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Complexation study of cinalukast and montelukast with cyclodextrines

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Abstract

A fluorimetric study on the spectral characteristics of two antileukotrienes, cinalukast and montelukast, has been performed. Ionization constants of both of them have been photometrically calculated. Cinalukast pK_a in ethanol:water 50:50 (v/v) medium resulted to be 2.2 ± 0.1. Because the spectral characteristics of montelukast are widely affected by the solvent nature, pK_a was estimated in two different ethanol:water media, 70:30 (v/v) and 10:90 (v/v) and the values calculated were p$K_a = 2.9 \pm 0.1$, and p$K_{a1} = 2.0 \pm 0.1$ and p$K_{a2} = 6.5 \pm 0.1$, respectively. It has been proven that the fluorescence of both, cinalukast and montelukast, is significantly intensified in the presence of cyclodextrins (CyDs). The host–guest complexation processes between cinalukast and α-CyD or heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (DIMEB) and between montelukast and DIMEB have been investigated by fluorescence spectroscopy. A 1:1 stoichiometric ratio was established for the three studied inclusion complexes. The changes produced on the fluorescence of cinalukast or montelukast, when they are included on the hydrophobic CyD cavity are used to calculate their association constants by a non-linear regression method. Semiempirical MO calculations using AM1 method were performed in order to characterize the studied inclusion complexes. A new method for cinalukast determination in human serum, based on the fluorescence of the complex cinalukast–DIMEB exhibiting limit of detection of 7.95 ng mL^{-1} has been proposed with satisfactory results. Adequate recovery values between 95 and 103% were calculated at five different concentration levels.

Keywords: Antileukotrienes; α-Cyclodextrin; Heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin; Host–guest complexation; Human serum; Spectrofluorimetry

1. Introduction

Leukotrienes (LTs) are potent lipid mediators that have been long implicated in the pathogenesis of asthma. They are end-products of arachidonic acid metabolism, formed from phospholipids cleaved from cell membranes by enzymatically activated phospholipases. Cysteinyl leukotrienes (CysLT) receptors mediate a range of pro-inflammatory effects and therefore they are implicated in a range of inflammatory diseases, notably asthma [1–3]. Newly released antiasthmatic medications include antileukotrienes (LT receptor antagonists), agents which function by blocking the interaction of LTs with receptors. Representatives of CysLT receptors antagonists are cinalukast and montelukast, whose structures are shown in Scheme 1.

Cinalukast, is a styryl-thiazol whose chemical name is (*E*)-4-[3-[2-(4-cyclobutyl-2-thiazolyl)ethenyl]phenylamino]-2,2-diethyl-4-oxobutanoic acid. Cinalukast at a dose of 1 mg/kg and in a single oral dose (10 mg/kg), produces a long-lasting inhibition of bronchoconstriction, with pharmacological effects being observed within 1 h and maintained for up to 36 h [4].

Montelukast, is a fast acting, potent and selective LTD_4 ($CysLT_1$) receptor antagonist [5,6] which is being used in the treatment of asthma [7]. It belongs to a styryl-quinolines series with the chemical name 2-[1-[1(*R*)-[3-[2(*E*)-(7-chloroquinolin-2-yl)vinyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)]phenyl] propylsulfanylmethyl]cyclopropyl acetic acid. It provides benefit to patients with chronic asthma by means of once daily oral administration [6,8], and is the first LT inhibitor approved by the Food and Drugs Administration (FDA) for use by children [9]. The most common adverse reactions in clinical trials were headache and abdominal pain, amongst others [10,11].

