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Assay of amoxicillin sustained release from matrix tablets containing different proportions of Carbopol 971P NF

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Abstract

The sustained release of amoxicillin is desired because of its short biological half-life. Particularly to treat *Helicobacter pylori* infections, the sustained release is desired to be confined to the upper gastrointestinal tract. In vitro dissolution of amoxicillin has been evaluated utilizing a direct UV-absorption method. However, UV-absorption has been reported as not useful to determine amoxicillin in acidic dissolution test medium. To clarify the suitability of the assay method, the stability and dissolution behavior of amoxicillin sustained release tablets was determined by HPLC, iodometric titration and UV-absorption. Stability of amoxicillin studied under dissolution test conditions of pH 1.2, 37 °C and 50 rpm and determined by HPLC and titration showed considerable degradation of amoxicillin. On the other hand, the UV-absorption increased progressively as amoxicillin degradation proceeded. Amoxicillin release curves determined by different analytical methods show different release profiles, which can be corrected for amoxicillin degradation and change in the UV-absorption to produce similar dissolution results. Release curves determined by UV-absorption and obtained from tablets containing 1017 mg amoxicillin trihydrate and Carbopol 971P NF in a range from 180 to 680 mg showed increasing values of the exponent indicative of the release mechanism (n) and decreasing release constant values (k) as the matrix polymer content increased. The release constant (k) and the exponent (n) were found to be logarithmically related.

Keywords: Sustained release; Amoxicillin; Carbopol 971P NF; Analytical methods; Release mechanism

1. Introduction

The convenience of administering a single dose of medication which releases active ingredient over an extended period of time as opposed to the administration of a number of single doses at regular intervals has long been recognized in the pharmaceutical art. However, oral sustained release formulations can be disadvantageous in that certain classes of active ingredients are poorly absorbed during passage through the gastrointestinal tract due to their physicochemical properties and/or favorable sites of absorption. In view of these considerations, some medicaments are not candidates for conventional sustained release formulations if they are not retained in a given part of the gastrointestinal tract, for instance the stomach (Sheth and Tossounian, 1979). Peroral dosage forms for gastric retention are particularly valuables for drugs that exhibit an absorption window in the upper part of the small intestine, dissolve better in the acidic environment of the stomach or exhibit local treatment of the stomach or pylorus (Zhenping et al., 2001).

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In the case of diseases involving peptic ulcers, it has been demonstrated that *Helicobacter pylori* is one of the major causative agents (Risbud et al., 2000). It is claimed that for gastric ulcer patients amoxicillin is ineffective even at high doses, apparently due to limited contact time with the target site when administered in a conventional oral dosage form (Acuña et al., 2001). The failure of amoxicillin has been proposed to be an outcome of sub-therapeutic bactericidal concentrations available at the site and instability of amoxicillin following oral administration (Risbud et al., 2000).

Some attempts have been made to develop a sustained release dosage form for amoxicillin to localize the antibiotic delivery in the acidic environment of the stomach (Patel and Amiji, 1996; Katzhendler et al., 1997; Hilton and Deasy, 1992, 1993; Whitehead et al., 2000). In several previously reported studies an aqueous acidic medium (pH 1.2) was used to evaluate the release profile. Amoxicillin has been determined by the ultraviolet absorption method, at or around 272 nm (Risbud et al., 2000; Patel and Amiji, 1996; Hilton and Deasy, 1992; Whitehead et al., 2000). On the other hand, Tokumura and Machida (2001) reported that according to their results, the UV-absorption method should not be used for amoxicillin determination under acidic conditions. Amoxicillin solutions were unstable at pH 1, with a half-life of 7.04 h.

The dissolution data will also be different although some other analytical method such as iodometric titration (Tsuji et al., 1978) and HPLC (Kimura et al., 1979; Hilton and Deasy, 1993) can be used selectively to determine intact amoxicillin molecules, because of degradation during the in vitro dissolution studies.

