was suitable for analytical purposes, was ascribed to irreversible electrooxidation of the phenolic functional group of cefadroxil on the 7 side-chain. Camacho *et al.* [29] reported in preliminary work on the electrochemical study of reduction of an unnamed cephalosporin with a thioether-linked

tetrazolyl substituent at position 3 and an unsubstituted amino group at position 7. They attributed the well shaped polarographic wave to a two-electron reduction of the C=N double bond rather than to reduction of the cephem C=C double bond, but pointed to insufficient data for confirmation of this hypothesis. However, determination of DPP parameters allowed them to use this technique for analytical purposes.

In an extensive electroanalytical study of several antibacterial and diuretic drugs at rotating gold and platinum disc electrodes, Bishop and Hussein [30] examined the cathodic and anodic activity of cephalexin and cephaloridine at both electrodes in acidic and alkaline media. Only cephalexin was reported to show two anodic waves at the gold electrode in 0.1 M sulphuric acid and at high concentrations (>10^{-3} M), but no activity was found at the platinum electrode. No attempts were made to elucidate the oxidation mechanisms.

In the first related paper by Şengün *et al.* [31], the DPP behaviour of ceftriaxone, cefuroxime, cefotaxime and ceftizoxime was described. The first three cephalosporins gave rise to two peaks, the more negative peak being ascribed to the known reductive cleavage of substituent at position 3; in contrast, ceftizoxime, with no side-chain at the 3-position, exhibited only a single peak. Well-shaped DPP peaks at −0.4 to −0.6 V (vs SCE), observed for all four cephalosporins examined, were believed to be due to a two-electron reduction of the azomethine (>C=N—) double bond in the methoxyimino group of the side-chain at position 7, present in all four compounds. However, no experimental or literature data were presented to support this hypothesis. In the second paper by Şengün *et al.* [32], the polarographic properties of cephalothin, cephacetrile, cefamandole, cefamandole nafate and cefoperazone were explored, employing cathode-ray polarography (CRP) and the DPP technique. All cephalosporins investigated exhibited a single polarographic signal as a consequence of the well known reductive cleavage of a substituent at position 3, in all compounds under study. The polarographic methods developed were applied to the determination of cephalosporins in dosage forms and a comparison was made with the nickel (II)-hydroxylamine (spectrophotometric) method. The relative standard deviations obtained for polarographic methods were 0.3–1.0% and were better than those for the spectrophotometric method.

A cefamandole synthetic intermediate with a tetrazolyl-thiomethyl substituent at position 3 was proved to be polarographically active; a buffer solution (pH 3) was found to be optimal for its polarographic determination in industrial production control, as reported by Kazandzhieva and Nedelchev [33]. Cai and Wu [34] then reported on the polarographic behaviour of two derivatives of cephalosporanic and penicillanic acid. The potassium salt of furoyl-ureido-phenylacetamido cephalosporanic acid gave a single-sweep polarographic peak at about −1.1 V that could be analytically exploited. Its adsorption character was also noted.

An electroanalytical study of cefazolin, ceftriaxone and cefotaxime was presented by Ogorevc *et al.* [35]. Whereas cefazolin, a cephalosporin with a thioether-linked thiadiazole substituent at position 3, gave rise to a well-defined single wave, ceftriaxone and cefotaxime gave rise to two reduction waves (Fig. 2). The analysis of polarographic and CV responses of cefazolin implied a two-electron, diffusion controlled and totally irreversible reduction process, influenced by certain adsorption phenomena. Without identification of the reduction product(s), no definite conclusion on the reduction mechanism of cefazolin was made. However, on the basis of experimental and literature data on the polarography of other thioether-bridging cephalosporins, the authors suggested that cefazolin reduction occurred at the Δ^3 double bond with cleavage of the corresponding substituent, as described above. In a second related paper by Ogorevc *et al.* [36], the DC, DPP and CV behaviour of the same three cephalosporins together with cefuroxime and moxalactam was reported. Besides cefazolin, moxalactam also exhibited only one polarographic wave, whereas cefuroxime gave rise to two waves. The reduction mechanism related to the more positive wave of ceftriaxone, cefuroxime and cefotaxime was investigated and discussed. A mechanism was proposed that involved a double two-electron reduction of the methoxyimino group, present in all three cephalosporins in the 7 side-chain, via a hydroxylamine intermediate to the corresponding amine and methanol. DPP methods for all five cephalosporins were established, providing methods with good sensitivity and excellent

precision (with a relative standard deviation of near 1.3% at 0.5 μg ml^{-1}).