No analysis method has been reported in the literature for the determination of cinalukast. Only a few chromatographic methods have been reported for the determination of montelukast in the bibliography. Furthermore, the determination of montelukast in human plasma using fluorescent detection [12], residual acetate analysis in bulk drug [13], its *s*-enantiomer in human plasma by stereoselective high performance liquid chromatogra-

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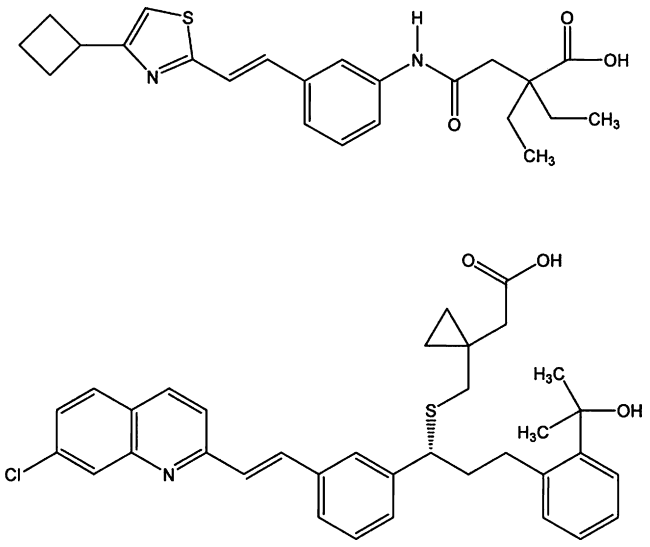

Scheme 1. Cinalukast (top) and montelukast (bottom) chemical structures.

phy (HPLC) using column-switching [14] and its determination in human plasma by column-switching HPLC method [15] have been described.

It is known that cyclodextrins (CyDs) have the property of forming inclusion complexes with guest molecules that have suitable characteristics of polarity and dimension [16–21]. The inclusion complex formation in the CyD systems is favoured by substitution of the high enthalpy water molecules located inside the CyD cavity, with an appropriate guest molecule of low polarity. An overview of the non-chromatographic analytical uses of CyDs has been presented by Szente and Szejtli [22]. Aspects covered in that review included: substrate/analyte solubilization and stabilization by CyD; sensitivity improvement by CyD; CyDs as enzimological analysis and development of CyD-based sensors and detectors with particular emphasis on biopharmaceutical and clinical analysis. The non-radiative decay processes of the analyte are often significantly attenuated and the fluorescence emission increases. This fact can be used as a resource for improving the performance of fluorimetric analytical methods.

In the present paper, the absorbent and fluorescent characteristics of cinalukast and montelukast have been investigated. Also their inclusion complexes with α-CyD and/or DIMEB have been fluorimetrically studied prior to the quantitative analysis. Based on the obtained results, the optimum working conditions were established and spectrofluorimetric methods for the determination of cinalukast and montelukast in both, the presence and absence of CyD, are discussed. Molecular structures based on AM1 semiempirical calculations are proposed for the three studied complexes. Cinalukast determination in the presence of DIMEB have been applied to human serum samples.

2. Experimental

2.1. Apparatus

Fluorescence measurements were made on a SLM Aminco Bowman, Series 2, luminiscence instrument, equipped with a 150 W continuous xenon lamp, interfaced by a GPIB card and driver with a PC Pentium III microcomputer. Data acquisition and data analysis were performed by the use of AB2 software. The excitation and emission slits were maintained at 4 and 8 nm, respectively. The scan rate of the monochromators was maintained at 5 nm s^{-1}. All measurements were performed in 10 mm quartz cells at 20 ± 0.1 °C, by use of a thermostatic cell holder and a thermostatic bath Selecta Model Frigiterm.

2.2. Software for AM1 calculations

Ground-state geometry optimization of the proposed structures for the inclusion complexes was performed with the AM1 method contained in the Chem3D package (CS Chem3D Ultra, ChemOffice).

2.3. Reagents

Cinalukast and montelukast sodium salt were obtained from Sigma and Merck, respectively, and α-CyD and DIMEB were obtained from Cyclolab (Cyclodextrin Research & Development Laboratory, Hungary) and used as received. Stock solutions of cinalukast and montelukast, containing 50 mg/L of each compound, were prepared in 100.0 mL volumetric flasks by dissolving 5.0 mg of each commercial product in ethanol, avoiding exposure to direct light and maintaining the solutions at 4 °C. Solutions of lower concentrations were prepared by appropriate dilution of the stock solutions with ethanol. 10^{-2} mol L^{-1} stock solutions of α-CyD and DIMEB were prepared in water. 0.50 mol L^{-1} acetic acid/sodium acetate buffer solutions (pH 4.6 and 6.0) were prepared from analytical reagents purchased from Panreac (Spain). All other chemicals and solvents were of analytical reagent grade.

