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Azithromycin loaded on hydrogels of carbomer: Chemical stability and delivery properties

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ABSTRACT

Hydrogels of carbomer (C) and azithromycin (AZI) were prepared by neutralizing with AZI 50% of the carboxylic groups of 0.25% C_{974} and C_{934} dispersions. The hydrogels exhibit pH close to 8 and are physically stable. Titration with NaCl revealed a high degree of counterion condensation C–AZI. The release of AZI in a Franz cell was almost negligible when the receptor compartment was filled with water but was increased about 20 times as water is replaced by NaCl solution. Two analytical methods were used to evaluate the effect of the counterionic condensation on the chemical stability of AZI, a microbial assay and an HPLC method. Degradation of AZI in buffered aqueous solution was used as reference. The stability of AZI was significantly improved in the hydrogels retaining more than 75% of the initial concentration along a period of 18–20 months evaluated and the self life (t_{90}) of the drug was increased 27 and 20 times over the reference. The improvement of AZI stability could be attributed to the high degree of counterion condensation in which drug molecules remain associated to the macromolecular phase having a high negative electrokinetic potential and higher viscosity and lower kinetic energy than those in the fluid phase.

1. Introduction

Azithromycin (AZI) is a member of the family of 15-membered macrolide antibiotics called azalides. AZI and the newer macrolides, such us clarithromycin, dirithromycin and roxithromycin, can be regarded as "advanced-generation" macrolides compared with erythromycin, the first macrolide used clinically as an antibiotic. AZI differs chemically from erythromycin in that a methyl-substituted nitrogen atom is incorporated into the lactone ring as indicated in Fig. 1.The addition of this group produces a dibasic molecule, in contrast to the monobasic nature of virtually all other macrolides (Amsdem, 2001; Kremer, 2002).

The replacement of the keto group in the lactone ring with the N-methyl group improves the stability of AZI over erythromycin in an acidic environment (pH 2.1) exhibiting $t_{1/2}$ 5.7 h versus 0.3 h, respectively (Fiese and Steffen, 1990). Nevertheless, the chemical stability of AZI in aqueous systems remains as an issue. In fact, oral suspensions and intravenous formulations are presented as extempore preparations.

Previously we have reported some articles dealing with the properties of aqueous dispersions of systems consisting of an acid polyelectrolyte (PE) partially or fully neutralized by a drug (D) having basic groups (Vilches et al., 2002; Jiménez Kairuz et al., 2004). The acid–base interaction between the PE and D yields a high proportion of counter ionic condensation forming ionic pairs PE–D. It has been shown that such interaction is useful to increase the aqueous compatibility of low solubility drugs as well as to improve the chemical stability of unstable drugs. Besides, due to the reversibility of the PE–D interaction, these systems behave as a drug carrier having the ability of delivering the drug through ionic exchange in contact with biological fluids. Thus, AZI having two aliphatic amino groups able to react with the acid groups of a PE is an interesting system to test the effect of such interaction in promoting the aqueous chemical stability of the drug.

In this work carbomer (C) was selected as the carrier PE to be loaded with AZI. Both, C–AZI hydrogels and aqueous solutions of AZI were evaluated determining the drug remaining during the storage under selected conditions. With that purpose, a microbial assay and an HPLC method were used.

2. Materials and methods

2.1. Chemicals

Carbomer 934P NF and 974P NF were kindly provided by BF Goodrich (OH, USA). Azithromycin dihydrate (Parafarm), sodium