In another study, by Peled *et al.* [37], a method was established for the determination of cephalothin by rapid-scan square-wave voltammetry (SWV) at a static mercury drop electrode, or after adsorptive accumulation on a stationary mercury drop electrode. The voltammetric behaviour of cephalothin employing the new SWV technique was thoroughly studied in order to optimize its reduction signal for analytical purposes. The authors stated that the adsorptive stripping method with SWV detection of cephalothin could not be applied to urine or plasma samples without prior solvent extraction or chromatography.

In work by Muñoz *et al.* [38], a study of the reduction of cefsulodin by means of DPP and DC polarography as well as CV, was carried out. It was found that the compound yielded three peaks in an acidic medium with the main peak (or wave) at the least cathodic potentials being twice as high as either of the other two. The electrode behaviour of cefsulodin was shown to be complex owing to its adsorption activity, affecting the linearity of the main peak current-to-concentration dependence. This effect was not pronounced, however, at cefsulodin concentrations lower than 60 ppm or, after addition of Triton X-100, in the investigated concentration range (1–200 ppm). The authors also discussed the electrode reaction pathway and attributed the main wave to the four-electron reduction of the isonicotinamide group at position 3. This was also supported by experiments with isonicotinamide itself. They did not consider the other two, more negative, peaks, because of their poor analytical significance.

In an electrochemical study, Muñoz *et al.* [39] examined adsorption and surface reduction parameters of cefazolin. The voltammetric behaviour, adsorption isotherm and reduction kinetic parameters of this compound were explored using CV with a fast potential scan (20 V s^{-1}) and chronocoulometry with voltammogram integration, under non-diffusion conditions. It was found that the isotherm describing the adsorption of cefazolin at the mercury electrode, at least at low concentrations, was of the Frumkin-type and that adsorption occurred in a monolayer. Furthermore, kinetic parameters of the heterogeneous reduction of cefazolin, considered as slow–fast, double, one-electron transfer, were shown to be dynamically dependent on the pH of the medium, since protonation was the rate-determining step at pH values higher than the pK_a of the compound. Muñoz *et al.* [40] later reported on the CV and LSV behaviour of cefazolin and cefmetazole, using the fast scan mode as in their previous study [39]. They explored the adsorption characteristics and discussed the kinetic parameters of the reduction process for both cephalosporins.

Cephaloridine and cefsulodin were also studied polarographically by Morgil *et al.* [41]. They found 2 and 3 DPP peaks yielded by cephaloridine and cefsulodin, respectively. The DPP methods developed for both compounds were found to be better in terms of precision than the USP XX hydroxylamine colorimetric method. Mohamed *et al.* [42] illustrated the potentialities of polarography for degradation and stability studies of drugs by their work on DP polarography and degradation of cephapirin, cephalothin and cefalexin antibiotics. The degradation processes and their DPP detection were examined and discussed.

Ogorevc *et al.* [43] described an adsorptive stripping voltammetric (AdSV) method for the trace determination of cefazolin, cefuroxime, ceftriaxone and cefotaxime, using the LSV or DPP mode. The optimum conditions (deposition potential and time, pH range, adsorption characteristics, etc.) were evaluated for each compound and analytical parameters established. Detection limits were found to be in the nanomolar range for all four compounds. Practical application of this method was demonstrated for the direct determination of cefazolin in dilute urine samples; the limit of detection was found to be 7 μg ml^{-1} of cefazolin in urine. The proposed AdSV method was stated to be equally or even more sensitive than HPLC methods for the same compound.