To examine the dissolution behavior of amoxicillin from a sustained release dosage form under acidic conditions, it is necessary to know the degradation rate of amoxicillin. In this way, dissolution data can be recalculated to obtain the actual dissolution profile of amoxicillin.

This investigation describes the use of different analytical methods to determine the amoxicillin degradation rate under conditions of a dissolution test and its application to correct amoxicillin release profiles. Moreover, a selected analytical method will be used to determine the effect of the Carbopol 971P NF proportion on the release profile of amoxicillin matrix tablets.

2. Materials and methods

2.1. Materials

The pharmaceutical excipient Carbopol 971P NF, a brand of a synthetic high molecular weight polymer of acrylic acid from B. F. Goodrich Co. obtained from Multiquim-México and Amoxicillin trihydrate obtained from FERSINSA-Mexico with a potency of 86%, were used as received.

2.2. Methods

2.2.1. Matrix preparation

Matrix tablets were produced with increasing polymer content and a fixed quantity of amoxicillin, 875 mg, equivalent to 1017 mg of the trihydrate. The polymer proportions corresponded to 15, 20, 25, 30, 35 and 40% of the tablet matrix weight. Fifty grams of the powders corresponding to each formulation were tumble mixed for 20 min. The powder mixtures were moistened in a mortar, kneading with a spatula. The agglomerates were dried at 50 °C for 2 h and size reduced in a mortar. The weight of granules corresponding to each tablet was compressed in a hydraulic press at 28 MPa during 10 s, with punches and die producing 7 mm width and 21 mm length capsule shaped tablets. No lubricant was used in the tablets.

2.2.2. Determination of the amoxicillin degradation rates

The studies for amoxicillin transformation were carried out under the same conditions as those of a dissolution test, utilizing buffer solution pH 1.2 (900 ml) as the dissolution medium. Degradation was initiated by the addition of a known weight of amoxicillin (450 mg) to the medium, which was preheated at 37 °C, to make a final concentration 0.5 mg/ml. The stirring rate was fixed at 50 rpm. Samples (2 ml) were taken at appropriate time intervals and assayed spectrophotometrically at 272 nm after dilution to 10 ml with buffer solution pH 1.2. The residual amoxicillin concentration was also determined by iodometric titration (Pharmacopoeia of the Mexican United States, 2000) and by the HPLC method, injecting two samples of 20 μl, after calibration with a known standard. The HPLC system consisted of a reversed phase with a C-18 column at a flow rate of 1 ml/min.

Amoxicillin was detected at a wavelength of 230 nm. The mobile phase used for the determination of amoxicillin was phosphate buffer pH 4.4/methanol (95/5); the retention time was about 7 min. The stability of amoxicillin solution at pH 7.4 was determined following the same procedure as a reference for the case when the tablets cannot be retained in the stomach. For stability studies at room temperature (22 °C) the procedure was the same but the amoxicillin solution (100 ml) was prepared at a concentration of 1.0 mg/ml and stirred with a magnetic bar.

2.2.3. Drug release

Dissolution studies were performed in triplicate using the USP apparatus 2 at 50 rpm. The medium was 900 ml buffer solution pH 1.2 which was maintained at 37 °C. Samples were taken at appropriate time intervals and assayed. Matrix tablets containing 60% amoxicillin and 40% Carbopol 971P NF were assayed by titration, HPLC and spectrophotometrically at 272 nm. All other formulations were assayed only spectrophotometrically at 272 nm.

3. Results and discussion

3.1. Stability of amoxicillin in aqueous solution

The degradation of amoxicillin at low concentrations has been reported to follow pseudo-first-order kinetics (Tsuji et al., 1978). Moreover, the cleavage of amoxicillin has been found to be subject of general acid–base catalysis. The stability of amoxicillin has been reported to show a U-shaped relationship between the rate constant and the pH of the medium, reaching a minimum at pH-values between 5 and 7 (Tsuji et al., 1978).