2.4. Procedures for the fluorescence determination of cinalukast and montelukast: calibration graphs of cinalukast and montelukast

For the fluorimetric studies of cinalukast and montelukast, suitable aliquots containing different amounts of the analytes, between 0.126 and 0.750 μg for cinalukast and between 0.054 and 1.080 μg for montelukast, were transferred into a 3.0 mL measure cell and diluted to volume final with variable volumes of absolute ethanol and water in order to maintain the composition of the medium ethanol:water 50:50 (v/v) for cinalukast and 70:30 (v/v) for montelukast. The fluorescence intensity of each solution was measured at $\lambda_{ex}/\lambda_{em}$ = 335 nm/400 nm for cinalukast and at $\lambda_{ex}/\lambda_{em}$ = 281 nm/392 nm for montelukast.

2.5. Calibration graphs for cinalukast and montelukast in presence of CyDs

2.5.1. Cinalukast–CyD complexes

Aliquots of cinalukast ethanolic solution containing between 0.099 and 0.444 μg (in presence of α-CyD) and between 0.099 and 0.492 μg (in presence of DIMEB) were transferred to the measure cell. Then ethanol, if necessary, to complete 1.5%,

0.60 mL of 0.50 M acetic acid/acetate buffer (pH 6.0 or 4.6), 2.0 mL of 10^{-2} M CyD stock solution and deionized water to complete 3.0 mL were added. The fluorescence of the complexes was measured at 403 by exciting at 336 nm.

2.5.2. Montelukast–DIMEB complex

Aliquots of montelukast ethanolic solution containing between 0.270 and 1.620 μg, were transferred to the measure cell. Then necessary volume of absolute ethanol to complete 2%, 0.60 mL of 0.50 mol L^{-1} acetic acid/acetate buffer solution pH 4.6, 1.0 mL of 10^{-2} M DIMEB stock solution and ultrapure water to complete 3.0 mL were added. The fluorescence was measured at 396 nm by exciting at 281 nm.

2.5.3. Procedure for the analysis of cinalukast in serum samples previous formation of cinalukast–DIMEB complex

0.030 mL of absolute ethanol and 1.0 mL of acetonitrile were added on 0.50 mL of a human serum pool. The mixture was centrifugated during 20 min, 0.30 mL of the supernant were tansferred into the measure cell and the proposed method to analyze cinalukast was applied.

3. Results and discussion

3.1. Fluorimetric study of cinalukast and montelukast

Both analytes present native fluorescence in ethanol, methanol, water, acetonitrile and acetone. The excitation and emission fluorescence spectra of cinalukast in ethanol:water (50:50) and of montelukast in different proportions of ethanol are summarized in Fig. 1. The excitation spectrum of cinalukast in ethanol:water 50:50 (v/v) shows two maxima located at 246 and 336 nm, and the emission spectrum shows only a maximun located at 402 nm. The intensity of fluorescence increases when increases the percentage of ethanol in the medium and it was found that fluorescence remains practically constant from a value of 50% and decreases for minor ethanol percentage. No shifts are observed in the maxima wavelengths.

Montelukast spectra are very influenced by the percentage of ethanol in the medium. The native fluorescence of montelukast in aqueous media is low and increases when the percentage of ethanol does. When this percentage is lower than 20%, the emission spectra present a maximum located at 485 nm, but when the percentage of ethanol is higher than 20%, this maximum shows a hypsochromic shift to 393 nm, as we can observe in Fig. 1B. On the other hand, the excitation spectra are very similar for all percentages of ethanol assayed, showing two maxima situated at 281 and 347 nm, respectively. Excitation wavelengths of 336 and 281 nm were selected for cinalukast and montelukast, respectively.

