Formation of inclusion complexes of monochlorotriazinyl-\(\beta\)-cyclodextrin with promethazine was studied, for establishing the optimal conditions of their formation, in view of subsequent grafting on cellulose textiles with potential medicinal use. The infrared spectral study of inclusion in solid state indicated that the complex can be even formed by physically mixing the two substances. By the co-precipitation method, one can obtain a complex with obvious spectral changes, compared to the spectra of the two substances viewed separately. The stability constants of the complex in aqueous medium, in both acid and alkaline environment, were established by means of UV spectra and Benesi-Hildebrand equation. The value of the stability constant obtained is ten times higher in acid than in alkaline medium, which indicates that the alkaline medium is more favourable for producing the complex.

**Keywords**: promethazine, monochlorotriazinyl-\(\beta\)-cyclodextrin, UV spectra, Benesi-Hildebrand equation

**INTRODUCTION**

Promethazine (\(N,N\)-dimethyl-1-(10 \(H\)-phenothiazine-10-yl)propan-2-amine) is a member of the family of phenothiazine derivatives and, unlike other substances from this group, has a reduced neuroleptic effect, being used mainly as an antihistamine (H1), sedative, antiemetic, anticholinergic agent, as well as against motion sickness. According to its chemical structure, promethazine belongs to the derivatives without substitutes on the phenothiazine core, the chain linked to the nitrogen atom from the heterocyclic ring being composed of two carbon atoms, which explains the biological activity indicated above. Promethazine acts as a monoprotic base, with low solubility in alkaline medium, which explains its utilisation as a hydrochloride salt, as it is more soluble.

Whereas some phenothiazine derivatives were analyzed as guests of cyclodextrins to obtain pharmaceuticals for oral or parenteral use and less for transdermal administration (with the exception of creams), it was considered interesting to study textiles grafted with monochlorotriazinyl-\(\beta\)-cyclodextrin (MCT-\(\beta\)-CD), in which phenothiazine derivatives with sedative and anti-histaminic action, such as promethazine (PM), are included. The applications of these textiles could be both as underwear for patients with skin diseases or allergies, and for local applications on limited areas of the body, in the form of patches. Promethazine is prescribed as an anti-emetic (for vomiting, nausea or motion sickness). The oral administration in these cases is inefficient because of vomiting, while injections or suppositories can be also unacceptable.

In such cases, the trans-dermal administration of promethazine appears the best solution, involving textiles grafted with products of inclusion from which the drug is released slowly, to provide an approximately constant concentration in blood for a relatively long period.

Cyclodextrins (CDs) are cyclic oligosaccharides formed by enzymatic hydrolysis of starch, frequently containing six, seven or eight units of D-(+)\(\alpha\)-glucopyranose linked by (1-4)-glycosidic bonds (\(i.e. \alpha\), \(\beta\) and \(\gamma\)-cyclodextrin). The CD molecules appear as a truncated cone with hydrophilic outer surface (due to the hydroxyl groups facing outwards) and hydrophobic interior (with carbon and hydrogen
atoms and bridges of oxygen whose electron lone pairs are directed towards the inner cavity, thus generating a high density of electrons). Due to their special conformation, CDs are able to include in their hydrophobic cavity various molecules, called guests, and to form inclusion complexes.2-5 Water plays an important role in the formation of these complexes in aqueous solution. In the absence of other substances, the water molecules penetrate the hydrophobic cavity of CD, their high enthalpy – explained by the unfulfilled hydrogen bonds – leading to the replacement of water molecules inside the hydrophobic cavity by hydrophobic molecules, which creates a lower energy state and, consequently, a higher stability. The return of the water molecules into solution leads to an increased number of hydrogen bonds. The inclusion of hydrophobic guest-molecules into the cavity of the CD-molecule involves the emergence of hydrophobic interactions or of van der Waals forces able to relax the cyclodextrin ring and to reduce the interactions of rejecting the guest-molecules by the aqueous medium. The ability of cyclodextrins to form inclusion complexes is influenced both by the thermodynamic interactions between the components tending to equilibrium (which means the lowest energy state) and the steric factors (size of guest-molecules, orientation of certain functional groups, local interactions between atoms, etc.). The interactions between the guest-molecules and the hydrophobic inner part of the CD are not covalent bonds in their nature. These bonds are not permanent, a dynamic balance occurring between their formation and dissolution. A CD molecule generally includes a guest-molecule, however, the small molecules may form inclusion complexes with two guest molecules. There are cases in which the guest molecules do not fit completely inside the CD, and only the more hydrophobic side of the molecule, fitting the cavity shape, is included. When the guest-molecules are large, they may form an inclusion complex with two molecules of CD.6 Permanent fixing of cyclodextrins on cellulose textile fibers represents a possibility to modify the textile surface, by means of the monochlorotriazinyl reactive group, as in the case of the MCT-β-CD derivative. The inclusion of different substances, drugs included, through the cyclodextrin attached to the fiber surface, allows valuable textile applications.7 The guest-molecules encapsulated in the cavities of cyclodextrins show changes in the physico-chemical properties, which can be evidenced by different methods, thus demonstrating the formation of inclusion complexes.8-9 Based on these changes, one can also quantify the stability of the formed inclusion complex using the stability constant.5 This constant was determined in both acid and alkaline media, to establish the more suitable pH conditions for including promethazine, the resulting data being used to obtain the inclusion complex on the fabric surface after MCT-β-CD grafting on cellulosic materials.