Fig. 1 shows the degradation profile of amoxicillin aqueous solution determined by HPLC and an iodometric titration. The figure also shows an increase of the UV-absorption with time, expressed as an increase in percentage of concentration calculated against the amoxicillin standard curve. The degradation conditions correspond with those being used for dissolution studies of amoxicillin sustained release tablets. The half-life obtained by HPLC and iodometric titration is 6.1 and 5.5 h, respectively which are in the range observed by Tokumura and Machida (2001), 7.04 h and by Erah et al. (1997), 5.2 h. The loss of 50% of the drug corresponds with an increase in UV-absorbance of about 27%. Table 1 shows the regression parameters of amoxicillin degradation under acidic conditions, at 22 and 37 °C, as well as those for the degradation at pH 7.4 and 37 °C. The results are similar for both iodometric titration and HPLC. The amoxicillin half-life at pH 7.4 determined by iodometric titration and HPLC is 38.3 and 37.1 h, respectively. Based on the degradation rate of amoxicillin it is necessary to correct the dissolution data to obtain the real dissolution profile of a given dosage form, particularly those showing sustained release.

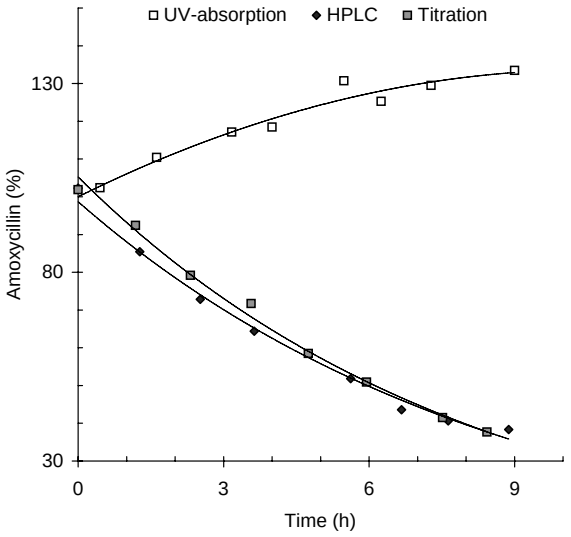

Fig. 1. Alteration of the $UV_{272\,nm}$ absorption, converted to concentration, and stability measured by HPLC and titration of amoxicillin in aqueous solution of pH 1.2 at 37 °C. Experimental points and calculated regressions.

3.2. Dissolution of amoxicillin from Carbopol 971P NF sustained release tablets

According to the above-mentioned results, dissolution of amoxicillin at pH 1.2 and determined by UV-absorption will display higher values than those corresponding to dissolution without degradation of the drug. On the other hand, if the dissolution process is determined by iodometric titration or HPLC the values obtained will display an opposite trend, lower values than the real dissolution data from a non-degrading drug.

Table 1
Regression equations for amoxicillin degradation profiles in aqueous solution at pH 1.2 and 7.4, temperature of 22 and 37 °C and stirring rate of 50 rpm (paddle), utilizing UV-absorption, iodometric titration and HPLC as analytical methods

Method; conditions	Equation	r^2	$t_{50\%}$
UV; 37 °C, pH 1.2	$y = -0.3017x^2 + 6.3734x + 99.99$	0.953	
Titration; 37 °C, pH 1.2	$y = 105.24\,e^{-0.1216x}$	0.996	5.70
HPLC; 37 °C, pH 1.2	$y = 98.697\,e^{-0.1141x}$	0.989	6.07
UV; 37 °C, pH 7.4	$y = -0.1008x^2 + 2.7647x + 102$	0.964	
Titration; 37 °C, pH 7.4	$y = 99.473\,e^{-0.0181x}$	0.991	38.30
HPLC; 37 °C, pH 7.4	$y = 99.834\,e^{-0.0187x}$	0.953	37.07
Titration; 22 °C, pH 1.2	$y = 98.744\,e^{-0.0424x}$	0.977	16.35
HPLC; 22 °C, pH 1.2	$y = 96.58\,e^{-0.0447x}$	0.961	15.51