A study of the influence of acidity of the medium on the fluorescence intensity of cinalukast and montelukast, has been carried out. The pH of all solutions was adjusted, over the range 1–12, by the addition of trace amounts of hydrochloric acid or sodium hydroxide. Fluorescence intensity of cinalukast increases until pH near to 3.0, being practically constant for pH > 3.0. Fluorescence intensity of montelukast in ethanol:water 70:30 (v/v) medium increases until pH 4.0 and remains constant between pH 4.0 and 5.5, then the signal decreases and becomes independent of pH for values higher than 7.0. The position of the emission maxima of montelukast in aqueous media is influenced by the pH, being centred at 485 nm in neutral and acidic media and at 407 nm in basic medium. The fluorescence signal of montelukast in aqueous media measured at 407 nm, increases until pH 7.0 and keeps constant for higher pH values, on the other hand, the signal measured at 485 nm increases until pH 4.0, remains constant until pH 5.5 and decreases drastically for values of pH higher than this, being practically negligible and constant for pH > 7.5.

The variation of the fluorescence intensity and the absorbance signal with the pH allowed the quantification of the pK_a of cinalukast and montelukast in different working media by both techniques, applying the Stenström and Goldsmith [23] method, adapted to fluorescence measurements [24]. The obtained results are summarized in Table 1. pK_a values calculated by both techniques are quite similar. This fact indicates that the pK_a

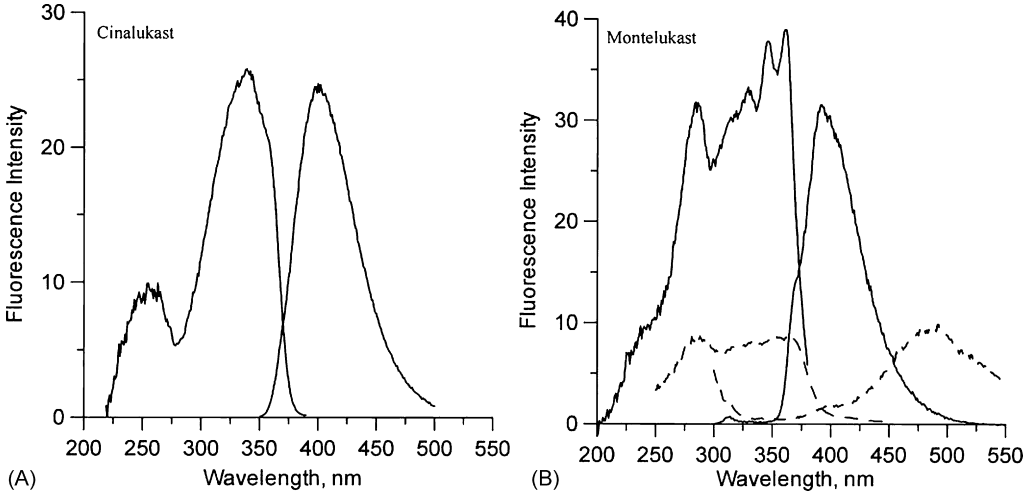

Fig. 1. Excitation and emission spectra of cinalukast in 50:50 (v/v) ethanol:water and montelukast in 70:30 (v/v) (—) and 10:90 (v/v) (– – –) ethanol:water solution. [Cinalukast] = 4.0 μg mL^{-1} and [Montelukast] = 5.5 μg mL^{-1}.

Table 1
Experimental acidity constants, pK_a

	pK_a values			
	Photometry		Fluorescence	
	pK_1	pK_2	pK_1	pK_2
Cinalukast 50:50 (ethanol:water)	2.2 ± 0.1	–	2.1 ± 0.2	–
Cinalukast–α-CyD complex	–	–	2.9 ± 0.2	–
Cinalukast–DIMEB complex	–	–	2.1 ± 0.1	–
Montelukast 70:30 (ethanol:water)	2.9 ± 0.1	–	3.1 ± 0.1	6.4 ± 0.1
Montelukast 10:90 (ethanol:water)	2.0 ± 0.1	6.5 ± 0.1	2.7 ± 0.3	7.0 ± 0.3
Montelukast–DIMEB complex	–	–	3.1 ± 0.1	6.5 ± 0.1

values fluorimetrically calculated corresponds to the ground states.