In another two papers by Muñoz *et al.* [44, 45], further theoretical and kinetic aspects of the electrochemical reduction of some cephalosporins were considered; these are important to comprehend in order to properly assess the voltammetric and analytical parameters. In the first [44], an explanation was presented for the unusual polarographic behaviour of cefazolin and cefmetazole at high concentrations ($>10^{-4}$ M) in acidic media, which was shown to be influenced by adsorption of the com-

pounds at the mercury electrode surface. In the second [45], the mechanism and kinetics of the reduction of cefsulodin (i.e. its isonicotinamide moiety), which depended on the pH of the medium and/or the potential scan rate, was exhaustively studied. In both studies, classical (DC, etc.) polarography and fast potential scan LSV were employed; the results contributed usefully to the understanding of the electrochemical behaviour of cephalosporins.

Indirect polarographic procedures, degradation studies and other electroanalytical methods

Those cephalosporins that do not contain a substituent at the C_3 carbon in the cephem dihydrothiazine ring and do not possess any other reducible or oxidizable group are electroinactive at mercury or solid electrodes. However, several degradation products after alkaline, neutral or acidic hydrolysis of these cephalosporins do give polarographic signals that can be used for their determination after forced and controlled hydrolysis procedures. Kinetics and mechanisms of the degradation of cephalosporins is described elsewhere [64, 65].

Fogg and co-workers have published several reports [46–48] on cephalosporin degradation studies where polarography and other methods were used for monitoring the degradation reactions, and, possibly, for determination purposes. In one paper [46], a DPP degradation study of cephalexin was reported. The advantages of DPP as a complementary technique for following its degradation process (over e.g. HPLC) were illustrated. In this process, as many as 12 peaks were detected and three of these were identified. In another publication, Fogg *et al.* [47] described a procedure for the DPP determination of cephalexin after its hydrolysis in phosphate buffer under controlled conditions (pH 9.4, 100°C, 60 min). Fogg and Martin [48] presented a comparison of cephaloglycin degradation in neutral medium (pH 7.4, 100°C, 60 min) with that of cephalexin, monitored by DPP detection of electroactive degradation products or intermediates.

Squella *et al.* [49] found by DC polarography that cephalexin was not capable of undergoing reduction and giving a polarographic signal without prior derivatization. Hence a procedure was developed in which the compound was hydrolysed in acidic medium (5 M HCl, 80°C, 15 min) yielding two polarographic waves, both of which were diffusion controlled. In similar work carried out by Nuñez-Vergara *et al.* [50], a DC polarographic method was developed for the quantitative analysis of cephradine and its dosage forms. The electroactive product was formed by acidic hydrolysis (5 M HCl, 80°C, 60 min), giving two waves, and the less cathodic wave was preferred for determination purposes. In another contribution by Nuñez-Vergara *et al.* [51], DC polarography of an acidic degradation product from cephalexin and a method for its indirect analysis was reported. An application of this procedure to the direct determination of cephalexin in human plasma was described. However, the analytical usefulness of such a method is limited, despite the advantages of such a procedure (e.g. no need of deproteination), since the lower limit of determination of the proposed method (40 $\mu g\ ml^{-1}$ of cephalexin in plasma) was about three times higher than the highest concentration of cephalexin in blood after normal therapeutic dosage, as stated by the same authors.

Another possibility for the indirect polarographic determination of cephalexin was reported by Hernández Méndez *et al.* [52]. A DPP method involving the nickel(II) catalytic pre-peak, caused by its complexation with cephalexin, was shown to be applicable to the determination of the compound at low concentrations (10^{-7} to 10^{-6} M). Other amine group-containing cephalosporins (e.g. cefadroxil, cefotiam, cefroxadine) behaved similarly. An analytical method for the determination of cefotiam in serum or urine samples by DPP, based on the catalytic pre-peak of nickel(II), after a thin-layer chromatographic and extraction procedure, was described by Schröder *et al.* [53]. The method was applied in a pharmacokinetic study on pregnant women.