EXPERIMENTAL
Materials and methods
Promethazine hydrochloride (Aldrich) was employed as a guest and monochlorotriazinyl-β-cyclodextrin (Wacker Chemie) as a host. To demonstrate the formation of the PM/MCT-β-CD inclusion complex, the following methods were used: infrared study of the complex formed by triturating and heating solid phase compared to physical mixture, co-precipitation and spectral method for determining the stability constant.

Trituration method
An equimolecular mixture of PM·HCl and MCT-β-CD was triturated in agate mortar for 30 min, with a small amount of alcohol, then left to dry at room temperature. The material was formed into tablets using KBr (1 mg triturated mixture to 300 mg KBr of spectral purity) and was analyzed with a Bruker Vertex 70 FT-IR spectrophotometer in the 4000÷600 cm⁻¹ frequency range.

Heating method
An equimolecular mixture of PM·HCl and MCT-β-CD was heated in the oven for 72 h at 70 °C, then stored for 24 h at 4 °C, after which it was formed into tablets, as shown above.

Co-precipitation method
2 g of MCT-β-CD were dissolved in 10 mL of distilled water, and 0.4113 g (equimolecular amounts) of PM·HCl were added while stirring. An adhesive paste, heated in a water bath at 50-60 °C, under stirring, was obtained. After about an hour, a spherical agglomeration was obtained, which was separated from the liquid and allowed to dry at room temperature in the dark, triturated and then formed into tablets.

Spectral method for determining the stability constant (Kₜ) in aqueous solution
The method was applied to obtain the UV spectra highlighting the changes of promethazine absorption at
the wavelength of maximum absorbance. This was done by keeping constant the concentration of promethazine (8 mg/L) and by varying the concentration of MCT-β-CD between 0 and 60 mg/L, at room temperature. The spectra were recorded between 200 and 300 nm, using a CAMSPEC Scanning Single Beam UV-VIS spectrophotometer and 1 cm quartz cuvettes.

Considering that promethazine has an exponent of acidity pKa = 9.1, the study was developed separately for the two pH ranges, namely for promethazine hydrochloride at pH 7, obtained with MERCK buffer, and for promethazine base at pH 10.8, obtained with the Britton-Robinson buffer.

The stability constant was calculated with Benesi-Hildebrand equation (1), which establishes a relationship between the variation of UV-VIS absorbance (∆ε) and cyclodextrin concentration:3,10

\[ \frac{1}{\Delta A} = \frac{1}{\Delta \varepsilon \cdot K_s \cdot [CD]} + \frac{1}{\Delta \varepsilon \cdot [PM]} \] (1)

where ∆ε is a constant representing the difference between the molar absorptivity of PM, in the absence and presence of MCT-β-CD, [PM] is the molar concentration of promethazine and [CD] is the molar concentration of MCT-β-CD; one can see that [PM] is also a constant, so that the equation becomes: y = ax + b, where b is expressed as 1/Δε⋅[PM] and is determined as the y axis interception, and a is 1/Δε⋅[PM]⋅[CD], i.e. the slope of the obtained line. By dividing the expressions of a and b, there follows that \( K_s = \frac{b}{a} \).

RESULTS AND DISCUSSION

Infrared study of the complex formed in solid state

The FTIR spectra of the mixtures (a heated and triturated mixture compared to a physical mixture), are presented in Figure 1. Some differences could be observed in band intensity, with no definite changes in the wavelength at which absorption occurs. The profiles of the curves remain generally the same, so that the changes can be attributed to the oxidation of promethazine during heating or trituration.

A comparison between the FTIR-ATR spectrum of the physical mixture PM·HCl/MCT-β-CD and the spectra drawn separately for the two substances (Fig. 2) evidenced, in most cases, additive absorption at 1450, 1325, 1224, 1123, 857 cm⁻¹, as well as some exceptions (784 and 649 cm⁻¹), when addition did not occur, which supports the idea that, even by simple physical mixing (grinding without heating or addition of alcohol), an inclusion process of PM·HCl in MCT-β-CD occurs.

