^{1}H-NMR Study of Famotidine and Nizatidine Complexes with β-cyclodextrin

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The aim of this study was to obtain and to characterize some inclusion complexes of famotidine and nizatidine with β-cyclodextrin (β-CD) in solution. The formation of famotidine- and nizatidine - β-CD complexes were evaluated by means of ^{1}H-NMR spectroscopy. Thereafter, the stoichiometry and association constants of the complexes obtained were calculated via a continuous variation method by using the chemical shifts of specific protons from both host and guest molecules. The association constants calculated are 179.6 M^{-1} for famotidine - β-cyclodextrin complex, and 74.9 M^{-1} for nizatidine - β-cyclodextrin complex, at 295 K. Due to their better stability, these complexes could be use as oral pharmaceutical preparations with better taste compared with that of free drugs.

Keywords: ^{1}H-NMR spectroscopy, β-cyclodextrin, famotidine, nizatidine

Famotidine [(N' -(Aminosulfonyl)-3-(((2-((diamino-methylene)amino)-4thiazolyl) methyl) thio) propanimid-amide] (M=337.43), and nizatidine [N-(2-(((2-((dimethylamino)methyl)-4-thiazolyl) methyl)thio)ethyl)-N'-methyl-2-nitro-1,1-ethenediamine], (M=331.45), belong to the class of competitive antagonists of the H2-histamine receptors and are widely used medications in the treatment of peptic ulcer, gastroesophageal reflux and of other disorders in which gastric hyperacidity occurs, such as Zollinger-Ellison syndrome [1].

Famotidine is very slightly soluble in water and has a bitter taste, and nizatidine has a bitter taste and a weak smell of sulfur, properties that make them extremely unpleasant for oral administration. Eliminating these inconveniences would increase patient's compliance with the treatment, which is an important objective of the pharmaceutical industry and of the producers since the design phase of the pharmaceutical product [2-6].

In order to increase therapeutic efficiency and compliance to treatment, several methods were tested in pharmaceutical practice, among which: addition of neutralizing excipients, prodrugs formation, and microencapsulation of some inclusion complexes (e.g. with cyclodextrin) [7, 8].

Cyclodextrins are natural cyclic oligosaccharides, widely used in pharmaceutical research and development for over 100 years. Their use has led to very good results in increasing the solubility and stability of medicines, as well as in improving the organoleptic characteristics [9-11].

In aqueous solutions, cyclodextrins are capable of forming inclusion complexes of guest-host type with more medicines by including in their own cavity of a whole molecule or a non-polar part [12]. The ability of cyclodextrins to form complexes has been widely exploited in the pharmaceutical field because, since no covalent bonds are formed during the formation of the complexes, they are in dynamic equilibrium with the free medicine. [7, 13]

The aim of this study was to obtain and characterize some inclusion complexes of famotidine and nizatidine with β-cyclodextrin (β-CD). The molecular association between these two substances and β-cyclodextrin as well as the stoichiometry of the inclusion complexes and the strength of the bonds were examined by ^{1}H-NMR spectroscopy.

The NMR spectroscopy technique was able, through the variation of the chemical shifts of the specific protons of both host and guest molecules directly involved in the interaction [14], to establish the stoichiometry of the obtained complexes. Thereafter, the association constants and percentage of drug involved in inclusion complex were calculated by using a continuous variation method.

Experimental part

β-Cyclodextrin (containing 8 mol water/mol) was bought from Sigma Aldrich GmbH, Germany, and used without any further purification. D_2O (99.7% atom D) was obtained from National Institute for Cryogenics and Isotopics Technologies (Ramnicu Valcea, Romania). Famotidine and nizatidine were aquired from S.C. Helcor S.R.L. Baia Mare, respectively from S.C. Sicomed/Zentiva S.A Bucuresti, Romania. Famotidine and famotidine containing samples were dissolved in 20 % (v/v) CD_3COOD in D_2O due to famotidine's low solubility, meanwhile nizatidine and its complexes were dissolved in D_2O, and the solutions were transfered to capillary. ^{1}H-NMR spectra were recorded on a Varian-Gemini spectrometer, at 300 MHz, at 295±0.5K, with TMS as standard, and spectra were obtained by co-addition of 32 or 64 scans.

In order to study the stoichiometry and the association constant (Ka) of the complexes obtained after mixing the drugs and β-cyclodextrin, the continuous variation method was applied. 10 mM stock solutions of famotidine or nizatidine and β-cyclodextrin in D_2O were used, in diverse volumetric ratios, and two series of nine specimens (i=1-9) were obtained, at a final volum of 2 mL.