The amperometric detection of some cephalosporins following their HPLC separation in biological fluids was presented by Fabre *et al.* [54, 55] and Blanchin *et al.* [56]. In one paper, the anodic amperometric behaviour of cefotaxime and its two main metabolites at the glassy carbon electrode was investigated [54]. An optimum signal-to-noise ratio was obtained at potentials of about +1.0 V (vs SCE) at pH 7.6 and the sensitivity was stated to be down to the picomolar range. This value, however, should be taken with caution since from the results presented, a detection limit of ~0.2 $\mu g\ ml^{-1}$ for cefotaxime (about five orders of

magnitude higher) can be calculated. As the aminothiazole group on the side-chain at position 7 was assumed to give the anodic response, oxidative detection of other cephalosporins containing this functionality (e.g. ceftizoxime, cefmenoxime, ceftazidime and ceftriaxone) was examined [55]. At a pH of 2 and at potentials of about +1.2 V (i.e. on the limiting current plateau), limits of detection were found to be five times lower than those obtained by UV detection. The capability of using amperometric detection for the determination of these cephalosporins in serum and urine samples was demonstrated on cefmenoxime. In another paper [56], two electrochemical detection modes for the HPLC determination of cephalosporins were described: a direct, anodic detection mode, similar in principle and design to that described in ref. 55, and an indirect mode via an on-line bromine oxidation of a cephalosporin and subsequent reductive amperometric detection of excess bromine. The latter detection method was tested on a variety of cephalosporins and was claimed to be applicable to all the cephalosporins since it was based on the general property of a β-lactam ring to be oxidized by bromine. Its sensitivity was shown to be equivalent to, and its selectivity better, than that of UV detection. A similar procedure, based on the liquid chromatographic determination of cephalosporins, involved an in-line coulometric titration with electrochemically generated bromine as the oxidizing agent, followed by reductive amperometric detection of the excess bromine was described in an article by Fabre and Kok [57]; this procedure was applied to the determination of cefotaxime in serum and urine samples.

An indirect method for oxidative electrochemical detection in the liquid chromatographic trace analysis of drugs and biologically active materials including some cephalosporins was described by Krull *et al.* [58]. A photolytic derivatization step was employed prior to detection to produce anodically active compounds. This type of indirect electrochemical detection was employed by McClintock and Cotton [59] for the determination of a modified neutral cephalosporin (L-658,758) using a HPLC separation with a post-column UV irradiation reactor. The resulting species produced was detected on a glassy-carbon electrode set at 1.1 V vs a Ag/AgCl reference electrode. The signal was linear over three orders of magnitude of the cephalosporin amount injected (1 ng–1 μg) and the amperometric detection was stated to be five times more sensitive than conventional UV detection but, unfortunately, insufficient data (e.g. injection volume) were given to calculate the concentration range and sensitivity of the method.

Only a few potentiometric procedures have been reported and are available for the determination of cephalosporins. Casalini *et al.* [60] described procedures for the automatic potentiometric titrations of a number of penicillins and cephalosporins (7-ACA, cefalexin, cephaloridine, cephacetrile, cephalothin, cefazolin and cephapirin) in non-aqueous media. Conditions, solvents and reagents were given for the compounds, depending on their basic or acidic nature. In two reports by Korbl and Pospišilová [61] and Pospišilová and Kubeš [62], mercurimetric determination of cephalosporin antibiotics was described. At pH 7, and after treatment by hydroxylamine, cephalosporins were titrated with $Hg(ClO_4)_2$ with potentiometric end-point detection. Whereas cefuroxime, cefsulodin, cefotaxime and ceftriaxone gave results that were identical with those obtained by spectrometric and microbiological methods, the method could not be applied to cefoperazone and cefoxitin. In one paper [61], interference of the 3 side-chain of cefazolin was mentioned, whereas in the other [62], the determination of degradation products of cephalexin, cefuroxime, cephalothin, cefsulodin and ceftriaxone were reported to be verified as well.

A microbial electrode sensor for the determination of cephalosporins was described by Suzuki and Karube [63]. A cephalosporin could be detected from the determination of proton concentration in a medium by using cephalosporinase-producing *Citrobacter freundii*, immobilized in a collagen membrane in combination with a pH electrode. The authors proposed that a cephalosporin produced in fermentation broth could be measured continuously by this method.

Conclusions and Compilation of Analytical Data

The published research work on the electroanalytical properties of cephalosporin antibiotics and on their applications have demonstrated the powerful capabilities of these tech-

niques, especially polarography and voltammetry, for the analysis of these compounds in either simple solutions or biological samples.

There are several advantages in the use of polarographic and voltammetric methods in the determination of cephalosporins.

