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Effect of cationic micelles on the decomposition of α-aminophenyl cephalosporins

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ABSTRACT

The intramolecular rates of degradation of α-aminophenyl cephalopsorins were determined with and without hexadecyltrimethylammonium bromide (CTAB). Micellar-derived spectral shifts were used to measure the bind of the ionic forms as well as to determine the effect of CTAB on the apparent dissociation constant of the antibiotics. The rate of the degradation of cephalexin (Cp), cefadroxil (Cf), and cephradine (Cph), increased with surfactant concentration reaching a plateau at high surfactant concentrations. In the plateau region, the rate constant was salt sensitive decreasing with NaBr concentrations. These effects were quantitatively analyzed within the framework of the pseudo-phase model with explicit considerations of ion exchange. All the experimental results were fitted to this model. The intramolecular degradation of Cf, Cp and Cph was catalyzed by 96-, 59-, and 29-fold, respectively. A working hypothesis to rationalize these effects was suggested. The obtained results demonstrate that the quantitative analysis can be used to assess, predict and control the effects of surfactants on the drug stability.

1. Introduction

Micelles, self-assembling nanosized colloidal supramolecular aggregates with a hydrophobic core and hydrophilic shell [1–3], are currently successfully used for drug solubilization and have been extensively studied as a potential carrier for poorly water-soluble drugs [4–9]. Furthermore the surfactants are the main stabilizing agents of a series of micelle-related structures used as drug carriers including emulsions and microemulsions [10–15].

Although the study of the effect of surfactants on the stability of drugs is a phenomenon studied for a long time [1,16–23], there are still many challenges in the detailed understanding of these effects in pharmaceutical formulations.

The widespread therapeutic use of α-aminopheny-lcephalosporins and their antimicrobial activity is related to the reactivity of the β-lactam ring makes the study of their degradation of particular importance [24,25]. In fact, the effect of surfactants on the stability of α-aminophenylcephalosporins is particularly interesting, because depending on the pH, these compounds become amphoteric and can exist in different ionic species [26–31] which differently interact with supramolecular aggregates [19–21,32,33]. Thus, the decomposition pathway of these antibiotics involves intra- and intermolecular reactions. The intramolecular degradation occurs at neutral pH by aminolysis involving the attack of the amino group at C-17 (N-18) on the β-lactam carbonyl producing piperazine-2,5-dione derivatives (Scheme 1). On the other hand, the intermolecular degradation takes place above pH 8.0 through nucleophilic attack of OH^- ions on the β-lactam ring [24,25,27–31].

It is widely known that the stability of these compounds may be changed in the presence of ionic micelles [22–24,26,31]. In previous work we have demonstrated that not only is the intramolecular decomposition of cefaclor catalyzed by positively charged [18], neutral and zwitterionic micelles [19], but also the origin of this catalysis is related to the stabilization of a more reactive molecular conformation of the antibiotic. Further studies on the effect of temperature on this reaction showed that the modification of the cefaclor reactivity by the hexadecyltrimethylammonium bromide (CTAB) micelles led to a change of 7 entropy units (e.u.) when the reaction was transferred from the aqueous homogeneous medium to the micellar one. This result is in agreement with the proposition of a change in the molecular conformation [20,21].

In this work the intramolecular decomposition that occurs in the neutral pH region (Scheme 1), was studied. The structurally related semi-synthetic oral cephalosporin antibiotics cephalexin (Cp), cephradine (Cph) and cefadroxil (Cf), with the different R′ substituent group in the side chain of C7 of the β-lactam ring, were used (Scheme 2). The study was designed in order to obtain additional information about the modification of the stability of this group of compounds induced by cationic micelles.

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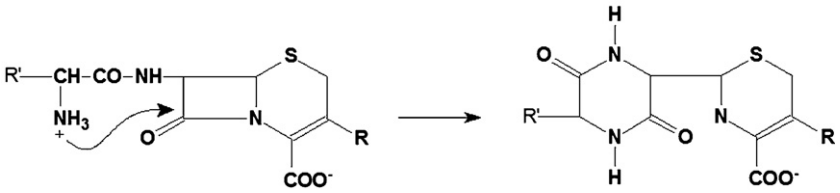


Scheme 1. Reaction of decomposition of the α-aminophenyl cephalosporins at neutral pH.

2. Materials and methods

2.1. Materials

Analytical grade cephalexin (Cp), CAS 156686-71-2; cephradine (Cph), CAS 38821-53-3 and cefadroxil (Cf), CAS 50370-12-2 were purchased from Sigma Chemical Co., USA. Stock solutions of the antibiotics were prepared daily in water, maintained at 4 °C, and discarded after use. Analytical grade hexadecyltrimethylammonium bromide (CTAB), from Merck S. A, Brazil, was recrystallized three times in ethanol:acetone (85:15, v/v) and dried under reduced pressure [34]. Hydrobromic acid and sodium bromide, and Tris-(hydroxymethyl) aminomethane were supplied by E. Merck, Darmastadt, and Aldrich Chemical Co., USA, respectively. Deionized, double-glass distilled water was used throughout. All other reagents were of analytical grade.