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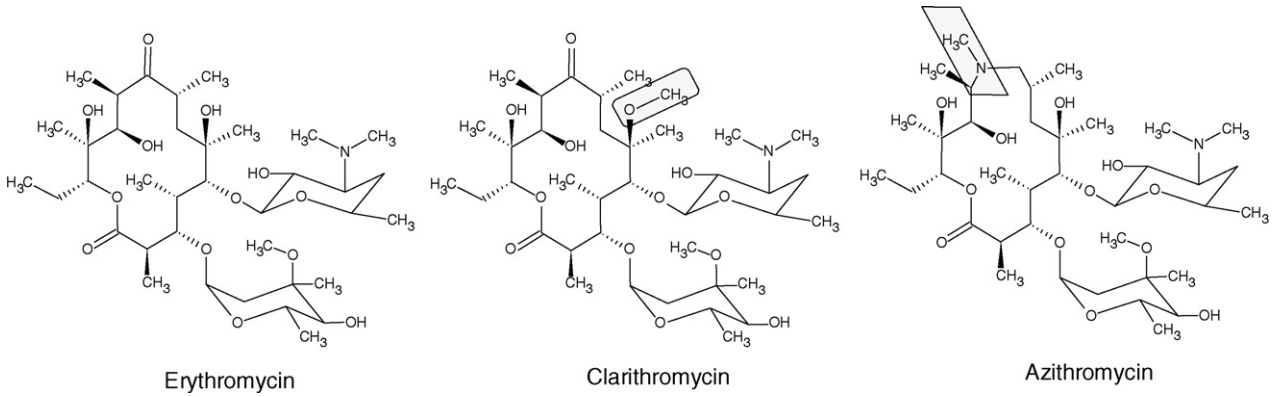

Fig. 1. Chemical structure of some macrolides.

chloride, dipotassium hydrogen phosphate and potassium dihydrogen phosphate (Cicarelli, Argentine). Methanol and acetonitrile, both HPLC grade (Cicarelli, Argentine). Grove Randall number 1 agar and Grove Randall number 11 agar were obtained from Merck (Bs. As., Argentine).

2.2. Preparation of hydrogels

Hydrogels designated as: C_{934}–AZI_{50} and C_{974}–AZI_{50} were prepared by addition of appropriate amount of AZI to neutralize 50% of carboxylic groups of 0.25% aqueous dispersions of each carbomer. AZI as a fine powder was gradually added while mixing at room temperature to get uniform gels having 11.8 mg/ml of AZI.

2.3. Electrokinetic potential (ζ)

Electrophoretic mobility (μ) and electrokinetic potential (ζ) were assayed in a Rank Brothers Mark II electrophoresis apparatus, equipped with a cylindrical cell with 10 cm between electrodes at 25 °C and ±50 V; ζ was calculated as: $\zeta = 128\,s/(cm^2\,\mu)$ (Martin, 1993). Samples were prepared by diluting the hydrogels 30 or 35 times with water.

2.4. Titration of ionic pairs

The effect of the addition of NaCl solution 3 M on ionic equilibrium of C–AZI dispersions was evaluated by plotting the pH of the dispersion against volume of NaCl added.

2.5. Drug delivery from C_{934}–AZI hydrogel

Experiments were performed in a modified Franz diffusion assembly at 37 ± 1 °C. Semi-permeable acetate cellulose membrane (Sigma® 12000) was placed between the donor and receptor compartments. Twenty milliliters of hydrogels were placed in the upper compartment while the receptor one was filled with 300 ml of either water or 0.9% NaCl solution and stirred at 260 rpm with teflon-coated magnetic stirring bar. At selected times, 3 ml aliquots were withdrawn and replaced by the same volume of fresh and pre-warmed receptor medium. The assays were done in triplicate. Concentration of AZI in the samples was assayed by HPLC.

2.6. Stability studies

The chemical stability of AZI in the hydrogels was evaluated during natural aging through a period of 18–20 months using a microbiological assay. In a comparative way, the behaviour of AZI in an aqueous solution of phosphate buffer pH 8 (1 mg/ml) was quantitatively assayed by HPLC at two temperatures (20 and 40 °C). Besides, the performance of both analytical methods (Bioassay and HPLC) was compared through the analysis of samples of an AZI solution of pH 8 progressively degraded.

2.7. HPLC assay

The chromatographic system (Brezee system) consisted of a Waters 1525 pump, a Waters 717 plus auto sampler and a Waters 2996 Photo Array Detector (PDA) (Waters Corp. Milford, USA) The wavelength of the detector was set at 210 nm. Data acquisition was performed by the Empower Software data registration™. The analytical column was a reversed-phase ACE 5 CN (25 cm L × 4.6 mm i.d., 5 μm particle size) maintained in the column oven at ambient temperature and protected by a Security Guard precolumn. The mobile phase consisted of potassium phosphate buffer 0.1 M (pH 7.5): methanol: acetonitrile (30:60:10). Elution was performed isocratically at 25 °C at a flow-rate of 0.5 ml/min. The mobile phase was filtered through a 0.45-μm Millipore™ Durapore filter and degassed by vacuum prior to use. Employing this HPLC system, AZI produces a peak with a retention time of approximately 16 min. Calibration curves in mobile phase were linear in the concentration range of 12.5–600 μg/ml.