Firstly, these techniques provide an inherently good sensitivity and usually a wide range of linearity of the signal with the concentration of the analyte. Since the reductive electrode process of nearly all cephalosporins involve either a two- or even four-electron transfer per cephalosporin molecule, low detection limits can be achieved owing to a high signal gain per analyte unit and good signal-to-noise ratios. An example of this is shown in Fig. 4, with DPP calibration curves of cefuroxime in both simple solutions and solutions containing serum ultrafiltrate.

Secondly, on the basis of the date presented, it can be concluded that the precision of measurements is high for the electroanalytical determination of cephalosporins. Because of well-defined and simple experimental characteristics of these techniques, the relative standard deviation of reported procedures seldom exceeded 1–2%, even at low concentrations of cephalosporins.

Thirdly, one of the benefits of polarographic and voltammetric measurements is the nature of their scanning mode that enables not only measurements of the signal itself to be made but also visualization of its environment or solution "context". This is of a great usefulness when dealing with real samples (e.g. body fluids, fermentation broths, production mixtures). The example of a DPP determination of a cefazolin in an ultrafiltered serum sample, shown in Fig. 5, demonstrates the possibility for control over the quality of polarographic signals.

Fourthly, one of the factors of great importance is the time scale of electroanalytical determinations for routine analytical work. From literature data and the authors' own experience, an average direct polarographic or voltammetric procedure in a research laboratory lasts 10–20 min; however, with application of modern, computerized equipment, automation and new techniques (SWV) this time can be drastically reduced to only a few minutes per sample.

As can be noted from the literature review, many authors have made experimental comparisons or evaluations of the results between polarographic or voltammetric and other

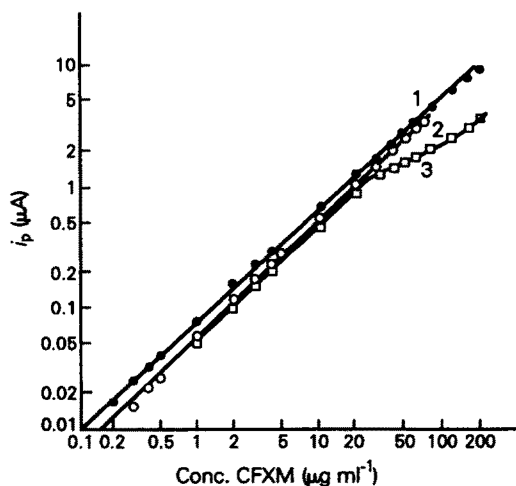

Figure 4
DPP calibration graphs for cefuroxime (CFXM): (1) in Britton–Robinson (BR) buffer (pH 2) (less cathodic peak I); (2) in BR buffer (pH 2)–ultrafiltered serum (4:1, v/v) (peak I); (3) in BR buffer (pH 2) (peak II). Instrumental settings: drop time 1 s; scan rate 4 mV s^{-1}; pulse amplitude 100 mV.

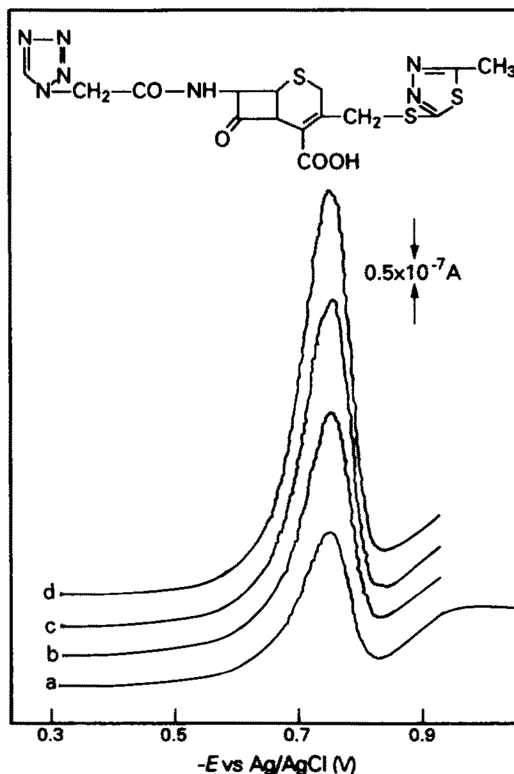

Figure 5
DPP determination of cefazolin in human serum: (a) ultrafiltered serum sample–BR buffer (pH 3) (1:4, v/v) (20 ml); (b–d) 20 μg of cefazolin standard additions. Instrumental settings: as in Fig. 4. Structural formula of cefazolin.

methods of cephalosporin determination or detection. They have claimed that in terms of not only sensitivity, precision and accuracy, but also selectivity and simplicity, electroanalytical methods were in many cases superior to other methods or detection modes including microbiological, iodometric, colorimetric, spectrophotometric, chromatographic and other techniques [25, 32, 41, 43, 46, 55, 56, 59].

