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Modification of fluorescent properties of norfloxacin in the presence of certain antacids

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Abstract

Modification of the fluorescent properties of norfloxacin samples in the presence of different antacids in terms of dissolution rates has been reported in a previous paper. The formation of chelates with Al^{3+} and Mg^{2+} ions has been previously suggested as a mechanism of interaction. In the present paper, the chelation was studied with different types and amounts of antacids and the stability of the non-absorbable chelates with each antacid was studied. Six dose fractions of each antacid were used in samples with the same norfloxacin concentration (9 μg ml^{-1}). All samples were measured using both UV/Vis-spectrophotometry and spectrofluorimetry, and compared to a standard solution of norfloxacin (10 μg ml^{-1}) without antacids, used as a reference in the calibration of the spectrofluorimeter. The results showed that the fluorescence signal features remarkable differences depending on the kind and the concentration of antacid, as well as on the time of contact. It was found that increasing amounts of antacids increased the fluorescence signal of norfloxacin samples. The evolution of the fluorescence signal in function of the antacid concentration showed a maximum and a posterior decrease. It was observed that, for a higher concentration of antacid in the medium, a higher signal was obtained and lower stability of the compound norfloxacin-antacid was observed. The data obtained strongly indicated that the binding of Al^{3+} and Mg^{2+} ions to the carboxylic groups of norfloxacin produced non-absorbable chelates. This effect might reduce the drug bioavailability.

Keywords: Norfloxacin; Non-systemic antacids; Chelate; Stability; Fluorescence; Ion-complexation

1. Introduction

The fluorquinolones in general constitute a group of synthetic antibiotics, which are widely used as broad-spectrum antibacterial agents due to their ability of inhibition of bacterial DNA-gyrase. Norfloxacin in particular is used in the treatment of complicated urinary tract infections because of the good penetration of the infected sites that this drug exhibits. Especially potent antibacterial activity has been found against *Pseu-*

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domonas aeruginosa and *Serratia* sp. [1,2]. It is known that about 30% of the oral dose is excreted unchanged in the urine within 24 h, thus producing high urinary concentrations [3].

The chemical formula of this drug is 1-ethyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid. It is known that certain antacid preparations containing aluminum and magnesium salts may reduce significantly oral absorption and bioavailability of norfloxacin. This phenomenon was previously observed for other drugs [4] and this interaction with quinolones in general and norfloxacin in particular is well documented [5–8].

Chelation between some metal ions such as Al^{3+}, Mg^{2+}, Fe^{3+} and Ca^{2+}, included in a variety of pharmaceuticals, and the 3-carboxyl and 4-oxo substituents on the quinolone nucleus resulting in a complex that is more polar and unable to be absorbed, has been suggested by many authors as the possible mechanism responsible for the reduced absorption of norfloxacin [9–11]. This effect was also studied from a physicochemical point of view in terms of modification of solubility and partition coefficients using different solvents [12].

The formation of a complex between norfloxacin and different ions results in a substantial increase in fluorescence intensity. This phenomenon has been used for elucidating the stoichiometry of metal-ion complexes and the influence of pH on the association constants [13]. Besides, some recent examples of utilisation of the chelation of norfloxacin with metal ions in order to develop analytical methods for norfloxacin in different matrices using the complex as a fluorophore were reported [3,14,15].

The modification of the fluorescent properties of norfloxacin in the presence of antacids in terms of dissolution rates of directly compressible norfloxacin tablets was previously reported [16]. In this study the fluorescent properties of norfloxacin in the presence of different dose fractions of four commercially available and widely used antacid preparations and the influence of the kind of antacid on the stability of the non-absorbable chelates are described.

2. Materials and methods

2.1. Chemicals and reagents

Norfloxacin was purchased from Copanor (Copanor S.A. Madrid) and all the reagents used were of analytical grade. Water was distilled and deionized (Milli-Q, Millipore). Four widely used antacid preparations in tablets containing Al and Mg derivatives and other salts were used in our study. These preparations are described in Table 1.