2.8. Microbial assay

A microbiological assay, adapted from the method described in the Argentinean Pharmacopoeia and USP applying the cylinder-plate diffusion technique was used to assay AZI in hydrogels and aqueous solutions (Farmacopea Nacional Argentina, 2003; US Pharmacopoeia, 2004). This assay was performed in 3 × 3 designs (three dose levels of reference and three dose levels of sample). The parallel-line model was chosen.

2.8.1. Microorganism and inoculum

2.8.1.1. Preparation of reference and sample solutions. Standard AZI solutions were prepared by dissolving an accurately weighted amount of drug in methanol (1000 μg/ml). Aliquots of this solution were diluted in a potassium phosphate buffer pH 8 to yield working solutions of 0.5, 1 and 2 μg/ml, which were used in the assay as reference solutions.

Samples: all preparations were maintained at room temperature, protected from light and periodically tested. Aliquots of the hydrogels and aqueous solutions of AZI were diluted in phosphate buffer pH 8 at the same concentrations that the solution of AZI used as reference.

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2.8.1.2. Cylinder-plate assay. Nine stainless steel cylinders of uniform size (8 mm × 6 mm × 10 mm) were placed on the solidified surface of the seeded agar. Three alternate cylinders were filled with 100 μl of reference solutions (three concentrations) or problem samples. Each assay was designed to evaluate in the same plate the reference solutions and two different samples (hydrogels or aqueous solutions of AZI). Four plates were used in each assay. After incubation (24 h at 35 °C) the zone diameter of the growth inhibition were measured using a calliper.

2.8.1.3. Calculation. The assay was statistically calculated by the linear parallel model and by means of regression analysis and verified using analysis of variance. Concentration of AZI in each sample was calculated according to Argentinean Pharmacopoeia (Farmacopea Nacional Argentina, 2003).

3. Results and discussion

3.1. The system C–AZI

Carbomer dispersions loaded with AZI as described in the experimental section yield physically stable hydrogels of pH close to 8.

It has been previously reported that the acid base interaction between C and monobasic model drugs (D) yields a high degree of counterion condensation through ion pairing according to:

$$R - COOH + D \rightleftarrows RCOO^- + DH^+ \rightleftarrows [RCOO^-DH^+] \quad (1)$$

in which R–COOH represents a carboxylic group of C (Vilches et al., 2002; Jiménez Kairuz et al., 2004).

In this case, the two basic groups of AZI, the one the amino sugar (pK_a 8.6) and the other at the lactone ring (pK_a 9.5), both have enough strength to react with the carboxylic groups of C giving rise to a greater number of possible interactions.

Previously we have shown that the fraction of ionic pairs of a C–D system can be titrated by ionic exchange with a neutral salt like NaCl. The protogenic effect arising from the displacement of DH^+ according to Eqs. (2) and (3) is easily measured in the range of pH in which equilibrium 3 is operating.

$$[RCOO^-DH^+] + NaCl \rightleftarrows [RCOO^-Na^+] + Cl^- + DH^+ \quad (2)$$

$$DH^+ \rightleftarrows D + H^+ \quad (3)$$

Titration with NaCl of a C–AZI hydrogel originated the plot of Fig. 2. Unlike the behaviour observed with monobasic C–D systems, in this case two differentiated steps were observed along the titration. At the beginning a quick decrease of pH that stops at about 0.8 mEq of NaCl respect to 1 mEq of AZI was observed. It was followed by a short plateau ending at about 1 mEq. After that, a slower decrease of pH was observed. Such pH decreasing remained even after having added 6.8 mEq of NaCl.