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All the authors had equal contribution at this original article

We used different ratios of β-CD (host, H) and famotidine or nizatidine molecules (guest, G), and the overall concentration of the two species was kept constant (10 mM), while $0 \le r \le 1$; r=[H]/([H]+[G]), [H] and [G] are the total concentrations of host, respectively guest (famotidine, nizatidine) molecules.

Results and discussions

Stoichiometry determination

The interaction between famotidine or nizatidine with β-CD was evaluated through ^{1}H-NMR spectroscopy. This technique and the continuous variation applied can propose the stoichiometry of the stable complex formation, so that the ability to include host molecules inside the hydrophobic cavity of β-CD, by comparing the peaks of specific protons in ^{1}H-NMR spectra of the starting materials and complexes obtained.

The structures and the proton numbers from β-cyclodextrin, famotidine, and nizatidine are depicted in figure 1.

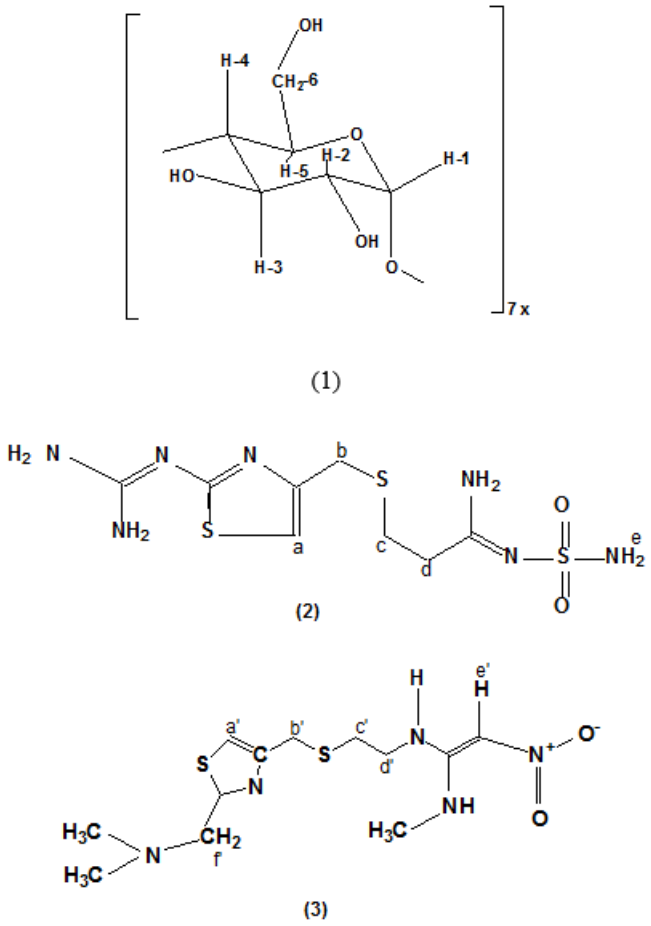

Fig. 1. Chemical structures of the investigated compounds used in the study: (1) β-cyclodextrin, (2) famotidine, and (3) nizatidine

NMR spectra of famotidine -β-CD complexes exhibit the peaks corresponding to H3 and H5 at the higher field, indicating that they are inside the molecule, H6 is outside the β-CD molecule, meanwhile H1, H2 and H4 are not shifted compared to free β-CD. Also it was observed a chemical shift at the lower field for Hd proton of famotidine after the complexation with β-CD. The signal observed for Hc and H5 from β--CD are overlaied, therefore it was not possile to use them for Ka calculations.

Similarly, NMR spectra of nizatidine - β-CD complexes exhibit shifted peaks for H3, H5, H6 from β-CD and for Hd' from nizatidine compared to that from free molecules.