In Table 1, some important analytical data are listed on the polarographic or voltammetric determination of cephalosporin antibiotics, compiled from various literature sources. Since data were given in different ways and forms, a certain measure of simplification was undertaken in order to make Table 1 concise. The data were compiled in order to facilitate an evaluation and comparison of different procedures, techniques and corresponding parameters for the cephalosporins examined.

In general, all cephalosporins containing a substituent at position 3 of the cephem nucleus

Table 1
Optimum parameters for polarographic or voltammetric determination of cephalosporin antibiotics, compiled from different literature sources

Cephalosporin	Medium	pH	*E* (*V*)	Method	Concentration range (μg ml^{-1}) or (M)	LoD (μg ml^{-1}) or (M)	Ref.
Cephaloridine	0.1 M HCl	~1	−0.9	DCP	0.01–2 mM	—	19
Cephaloridine	BR	4.0	−1.06	DPP	0–2	0.1	26
Cephalothin	BR	2.0	−1.00	DPP	0–2	0.1	26
Cephalothin	CI + HCl	2.0	−1.02	DPP	14–70	0.06	32
Cephalothin	0.1 M $HClO_4$	~1	−1.08	SWV	10^{-7}–19^{-5} M	—	37
Cephalothin	0.1 M $HClO_4$	~1	—	AdSV	10^{-8}–10^{-7} M	—	37
Cephalosporin *C*	BR	2.0	−1.00	DPP	0–2	0.1	26
Cephalexin	0.1 M HCl	~1	−1.25	DPP	4–400	2	28
Cephalexin	Ni + SA	8.3	−0.72	DPP	10^{-7}–10^{-5} M	2×10^{-7} M	52
Cephalexin*	5.0 M HCl		−0.50	DCP	10^{-5}–10^{-2} M	—	49
Cephalexin*	PH	7.4	−1.26	DPP	0.2–3	—	47
Cephaloglycin	1 M H_2SO_4		−0.95	DPP	1–5	<1	15
Cephaloglycin	BR	2	−0.90	DPP	—	—	48
Cefamandole	MI	2.3	−0.8	DCP	—	—	25
Cefamandole	CI + HCl	3.0	−0.74	DPP	12–60	0.07	32
Cefamandole N	CI + NaOH	6.0	−0.76	CRP	10–50	0.1	32
Cephalonium^{I}	Br	3.0	−0.72	DPP	0–2	0.1	26
Cefotiam	Ni − Sa	7.5	−0.98	DPP	—	—	53
Cefuroxime^{I}	BR	2.5	−0.39	DPP	0–2	0.1	26
Cefuroxime^{I}	CI	2.0	−0.38	DPP	5–27	—	31
Cefuroxime^{I}	BR	2.1	−0.35	DPP	0.1–200	0.01	36
Cephoxazole	BR	2.0	−0.95	DPP	0–2	0.1	26
Cefadroxil	0.1 M HCl	~1	−1.25	DPP	0.7–700	0.7	28
Cefadroxil	BR	7.3	+0.8	LSV	0.8–200	1×10^{-6} M	28
Cephapirin	BR	7.3	−1.22	DPP	0.2–400	—	28
Cephradine*	5.0 M HCl		−0.46	DCP	10^{-5}–10^{-2} M	—	50
Ceftriaxone^{I}	CI	2.0	−0.50	DPP	9–46	—	31
Ceftriaxone^{I}	BR	4.2	−0.63	DPP	0.2–20	0.02	36
Ceftriaxone^{II}	BR	3.0	−0.82	DPP	4–100	0.5	36
Cefotaxime^{I}	CI	2.0	−0.54	DPP	6–32	—	31
Cefotaxime^{I}	BR	2.0	−0.44	DCP	5–350	3.0	36
Cefotaxime^{II}	BR	2.0	−0.93	DPP	0.2–15	0.02	36
Cefotaxime	MI	7.6	+0.95	AMD	—	~0.2	54
Ceftizoxime	CI	2.0	−0.58	DPP	15–75	—	31
Cephacetrile	GL + HCl	1.0	−0.98	DPP	10–50	0.07	32
Cefoperazone^{I}	PH	7.0	−1.09	DPP	7–37	0.07	32
Cefazolin	BR	3.0	−0.64	DPP	0.1–100	0.01	36
Cefazolin	0.6 M APH	4.8	−1.1	AdSV	10^{-9}–10^{-7} M	10^{-9} M	40
Moxalactam	BR	2.2	−0.77	DPP	0.3–150	0.04	36
Cefsulodin^{I}	BR	3.0	−0.7	DPP	1–200	—	38
Cefmetazole	0.6 M APH	4.8	−1.1	AdSV	10^{-8}–5×10^{-7} M		40