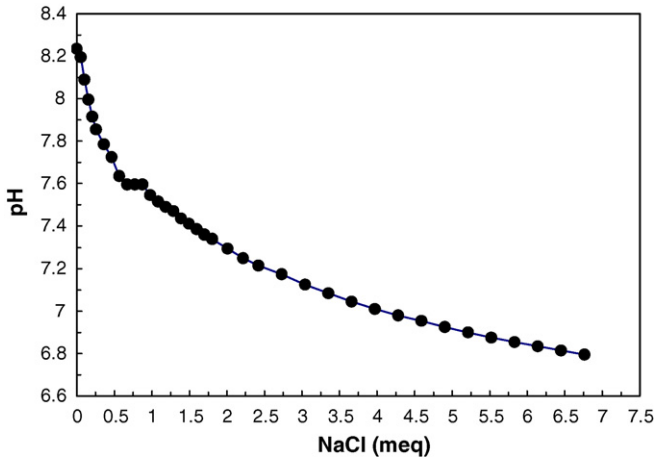

Fig. 2. Titration of ionic pairs in hydrogel C_{934}–AZI.

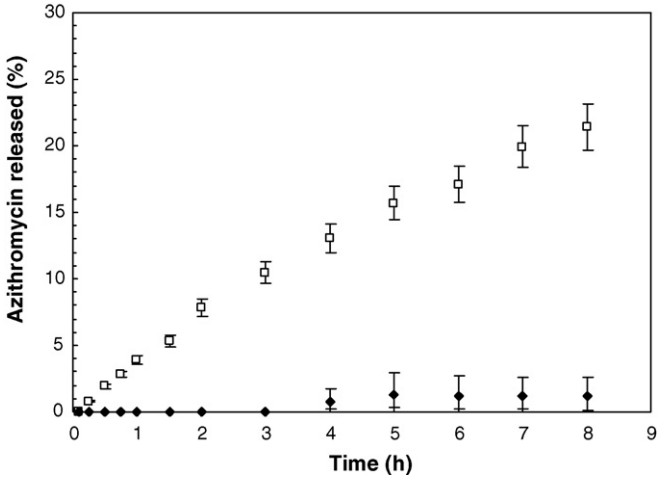

Fig. 3. Release profile of azithromycin from the hydrogel C_{934}–AZI in a Franz cell against water (♦) or NaCl solution (□) as receptor media.

This behaviour reveals that both basic groups of AZI are compromised in the interaction with C. The first step would be associated with the titration of a weaker ionic interaction relative to the second one, which exhibits a higher affinity between AZI and C.

In line with this view two different interactions would be present in this system, one in which both groups of AZI interact with two carboxylic groups of the same chain of C, and other in which AZI acts as a bridge between two different chains generating a cross linking effect.

It was expected that the ionic interaction C–AZI revealed by the titration would prevent the diffusion of AZI from the hydrogel. In fact, when the hydrogel (C_{934}–AZI) was placed in the upper compartment of a Franz cell limited by a semi permeable membrane with the lower compartment loaded with distilled water, a very slow release of AZI was observed. Fig. 3 shows that after 8 h the release of AZI is almost negligible.

Since diffusion of AZI from the hydrogel should occur through the free fraction of molecules in solution, this is a kinetic evidence of the strong degree of counterion condensation. Fig. 3 also shows the delivery of AZI when distilled water in the receptor compartment is replaced by 0.9% NaCl solution. The diffusion of the salt ions to the upper compartment promotes delivery through the ionic exchange mechanism depicted in Eq. (2) and the rate of AZI release is increased about 20 times.

3.2. Stability of azithromycin in C–AZI hydrogels and aqueous solution

To examine the effect of the counterionic condensation on the chemical stability of AZI two analytical methods were used. On one side the HPLC technique was useful to follow the chemical degradation of AZI in aqueous solutions. However, the low sensitivity derived from the poor UV absorption of AZI (quantification limit 12.5 μg/ml) renders the method inadequate to follow AZI degradation in the viscous hydrogels of C. Then, the microbial assay was examined as an alternative method to overcome such shortcoming considering previous reports in which a microbial assay was used for AZI determination in pharmaceutical formulations (Breier et al., 2002; Salgado and Roncari, 2005).