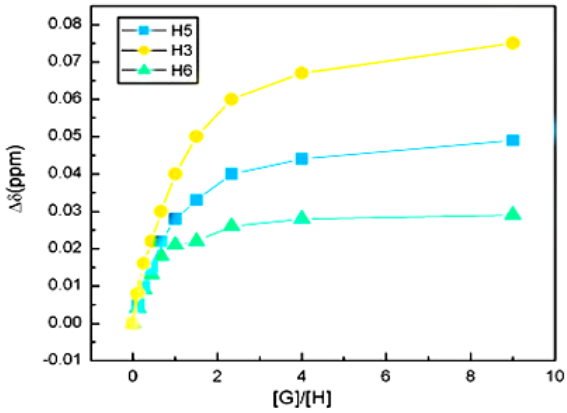

Fig. 2. Variation of the chemical shifts of specific protons of β-CD (H3, H5 and H6) after adition of diverse concentrations of nizatidine, where $\Delta\delta = \delta_{free} - \delta_{obs}$, [G] = concentration of nizatidine, [H] = concentration of β-CD

Variation of the chemical shifts of specific protons of β-CD (H3, H5 and H6) after the addition of diverse solution concentrations of nizatidine is presented in figure 2. Similar behaviour was observed in the case of the addition of famotidine solutions to β-CD.

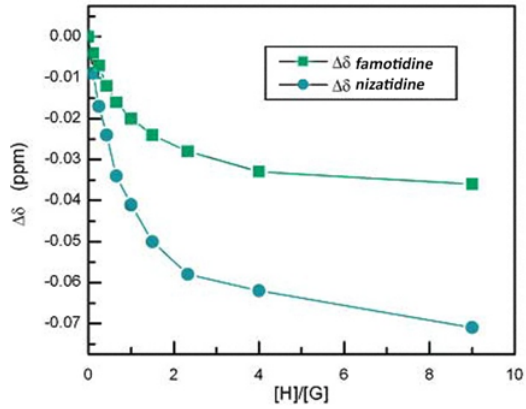

Fig. 3. Variation of the chemical shifts of specific protons: Hd from famotidine and Hd' from nizatidine, respectively, in the presence of diverse concentrations of β-CD, where $\Delta\delta=\delta_{free}-\delta_{obs}$, [G]=concentration of famotidine or nizatidine, [H]=concentration of β-CD.

In figure 3 is depicted the variation of the chemical shifts of specific protons: Hd for famotidine and Hd' for nizatidine with addition of different volumes of solution of β-CD.

The absence of new peaks in ^{1}H NMR spectra for the drugs - β-CD complexes compared to that of the starting materials demonstrate that the process is dynamic in the experimental conditions used in the determinations, the host molecules being in rapid interconversion between free and bonded species.

The determination of the stoichiometry of the two components from each complex was assigned by using the continuous variation method [15]. Mixtures of famotidine or nizatidine with β-CD were obtained in which the concentrations of the two components were between 1 and 0, and then ^{1}H NMR spectra were recorded.

The variation of the chemical shifts of the H3, H5 and H6 protons of β-CD that are the most affected after complexation with a drug, Δδ[β-CD], and r_1=m/(m+n), where m and n are the volumetric ratios of β-CD and G (G = famotidine or nizatidine) in the complex X_n: $(\beta\text{-CD})_m$, are shown in figure 4. Similar behaviour was observed in the case of the addition of famotidine solutions to β-CD.

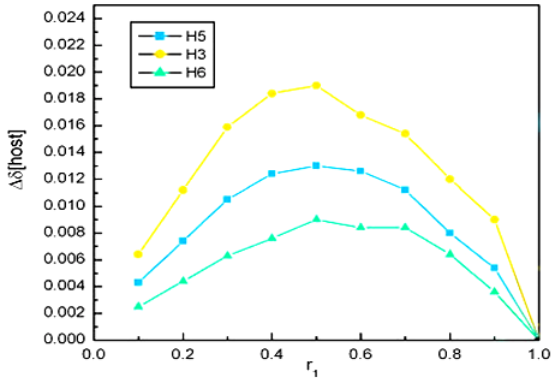

Fig. 4. Job plots for the protons: H3, H5 and H6 of β-CD in the presence of diverse concentrations of nizatidine

The protons H3 and H5 from β-CD that are inside of the cavity are considerable shifted after the complexation with the drugs, which demonstrates that famotidine and nizatidine interacts with β-CD inside its cavity.

Similary, the representation of the variation of the chemical shifts Δδ [G] of the specific protons of the drugs (famotidine, nizatidine) before and after complexation with β-CD as a function of $r_2 = n/(n+m)$ are depicted in figure 5.