In the pH column, the pH of a medium is shown, in which the subsequent half-wave or peak potential is given. With *E*, a rounded half-wave or peak potential is quoted in V against employed reference electrode (SCE or Ag/AgCl). Concentration range and limit of determination or detection (LoD) are given in either μg ml^{-1} or mol l^{-1} (assigned as M).

Legend: — no, obscure or uncertain data; *, indirect method; I, II, assignment of reduction peak (wave) — the higher the number, the more cathodic its position.

Abbreviations: cefamandole N = cefamandole nafate; BR = Britton–Robinson buffer; PH = phosphate buffer; MI = McIlvaine buffer; CI = citrate buffer; GL = glycine; Ni = nickel(II) solution; SA = sodium acetate; APH = acetic–orthophosphoric acid–sodium hydroxide buffer with sodium nitrate; AMD = amperometric detection.

(Fig. 1) were shown to be amenable to undergo reduction of the cephem Δ^3 double bond with subsequent cleavage of the related substituent. This mechanism seems to be elucidated [21, 23] although questions about the reduction products are still not fully answered. However, some disagreement about this reduction exists in the literature. It is uncertain whether or not cephalosporins with only a methyl-substituted C_3 carbon of the cephem thiazine ring, but containing no other reducible group, are polarographically active. It is still not clear whether these compounds undergo reduction [23, 24], and whether they are only catalytically active [28] or not active at all [21, 26, 27, 46, 47, 49, 50].

The published data show that cephalosporins with a thioether linkage in the side-chain at position 3 (i.e. cefazolin, moxalactam, cefamandole, ceftriaxone, etc.) form a separate group, according to their corresponding wave (peak) position. This wave is shifted anodically by about 200–300 mV, compared with that of ether-bridging cephalosporins. Consequently, it seems that the pathway of this reduction is still under discussion [22, 25, 29, 35, 36, 39].

The adsorption of cephalosporins on mercury or solid electrode surfaces has been reported to play an influential rôle in the electrochemical behaviour of many (or probably most) cephalosporins [23, 28–30, 34–40, 43–45]. Such adsorption on electrodes has been thoroughly studied [37, 39, 40, 43–45] and has also been employed as an analytical tool [37, 43].

The developments in the last few years in this field suggest possible future directions for further work.

Firstly, further application of modern, computer-driven, direct polarographic or voltammetric techniques (e.g. DPP, SWV), either to simple solutions (for pharmaceutical analysis) or to biological samples (for pharmacological or clinical application) after appropriate separation (e.g. extraction, chromatography and ultrafiltration) should be encouraged. There is still much work to be done, especially with new cephalosporins.

Secondly, amperometric detection (anodic or cathodic, direct or indirect) in conjunction with HPLC or other separation methods and in flow injection assemblies, appears to be promising and hence deserves more attention. Different solid and coated or modified electrodes have not yet been explored although the value of such sensors is becoming widely appreciated.

Thirdly, for determination of extremely low concentrations, e.g. after dilution of biological fluids, the AdSV technique is highly powerful in the case of cephalosporins with strong specific adsorptive properties.

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