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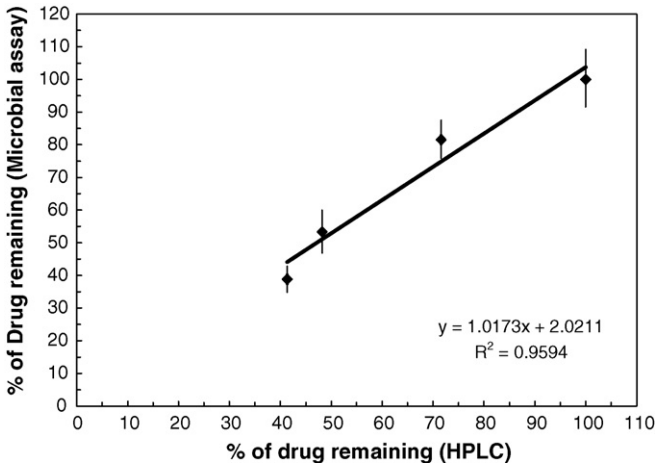

Fig. 4. Correlation of azithromycin stability data obtained by HPLC and Bioassay.

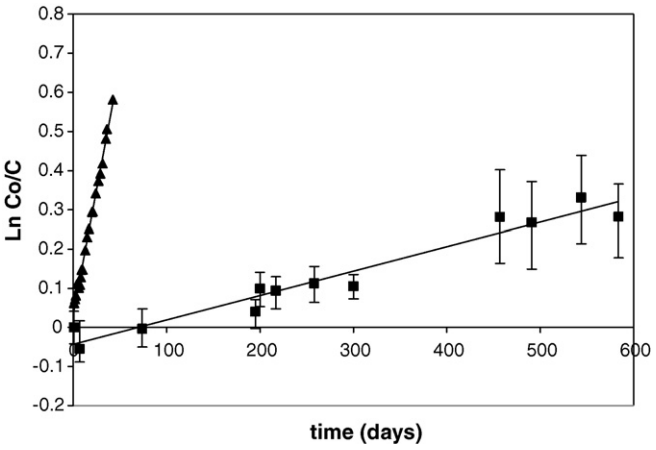

Fig. 6. Stability of azithromycin in hydrogel C_{934}–AZI_{50} (■) and phosphate-buffered solution (▲) as a function of time.

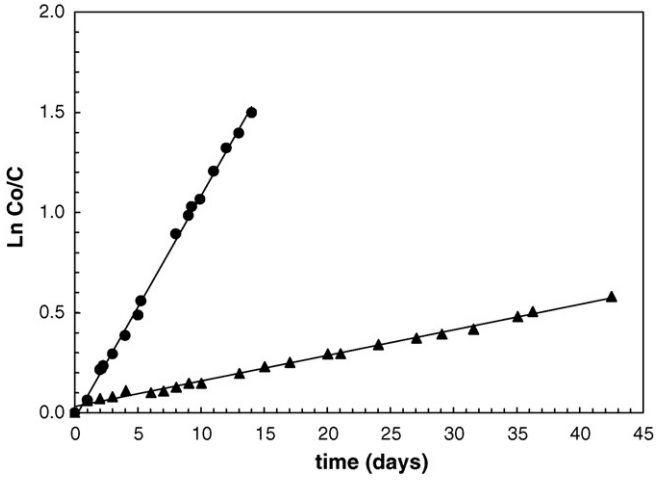

Fig. 5. Stability of azithromycin in phosphate-buffered solution pH 8 at 20 °C (▲) and 40 °C (●) as a function of time.

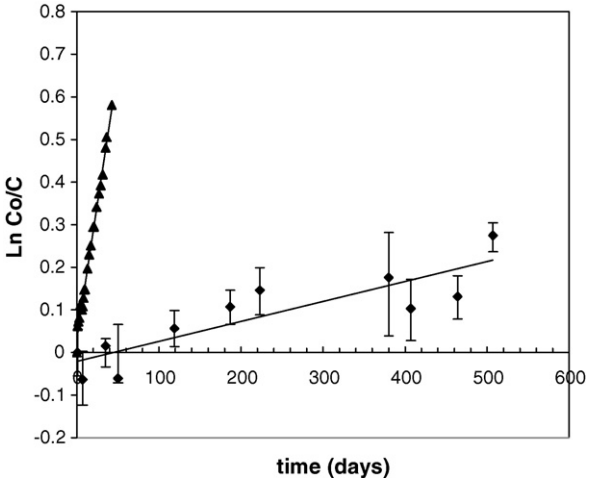

Fig. 7. Stability of azithromycin in hydrogel C_{974}–AZI_{50} (♦) and phosphate-buffered solution (▲) as a function of time.