After application of the corresponding degradation equation (Table 1), the real dissolution data can be calculated. The amoxicillin quantity obtained with the first sample will increase or decrease with time, according to the method employed for its determination. The second sample corresponds with the total amoxicillin dissolved plus/minus the change due to its degradation. The change produced in the amoxicillin dissolved after degradation, in the time from the first to the second sample can be added or subtracted from the quantity obtained in the second sample; the result equals the amoxicillin dissolved after the first sample. If this procedure is repeated for each time interval, adding or subtracting the change calculated for amoxicillin degradation, the cumulative dissolution curve can be obtained. To avoid miscalculations, it is better to calculate the regression curve of the experimental data to standardize the times and intervals to obtain the corrected dissolution data.

Experimental data were fitted to the power law expression shown in Eq. (1) to examine the kinetics and mechanism of drug release of each formulation (Mandal, 1995; Vigoreaux and Ghaly, 1994):

$$\frac{M_t}{M_{\text{inf}}} = kt^n \quad \text{or} \quad \ln\left(\frac{M_t}{M_{\text{inf}}}\right) = n\ln(t) + \ln(k) \qquad (1)$$

The terms in this equation are as follows: M_t, the amount of drug released at time t; M_{inf}, the total drug released over a long time period; k, the kinetics constant; and n, the mechanism of drug release. The value of n ranges from 0.5 ($t^{1/2}$ dependence, generally referred to as Fickian release) to 1 (representing the case-II transport which is purely relaxation controlled). The values in between indicate an anomalous behavior corresponding to coupled diffusion/relaxation. When the value of n is greater than that of the case-II transport ($n > 1.0$), the release is said to be Super case-II transport (Brazel and Peppas, 2000; Ranga Rao et al., 1988). In the case of a matrix with cylinder form, n is said to be 0.45 instead of 0.5 and 0.89 instead of 1.0 (Kim and Fassihi, 1997).

Fig. 2 shows the corrected dissolution profile of amoxicillin/Carbopol 971P NF (60/40) matrix tablets. The cumulative dissolution curves were calculated correcting the experimental data for the changes produced by amoxicillin degradation. Each curve

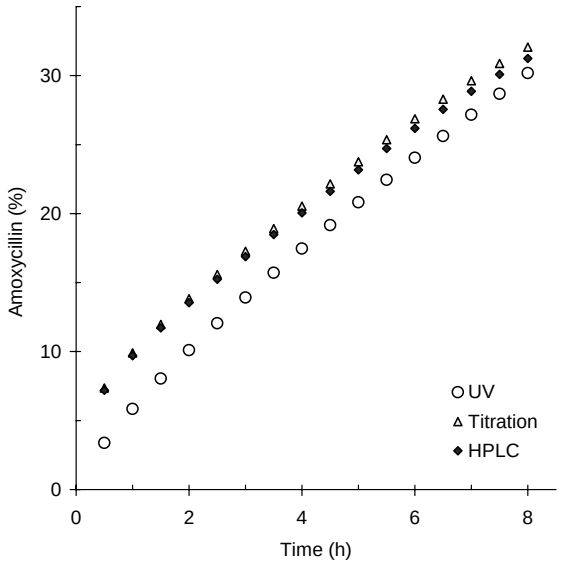

Fig. 2. Calculated dissolution profile of amoxicillin/Carbopol 971P NF (60/40) matrix tablets from data corrected for amoxicillin degradation, determined by different analytical methods.

corresponds with data corrected by each different amoxicillin degradation equation obtained from iodometric titration and HPLC data as well as from the equation describing the UV-absorption change with time.

The amoxicillin dissolution profile corrected with the degradation equation obtained from HPLC data is practically the same as that obtained with the degradation equation obtained from iodometric titration data. The dissolution profile corrected with the degradation equation obtained from UV-absorption data is something different. This is attributed to a greater scattering of experimental data obtained by UV-absorption and to a lesser correlation of the analytical response (Table 1). However and from a practical point of view, the amoxicillin dissolution curve corrected with UV-absorption data is comparable to the other two methods. The UV-absorption can be used to determine the effect of different formulation variables on the release behavior of amoxicillin sustained release tablets.