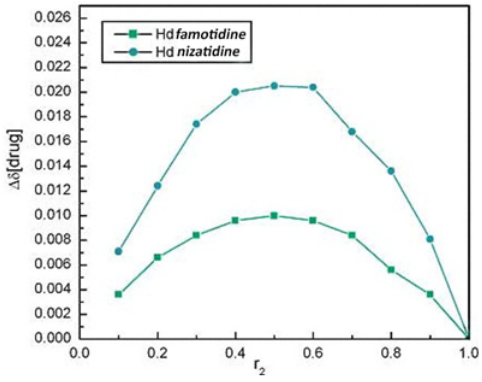

Fig. 5. Job plots for protons Hd from famotidine and Hd' of nizatidine, respectively, in the presence of diverse concentrations of β-CD

The variation of the chemical shifts is represented by the difference between chemical shifts of the specific protons in the absence or in the presence of the additional solution of the drug.

The variations of the chemical shifts for the specific protons of the drug - β-CD complexes exhibit a maximum at $r_1 = 0.5$ confirming that both complexes (famotidine - β-CD and nizatidine - β-CD) have a stoichiometry of 1:1.

Stability studies

Association constants (K_a) were calculated for famotidine - and nizatidine - β-CD complexes by a non-linear least squares regression analysis, in order to evaluate the degree of intermolecular bonds, by using the equation (1):

$$\Delta\delta^{(ij)} = (\Delta\alpha_c^{(j)}/2[X])\{[M]+(1/K_a)-[([M]+(1/K_a))^2-4[H]^{(i)}[G]^{(i)}]^{1/2}\} \quad (1)$$

where $\Delta\delta^{(ij)}$ is the difference between the chemical shift of a specific proton of the free drug molecule and the observed value for a specific ratio r of the drug - β-CD complex, i and j are the number of specimen sample and specific protons for each drug molecule, respectively, [X]=[G] or [H] are the concentrations of the guest or host molecules.

Equation (1) does not take into consideration and does not correlate the total concentrations of guest and host molecules observed in $\Delta\delta^{(ij)}$. Therefore, we applied a programm based on the repetition of the process by using special logarithms in order to be able to fit the experimental values of $\Delta\alpha^{(ij)}$ into the equation. [15,16]. Each repetition establish a quadratic programm for the determination of the errors until the results are similar. Each iteration establish a quadratic programm that determine the direction of search and loss function till the search converges. The search is finished when the difference between two consecutive values of E are smaller than 10^{-6}.

$$E = \Sigma_i \Sigma_j (\Delta\delta^{(ij)} - \Delta\delta_{calc}^{(ij)})^2 \quad (2)$$

This programm lead to a single value of K_a for the process and a set of calculated values of $\Delta\delta^{(ij)}$. K_a calculated for famotidine - β-CD complex is 179.6 M^{-1} at 295 K, while K_a for the nizatidine - β-CD complex is 74.9 M^{-1}.

The estimation of the drug that is complexed with β-CD can be made by using the equation (3):

$$[HG]=\{D-(D^2-4K_a^2[G]_t[H]_t)^2\}(2K_a)^{-1} \quad (3)$$

where: $D=K_a([G]_t+[H]_t)+1$

If we take into consideration the stoichiometry of the complexes 1:1, that means $[G]_t=[H]_t=5$ mM, the percentage of the host molecule [q] that form the inclusion complex can be calculated by using equation (4):

$$q(\%)=100([HG]/[G]_t) \quad (4)$$

Variation of the chemical shifts of the specific protons, the association constants K_a and q(%) for famotidine - and nizatidine - β-CD complexes are presented in table 1.

Table 1

VARIATION OF THE CHEMICAL SHIFTS (PPM) OF THE SPECIFIC PROTONS, ASSOCIATION CONSTANTS K_a (M^{-1}) AND q(%) FOR THE DRUG - β-CD COMPLEXES

Protons ($\Delta\delta_c$)	Complexes	
	Famotidine - β-CD	Nizatidine - β-CD
H3	0.0705	0.1243
H5	0.1041	0.1866
H6	0.0429	0.0082
Hd	-0.0577	-
Hd'	-	0.1820
K_a (M^{-1})	179.6	74.9
E	$1.041.10^{-4}$	$8.57.10^{-5}$
q (%)	36.4	22.5

Conclusions

Both famotidine and nizatidine form with β-CD complexes with stoichiometry 1:1 in aqueous solution, and it can be suggested that the drug molecules are inserted preferentially in the larger side of the host. The calculated values of the Ka for famotidine- and nizatidine -β-CD complexes are in good agreement with the higher hydrophobicity of famotidine compared to that of nizatidine. β-CD may be used to mask the bitter and unpleasant taste of the drugs by forming drug - β-CD complexes. Also this is a novel approach to increase the drug stability.

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