2.2. Apparatus

All pH measurements were made using a previously calibrated Crison 2002 pH-meter. Fluorescent measurements were performed using a Perkin Elmer-204 Spectrofluorimeter and ultraviolet absorbance a Beckman DU-6 spectrophotometer respectively. Both spectrophotometric and spectro fluorimetric methods were previously validated [16]. Briefly, the drug concentration range used for both methods was 0–10 μg ml^{-1} in 0.1 N hydrochloric acid and simulated gastric fluid (USP-23) [17] with a pH value of about 1.2. For all spectrophotometric assays, a $\lambda_{max} = 276$ nm was used. For the spectrofluorimetric measurements the $\lambda_{ex.} = 330$ nm and $\lambda_{em.} = 445$ nm were selected, calibrating previously the 100% fluorescence value by using a 10 μg ml^{-1} norfloxacin standard solution. All λ_{maxima} were checked in the presence of the four antacids and no modifications in the spectra were found.

2.3. Interaction studies

The binding of metal ions to norfloxacin was studied spectrofluorimetrically at ambient temperature (22 ± 1°C). Six concentrations (dose fractions) of each antacid were used in samples: 1/4, 1/2, 3/4, 1, 5/4 and 6/4 (solutions 1, 2, 3, 4, 5 and 6) using as unitary value the average weight obtained for 20 tablets and showed in Table 1. For each measurement 10 tablets were powdered and a sieve fraction below 100 μm screen was used. In all cases a norfloxacin concentration of 9.0 μg ml^{-1} was used.

Table 1
Detailed composition of the antacid tablets used, and the average weight values (using 20 tablets) used as a 1 dose-fraction

Trade Name	Code	Composition of each tablet	Average weight
Almax® (Almirall)	A	Almagate (INN)[a] ... 0.500 g Saccharin calcium ... 0.003 g Other excipients	1.218 g
Maalox® (Rhône Poulenc-Rorer)	M	$Al(OH)_3$ ... 0.600 g $Mg(OH)_2$ ... 0.300 g Saccharose ... 0.050 g Other excipients	1.781 g
Bemolan® (Boehringer Mannheim)	B	Magaldrate (INN)[b] ... 0.400 g Other excipients	1.103 g
Aligest Plus® (Schering-Plough)	L	$Al(OH)_3$ ... 0.298 g $Mg(OH)_2$ ... 0.328 g $CaCO_3$ ... 0.410 g Simethicone ... 0.025 g Saccharin sodium ... 0.005 g Aspartame ... 0.010 g Other excipients	1.623 g

[a] Almagate (INN): $[Al_2Mg_6(OH)_{14}(CO_3)_2\,4H_2O]$.
[b] Magaldrate (INN): $[Al_5Mg_{10}\,(OH)_{31}(SO_4)_2 \cdot x\,H_2O]$.

Table 2
pH values obtained using different dose-fraction of the antacid preparation (A, M, B and L) in simulated gastric fluid (mean ± SD)

Solutions	Dose fraction	A	M	B	L
0	0	1.10 ± 0.10	1.10 ± 0.10	1.10 ± 0.10	1.10 ± 0.10
1	1/4	1.29 ± 0.10	1.50 ± 0.14	1.12 ± 0.08	2.06 ± 0.12
2	1/2	1.78 ± 0.15	2.98 ± 0.12	1.34 ± 0.11	4.98 ± 0.21
3	3/4	4.36 ± 0.11	4.14 ± 0.13	2.13 ± 0.08	6.56 ± 0.35
4	1	4.55 ± 0.11	5.03 ± 0.28	3.96 ± 0.15	6.68 ± 0.28
5	5/4	4.89 ± 0.20	6.16 ± 0.25	4.20 ± 0.14	7.51 ± 0.32
6	6/4	4.92 ± 0.05	6.96 ± 0.16	4.32 ± 0.16	7.62 ± 0.12

A previously determined amount of antacid was added to 100 ml simulated gastric fluid and stirred at 1100 rpm during 20 min. The pH value of the resulting previously filtered solution was determined. 45 mg accurately weighed norfloxacin was added to the solution and treated in an ultrasonic bath during 10 min in order to obtain a final concentration of 450 μg ml^{-1} norfloxacin. 1 ml of this solution was transferred to a 50 ml volumetric flask and diluted having a final concentration of 9.0 μg ml^{-1}. There was no influence of filtration and of the norfloxacin addition on the pH value.