The effect of amoxicillin degradation on its dissolution profile is variable and dependent on the amoxicillin release rate. The lower release rate of tablets obtained with a higher polymer content produce less important differences between the experimental and the corrected curves. A smaller quantity of dissolved amoxicillin allows less degradation and lesser changes in the release profile. This can be seen in Fig. 3.

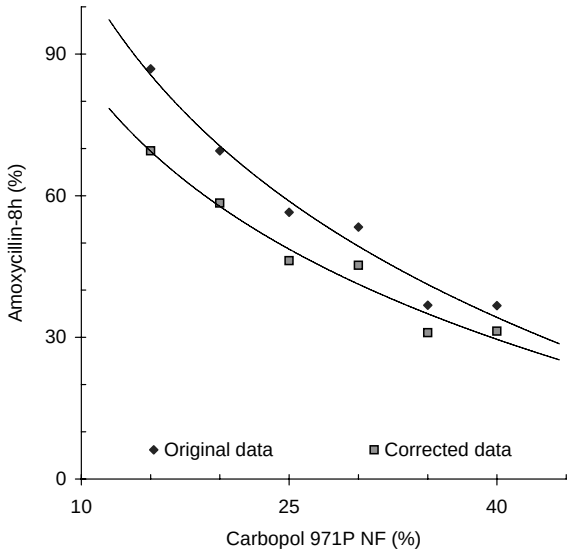

Fig. 3. Effect of polymer content on the amoxicillin released after 8 h from Carbopol 971P NF matrix tablets, before and after correction for amoxicillin degradation, determined by UV.

3.3. Effect of polymer content on the amoxicillin release profile from matrix tablets

As by other hydrophilic matrices, increasing polymer content decreases the amoxicillin release rate. Fig. 4 shows that increasing proportions of Carbopol 971P NF reduce in a logarithmical relationship the amoxicillin release constant. The reduction of the release constant produced by increasing proportions of Carbopol 971P NF increase the time needed to release a given quantity of amoxicillin, allowing a greater hydration and relaxation of the polymer matrix before this quantity of drug is released. The increased hydration and relaxation of the polymer matrix shift the release mechanism toward relaxation-erosion. This can be seen in Fig. 5. Increasing proportions of the polymer in the matrix tablet increase the value of the exponent *n*. Polymer proportions of 30–40% produce matrices in the vicinity of an apparent zero-order release. The effect of reducing the release rate on the mechanism controlling the dissolution process can be seen in Fig. 6. There is a logarithmic relationship

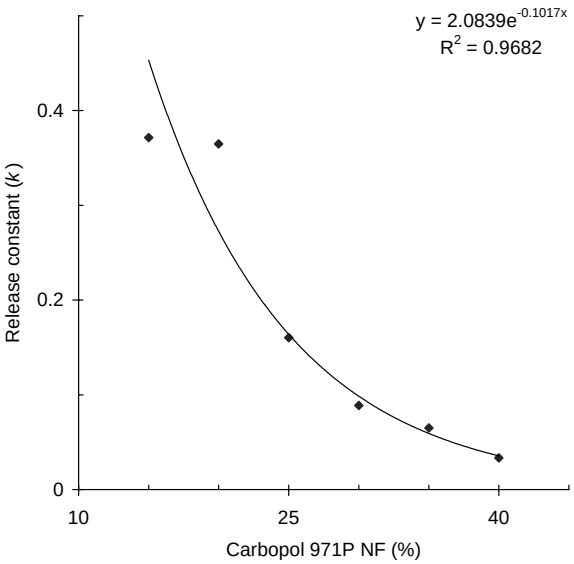

Fig. 4. Effect of polymer content on the release constant (*k*) of amoxicillin from Carbopol 971P NF matrix tablets. UV-experimental data corrected for amoxicillin degradation.