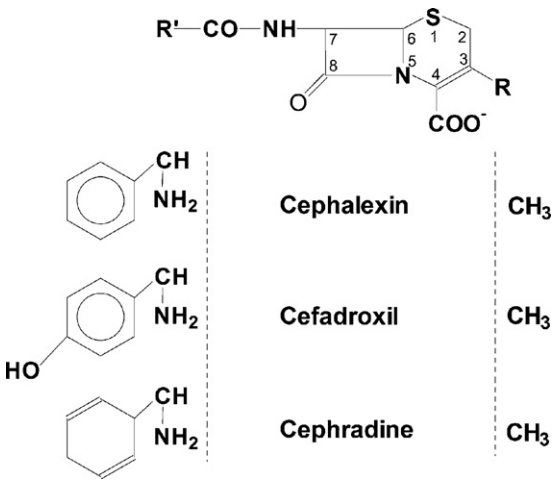
3. Methods

3.1. Micelle preparations

Micelles were prepared dissolving adequate weights of CTAB in 0.01 mol L^{-1} Tris–HCl buffer solution, pH 7.8, or in another solvent system specifically described.

3.2. Kinetic determinations

Micelles were temperature equilibrated in a cuvette (37 ± 0.1 °C), in the sample compartment of a HP 8453 diode array UV–vis Spectrophotometer using an HP ChemStation software for data analysis. Reported temperatures were measured in the cuvette with a microprobe and a calibrated digital thermometer. The reaction was started by addition of 50 μL of an aqueous standard solution of the antibiotics (10^{-3} mol L^{-1}) into 2 mL of Tris–HBr buffer, 0.01 mol L^{-1}, pH 7.8, ionic strength 0.01, and variable volumes of 0.1 mol L^{-1} buffered surfactant solution. The final concentration of the antibiotics was 2.5×10^{-5} mol L^{-1}. The intramolecular decomposition of the antibiotics was followed by a decrease of the absorbance at 262 nm against the time. The first-order rate constants (k) were calculated from kinetics data with a first-order fitting routine. In all cases, the reactions followed first-order kinetics for at least four half-lives. The reported values of k are the average of three independent experiments, with the standard deviation not exceeding 5%.



Scheme 2. Chemical structures of the α-aminophenyl cephalosporins used in this work.

3.3. Dissociation constants determination

The dissociation constants of the α-aminophenylcefalosporins were determined from the variation of the absorbance against the pH. The spectra of a solution at 6×10^{-5} mol L^{-1} of the antibiotics in HBr 0.1 mol L^{-1}, in formate buffer pH 3.5, and in Tris–HBr buffer pH 8.4, with ionic strength adjusted to 0.01 mol L^{-1} were recorded. The largest variations in the absorbance for the C4 carboxylate and the α-aminophenyl group were observed in the regions of 288 and 230 nm, respectively. The largest variations of absorbance were observed in the regions of 288 and 230 nm, for C4 carboxylate and amino group of the side chain of C7, respectively. Thus, these wavelengths were used to determine the variations of the absorbance with pH. The pK_a's values were obtained by iteration using curve-fitting software from the absorbance curves.

4. Results and discussion

Above the critical micellar concentration (CMC) of the used surfactant, the self-organized micelles exist as a dispersed pseudo-phase with a specific local volume, which moves in the aqueous medium with a diffuse layer of counter ions, allowing the binding of ionized drugs. In aqueous medium, the dissociation constants of the amine group of the lateral side chain of cephalosporins were determined from the variation of the absorbance versus pH. In the maximum absorption regions of the antibiotics (262–264 nm) the absorbance was very similar at pH 4.5, in which it is expected that the zwitterionic species exists, and at pH 8.5, where the negatively charged form predominates. However, the biggest spectral difference obtained in our experimental conditions was observed at 237 nm. At this particular wavelength the obtained values for the pK_a's of the amine group in the side chain of Cp, Cf, Cph and were 6.71, 6.92, and 6.95, respectively. These values are in agreement with other results described in the literature for this group of compounds [18,29]. As an example, the pK_a determination of cephalexin (Cp) was shown in Fig. 1.

In previous work we have determined that the pK_a value of the α-aminophenyl cephalosporin cephaclor decreases about one pH unity with the increase of the CTAB concentration [18]. Thus, it was possible to define the experimental conditions in which only the negatively charged cephalosporin species exists in the presence of the cationic aggregates.

In these experimental conditions the effect of positively charged micelles such as CTAB on the intramolecular aminolysis of this antibiotic type can be described in Scheme 3.