The evolution of the fluorescence signal (F) of the solutions was studied kinetically assuming a first-order reaction and the rate constant (k) calculated by using the following equation were F_0 is the fluorescence signal of the freshly prepared solution (time, $t = 0$).

$$\ln F = \ln F_0 + kt$$

3. Results and discussion

It was observed that the acid neutralizing capacity was different for each antacid preparation. The average pH data ($n = 3$) are summarised in Table 2. As can be seen, the pH value was lower than 5.0 in those cases where antacids consisted of Al and Mg in a complex structure as in almagate

Table 3
Mean values of UV-absorbance and fluorescence for freshly prepared norfloxacin solutions with different dose-fractions of the antacid preparation A, and mean percent recoveries obtained using both analytical methods

Solution number	Absorbance (AU)	Recoveries (%)	Fluorescence (%)	Recoveries (%)
0	1.171	99.9	95.5	100.1
1	1.126	96.2	215.0	237.9
2	1.127	96.3	230.0	255.3
3	1.186	101.2	490.0	555.3
4	1.230	104.9	500.0	566.9
5	1.172	100.1	470.0	532.3
6	1.124	96.1	320.0	359.1

and magaldrate (preparations A and B), whereas the pH values were higher than 5.0 even with only one dose for those preparations in which Al and Mg were available as $Al(OH)_3$ and $Mg(OH)_2$ (preparations M and L).

All norfloxacin solutions were assayed using a spectrophotometric and a spectrofluorimetric method. The UV-absorbance and fluorescence data of freshly prepared solutions with a constant norfloxacin concentration (9 μg ml^{-1}) and with increasing antacid A concentrations as well as the theoretical resulting percent recoveries are given in Table 3. No influence in the ultraviolet absorbance of the norfloxacin solutions in the presence of growing concentrations of the antacid preparation A was seen but a dramatic increase of the fluorescence signal was observed, leading to the obtaining of percent recoveries over 550% in some cases. These results strongly suggested that norfloxacin formed a chelate with Al^{3+} and Mg^{2+}. This association was not detectable using UV-spectrophotometry but it was observed that these ions could work as a fluorophore, increasing significantly the fluorescent signal, in simulated gastric fluid. This phenomenon was observed with all antacid preparations used in this study. All solutions were assayed by both analytical methods but for simplicity, later in the text and plots, only the fluorescence data are reported due to the fact that no significant modifications in the UV-absorbance were found in the presence of antacids and over a long time.

Table 4
Evolution of the fluorescent signal (F) of norfloxacin solutions with increasing the antacid A concentration and along time in days (mean values)

Time	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)
0	215	230	490	500	470	320
1	170	200	250	260	255	210
2	140	175	200	200	175	165
3	103	170	175	170	165	155
6	104	160	175	170	160	150
13	100	108	130	125	105	100

Table 5
Evolution of the fluorescent signal (F) of norfloxacin solutions with increasing the antacid M concentration and along time in days (mean values)

Time	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)
0	240	460	460	250	210	100
1	190	255	250	180	108	94.5
2	170	200	150	110	98	96
5	150	160	99	97	93	95
9	140	160	97	95	92	94

Table 6
Evolution of the fluorescent signal (F) of norfloxacin solutions with increasing the antacid B concentration and along time in days (mean values)

Time	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)
0	102	280	320	445	455	485
1	102	260	320	350	350	360
2	101	210	230	230	220	220
5	100	210	210	210	200	195
9	101	210	210	210	200	195

Table 7
Evolution of the fluorescent signal (F) of norfloxacin solutions with increasing the antacid L concentration and along time in days (mean values)

Time	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)
0	230	200	94	94	96	93
5	145	93	94	93.5	96	92
8	108	94	93	94	95.5	93
12	110	92.5	94	92.5	95.5	93
15	107	93	93	90	94	92