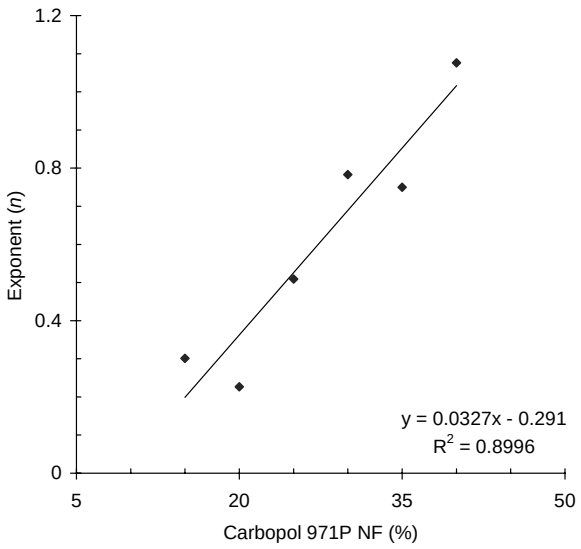

Fig. 5. Effect of polymer content on the exponent indicative of the release mechanism (n) of amoxicillin dissolution curves from Carbopol 971P NF matrix tablets. Calculated from UV-data corrected for amoxicillin degradation.

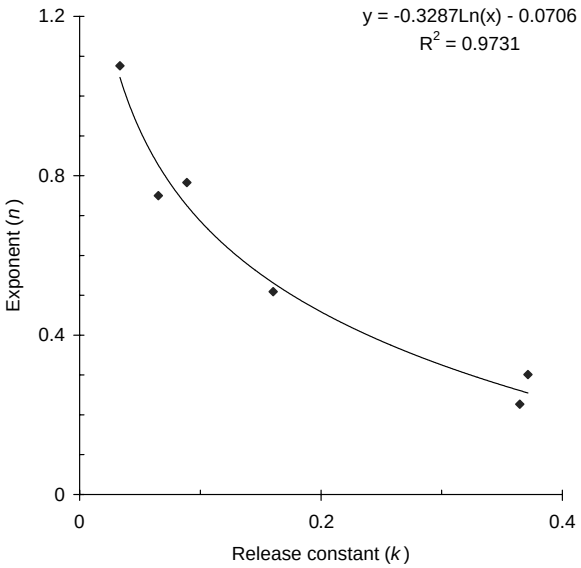

Fig. 6. Relationship between the release constant (k) and the exponent indicative of the release mechanism (n) of amoxicillin dissolution curves from Carbopol 971P NF matrix tablets.

between decreasing release constant values and increasing exponent n values.

4. Conclusion

The instability of amoxicillin aqueous solutions, under tablet dissolution conditions, makes it necessary to adjust the dissolution data to get a real tablet dissolution profile. This is particularly necessary by the dissolution test of amoxicillin tablets showing sustained release. The adjustment can be made applying equations that describe the degradation kinetics of the drug. Drug-specific analytical methods such as iodometric titration and HPLC produce the same results for degradation kinetics. Although the increase of UV-absorption is not a specific method to follow amoxicillin degradation, it is useful to correct dissolution data, producing similar dissolution profiles as the above-mentioned specific analytical methods. The experimental and the corrected dissolution data show increasing differences as the amoxicillin release rate increases.

Release curves obtained from tablets containing 1017 mg of the tridydrate, equivalent to 875 mg of amoxicillin and Carbopol 971P NF in a range from 180 to 680 mg show increasing values of the exponent indicative of the release mechanism (n) and decreasing release constant values (k) as the matrix polymer content increases. Decreasing release constant values (k) are logarithmically related to increasing values of the exponent indicative of the release mechanism (n). Tablets with Carbopol 971P NF proportions of 30–40% produce matrices in the vicinity of an apparent zero-order release.

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