In order to compare the performance of both methods in determining AZI degradation, concentration (HPLC) and potency (Bioassay) of samples of a progressively degraded aqueous solution was determined. Fig. 4 shows the linear correlation ($R^2 = 0.9594$) arising from the results obtained by HPLC and microbial assay. This correlation is indicative that both methods render potency related results. So, data obtained by the bioassay in the present report is also indicative of chemical stability.

Therefore, although the microbial assay exhibits lower precision than HPLC, the former was selected to follow AZI degradation in C–AZI hydrogels owing to its higher sensitivity.

Fig. 5 shows that AZI degradation in aqueous buffered solution pH 8 at 20 and 40 °C follows a first order kinetics. The decay rate constants (k) and times for 10% (t_{90}) and 50% ($t_{1/2}$) degradation are listed in Table 1. Besides, the activation energy (19.6 kcal/mol) was calculated through Arrhenius equation.

Degradation of AZI in the hydrogels was evaluated along the natural aging at room temperature using the bioassay. No change was observed in the physical appearance of the hydrogels along 18–20 months. The analytical results revealed that the stability of AZI was significantly improved in the hydrogels (C_{974}–AZI_{50} and C_{934}–AZI_{50}) since they retained more than 75% of the initial activity along the period evaluated. Such data were processed according to first-order kinetic yielding rate constants for degradation and times for 10% decay (t_{90}) which are reported in Table 1 and plotted in Figs. 6 and 7 together with the data obtained in aqueous solution

Table 1
First-order decay rate constants, shelf life and half-life values for azithromycin in hydrogels and aqueous solutions pH 8

	pH initial	Rate constant (k, days^{-1})	Regression coefficient (R^2)	$t_{1/10}$ (days)	$t_{(1/2)}$ (days)
C_{974}–AZI_{50}	8.28	4.69×10^{-4}	0.7624	224.73	1477.6[a]
C_{934}–AZI_{50}	8.57	6.236×10^{-4}	0.9474	169.02	1111.3[a]
(I)[b]	8.0	1.2731×10^{-2}	0.9937	8.3	54.4
(II)[c]	8.0	1.111×10^{-1}	0.9984	0.95	6.24

[a] Extrapolated data.
[b] AZI en phosphate buffer pH 8 at 20 °C.
[c] AZI en phosphate buffer pH 8 at 40 °C.

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at 20 °C. The ratio between hydrogels and aqueous solution rate constants indicate an increase of AZI stability as high as 27.1 and 20.4 times for C_{974}–AZI_{50} and C_{934}–AZI_{50}, respectively.

As it was early mentioned, the improvement of the aqueous stability of procaine in a model system carbomer–procaine was attributed to the acid–base interaction between the polyelectrolyte and the basic group of the drug. Such interaction yields a high proportion of ionic pairs in which drug molecules remain associated to the macromolecular phase of higher viscosity and lower kinetic energy than those in the fluid phase (Jiménez Kairuz et al., 2004). Analogously, the improvement on the AZI stability observed in this study would also be related to the same phenomenon.

It was also suggested that in the model system carbomer–procaine, the strong negative electrokinetic potential exhibited by the complex, which provides a microenvironment of lower pH than that of the bulk, would also be a factor to account for the stability improvement observed. Analogously, samples of two hydrogels C–AZI previously diluted 30–35 times exhibited high negative electrokinetic potentials ζ that were in the range −73 to −75 mV.

In summary, the results presented with two aqueous dispersions of carbomer–azithromycin shown a notorious improvement in the shelf life of the drug over the reference buffered solution. Therefore, AZI loaded on the acidic PE Carbomer yields a system with improved stability in which the release of the drug is modulated by the ionic interaction drug–polyelectrolyte.

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