Tables 4–7 show the mean fluorescence signal obtained using the four antacids previously described versus time. The evolution of the fluorescence versus antacid concentration and versus time is also shown in three-dimensional plots (Figs. 1–4). Increasing progressively the concentration of the antacid preparation increased the fluorescence signal of the norfloxacin solutions up to a maximum, then the signal decreased. This effect could be influenced by the lack of linearity of the fluorescence signal at high concentrations and due to the pH value of the solutions. It was also found that the increase of the fluorescence signal, due to the formation of the norfloxacin—Al^{3+} and Mg^{2+} complexes, was different with each antacid preparation. In this way, using antacids in which Al^{3+} and Mg^{2+} ions were included in a complex structure like A and B, higher fluorescence values were observed. These results strongly suggested that the delivering ability of Al^{3+} and Mg^{2+} to the medium was higher. Besides, the influence of the pH on the stability of the chelate should be also taken into account Studying the evolution of the fluorescence signal along time (Z-axis of Figs. 1–4), a dramatic decrease of the response was seen, leading to a decrease in the mean percent recoveries to reach values near 100% in some cases. Kinetic analysis of the evolution of fluorescence data using a first-order reaction rate equation allowed the calculation of the rate constant (k). These values are shown in Table 8 and the graphic evolution of k versus number of solution and antacid concentration was plotted in Fig. 5.

As can be seen, for a higher antacid concentration in the medium, a lower stability of the norfloxacin-metal ion complex was observed. Besides, the stability of the complex detected by fluorimetry was also found to be influenced by the kind of antacid. The complex concentration detected was significantly lower when the L

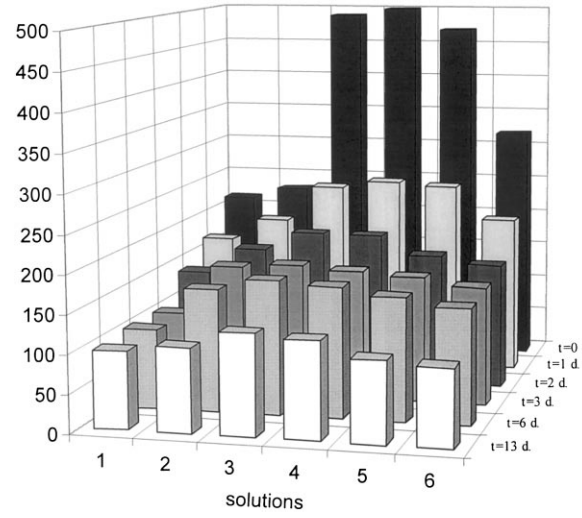

Fig. 1. Plot showing the graphic evolution of fluorescence signal of norfloxacin vs. time and vs. concentration of the antacid preparation A.

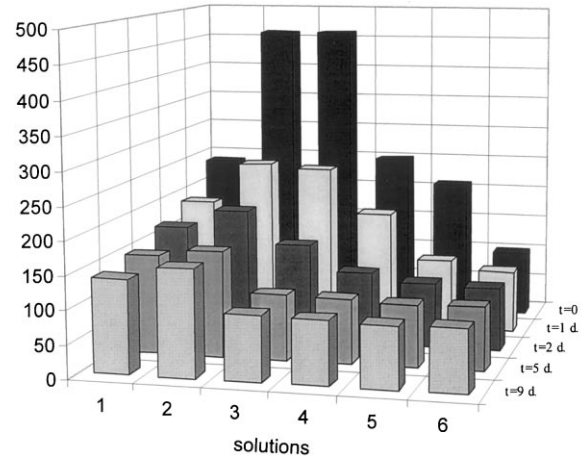

Fig. 2. Plot showing the graphic evolution of fluorescence signal of norfloxacin vs. time and vs. concentration of the antacid preparation M.