The FTIR-ATR spectrum of the complex obtained by co-precipitation (Fig. 3) shows obvious changes compared with the spectra of the two substances, viewed separately. These are shifts of the absorption bands (from 1005 to 1026 cm⁻¹) or disappearances of bands (805, 784, 704, 671 and 649 cm⁻¹).

The FTIR spectra of the MCT-β-CD/PM·HCl mixture: a) physical mixture; b) heated mixture; c) triturated mixture.

Figure 1: FTIR spectra of the MCT-β-CD/PM·HCl mixture: a) physical mixture; b) heated mixture; c) triturated mixture.

Determination of stability constants of the complex in acid and alkaline media

Although the solutions analyzed by UV spectroscopy always contain the same concentration of promethazine, an increase in UV absorption at λ<sub>max</sub> = 249 nm (Fig. 4) was found at pH 7.
The chart (Fig. 5) was plotted according to equation (1) and the stability constant was determined in an acid medium, $K_a = 26.3 \times 10^3$ mol$^{-1}$.

For the same concentrations of promethazine and MCT-$\beta$-CD in alkaline medium at pH 10.8, the spectra shown in Figure 6 were obtained.

The observation may be made that the first three curves (a, b, c) – corresponding to concentrations of MCT-$\beta$-CD inferior to the promethazine equivalence point – indicate a decrease in its absorbance, comparatively with pure promethazine, while the following curves (d, e, f) indicate an increase in its absorbance. This could be interpreted as an inclusion of the side chain of promethazine, free of its positive charge existing in acid medium, until it reaches the equivalence point, and also after the equivalence point, presumably including one of the benzene cycles of promethazine in the additional molecules of MCT-$\beta$-CD entering the system. The opposite behaviour in acid medium before the equivalence point indicates inclusion of the side chain in the first stage. During the second stage, that of benzene cycle inclusion, the effect of increasing absorbance appears, as in an acid medium, even if the 2:1 complex formed is not subject to Benesi-Hildebrand equation and does not have relevance for cyclodextrin inclusion complex formation on the grafted chain, because the cellulose macromolecular distances between the grafted molecules are too large to allow
formation of a 2:1 complex. Therefore, the calculation of a stability constant corresponding to the alkaline environment (Ksb) takes into account the first stage of inclusion. The graph in Fig. 7 allows the calculation of Ksb = 271.4·10³ mol⁻¹.

One can notice that the value of the stability constant is about 10 times higher in alkaline medium which, theoretically, should be more favourable to the formation of inclusion complexes. This will be experimentally verified by UV spectra. Two identical fabric samples, grafted with MCT-β-CD, will be treated in two solutions of 8 mg/L promethazine (liquor ratio 1:10), one at pH 7 and the other at pH 10.8, both buffered. Other two samples of the same fabric will be simultaneously prepared in the same way, but un-grafted, and will be treated identically. The four samples will be maintained under identical conditions (in the dark, at room temperature, without stirring) for 24 h, after which they will be removed from the solutions, and squeezed to a squeezing degree of cca. 100%. The UV absorbance of the recovered solutions will be then measured, taking as reference the initial solutions. Change in absorbance of the two series of solutions will indicate which of them favoured inclusion.

**CONCLUSIONS**

- The FTIR spectra of the mixture of PM·HCl with MCT-β-CD, heated and triturated, compared to those of the physical mixture, show small differences in band intensity, and no significant changes in the wavelength at which absorption occurs. This leads to the idea that the influence of the mode of mixing in solid state is low.
The FTIR-ATR spectra determined for the physical mixture of PM·HCl and MCT-β-CD, and also for the two substances taken apart, indicate the initiation of a process of PM·HCl inclusion into MCT-β-CD.

The FTIR-ATR spectrum of the PM·HCl/MCT-β-CD complex obtained by co-precipitation presents obvious changes, compared to those of the two substances taken apart, which is an evidence of inclusion complex formation.

The study of complexation in aqueous solution at pH 7 permits to establish the linearity of the graphical representation of Benesi-Hildebrand equation (1), which allows the calculation of the stability constant of the PM·HCl/MCT-β-CD complex (K_{sa} = 26.3\times 10^3 \text{ mol}^{-1}).

In an alkaline medium (pH 10.8), a decrease in absorbance for promethazine-base may be observed, depending on the addition of MCT-β-CD until it reaches the equivalence point, followed by an increased absorbance. Until reaching equivalence, one may suppose an inclusion of the promethazine side chain, which has lost its positive charge existing in acid medium, followed, after the equivalence point, by inclusion of one of the benzene cycles belonging to promethazine into the added MCT-β-CD molecules, as in the case of the acid medium. In the second stage, a 2:1 complex is formed, a phenomenon not subject to Benesi-Hildebrand equation. The calculation of the stability constant in alkaline medium (K_{b}) took into account the first inclusion stage (K_{b} = 271.4\times 10^3 \text{ mol}^{-1}).

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REFERENCES