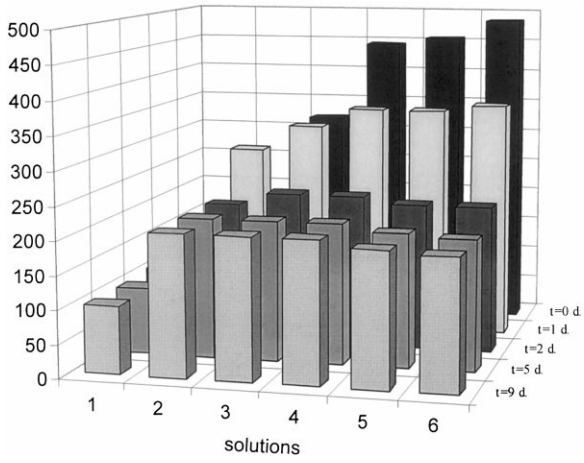

Fig. 3. Plot showing the graphic evolution of fluorescence signal of norfloxacin vs. time and vs. concentration of the antacid preparation B.

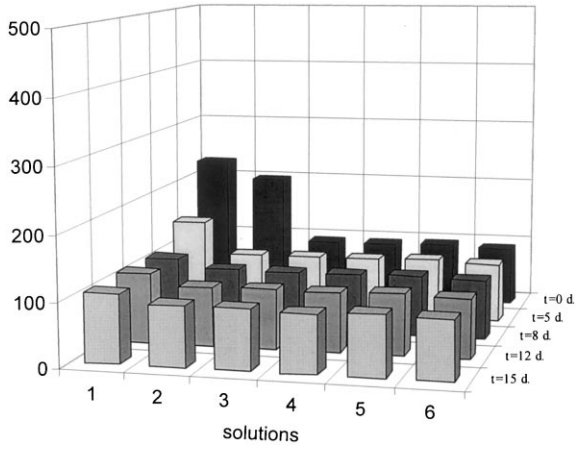

Fig. 4. Plot showing the graphic evolution of fluorescence signal of norfloxacin vs. time and vs. concentration of the antacid preparation L.

antacid formulation was used. It is known that a clear influence of pH on the complexation exists due to the ability of norfloxacin to change its structure to a zwitterionic form [13]. In the present study, an increase in the constant k, which leads to a decrease in the stability of the complex, was found in a range of pH between 2.5 and 5.0, but a clear relationship between pH values and stability of the complex was not found. These

Table 8
Calculated k-values for the different solutions with each antacid preparation

Solution	Antacid preparation			
	A	M	B	L
1	0.0484	0.0513	0.0015	0.0502
2	0.0519	0.0940	0.0240	0.0625
3	0.0726	0.1530	0.0480	0.0012
4	0.0776	0.1339	0.0730	0.0026
5	0.0864	0.1270	0.0801	0.0012
6	0.0701	0.0256	0.0891	0.0009

results strongly suggested that the kind of antacid preparation also affected the interaction.

The results obtained in the present work indicated that norfloxacin was able to form non-absorbable chelates with the Al and Mg ions present in the commercially available antacid preparations studied. This could lead to a dramatic decrease in the oral absorption of the drug. It was also found that the binding of norfloxacin to Al and Mg varied with pH and that the formation of these chelates was higher when the Al^{3+} and Mg^{2+} ions were included in a complex structure like almagate or magaldrate. Nevertheless, these results should be confirmed in vivo.

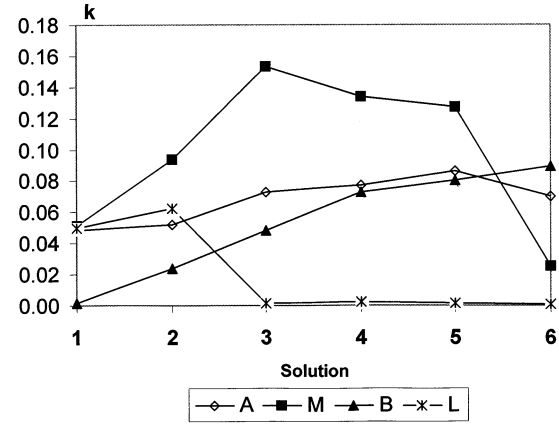

Fig. 5. Comparative evolution of the k values increasing the concentration of antacid in the medium (solutions 1–6), with the four antacid preparation studied.

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