3.2. Study of the inclusion complexes Cinalukast–α-CyD, Cinalukast–DIMEB and Montelukast–DIMEB

Several preliminary studies were performed with the aim to investigate the possible formation of inclusion complexes of cinalukast and montelukast with α-, β-, γ-, 2-hydroxipropil-β- and DIMEB. The different inner cavities of the CyDs enable them to discriminate among guest molecules on the basis of their sizes. The influence of several CyDs at different pH values on the fluorescence intensity of cinalukast and montelukast was studied. An important increase in the fluorescence of cinalukast is observed in the presence of α-CyD or DIMEB. Also, the similar effect was observed between montelukast and DIMEB. It is apparent that α-CyD possesses the optimal conditions for the partial inclusion of cinalukast and DIMEB does for the partial inclusion of both, cinalukast or montelukast.

3.3. Influence of CyDs concentration

The influence of the CyDs concentration on the fluorescence spectra of aqueous solutions 2.4×10^{-6} and 1.8×10^{-6} M of cinalukast and montelukast, respectively, was studied in the range 1.6×10^{-2} to 8.17×10^{-2} M. The obtained results are shown in Fig. 2. As may be appreciated, the changes in the fluorescence intensity are significant in the three complexes. Upon inclusion of a fluorophore, in the CyD cavity, generally the fluorescence of the guess molecule is enhanced by shielding the excited species from non-radiative processes occurring in the bulk solution. It can be observed that in the three complexes, the intensity of fluorescence increases when increasing the concentration of CyD. Cinalukast and its CyDs complex show quite similar emission wavelength values. The emission maximum of cinalulast–α-CyD and cinalukast–DIMEB complexes show a slight red shift of 3 nm as the concentration of CyDs is increased. An important hypsochromic shift of the emission is observed in the emission maxima of montelukast–DIMEB complex. In this case, the emission maximum of free montelukast is located at 489 nm and in presence of DIMEB is displaced to 395 nm (Fig. 2C). Values of the molar ratios CyD/analyte of 2750, 2740 and 2800 were selected for cinalukast–α-CyD, cinalukast–DIMEB and montelukast–DIMEB, respectively, for subsequently experiments, in order to guarantee the full complexation of analytes.

3.4. Influence of the pH

The effect of pH on the fluorescence intensity of the complexes shows that the fluorescence is maxima and practically constant for pH values higher than 5.0 for cinalukast–CyDs complexes and between pH 4.0 and 5.5 for Montelukast–DIMEB complex. This study shows that the change of the fluorescence with the pH is similar in both cases, free analytes and complexed analytes. The pK_a values of the inclusion complexes are presented in Table 1. The fact that the values of the pK_a found in the absence and in the presence of CyD are not significantly different is an indication that, in the inclusion complex, the groups responsible of the these ionizations must be located outside the CyD cavity.

A value of optimum pH 4.7 was selected for the complexes formation of both analytes with DIMEB. pH 6.0 was selected as optimum for the formation of the complex cinalukast–α-CyD. An acetic acid/acetate buffer solution of pH 4.7 for the complexes of both analytes with DIMEB and of pH 6.0 for the complex cinalukast–α-CyD, was selected for later studies. The complexes are stable for at least 60 min after the mixture of the reagents.

3.5. Stoichiometry of the inclusion complexes

The application of the Scatchard and Benesi-Hildebrand methods [25] allows one to graphically determine the stoichiometry of the system under study. Typical double-reciprocal plots for the studied complexes are shown in Fig. 3. A linear relationship is obtained when $1/(F - F_0)$ is plotted against $1/[\mathrm{CyD}]_0$ ($r = 0.999$, 0.999, and 0.997, for cinalukast–α-CyD, cinalukast–DIMEB and montelukast–DIMEB, respectively), indicating that the stoichiometry of the complexes is 1:1. In contrast, a downward concave curvature is obtained when these data are fitted to a 2:1 stoichiometry. This stoichiometry has been verified by Scatchard method.

3.6. Association constants of the inclusion complexes

Once the stoichiometry of the system is known, the association constant can also be calculated. In the case of the

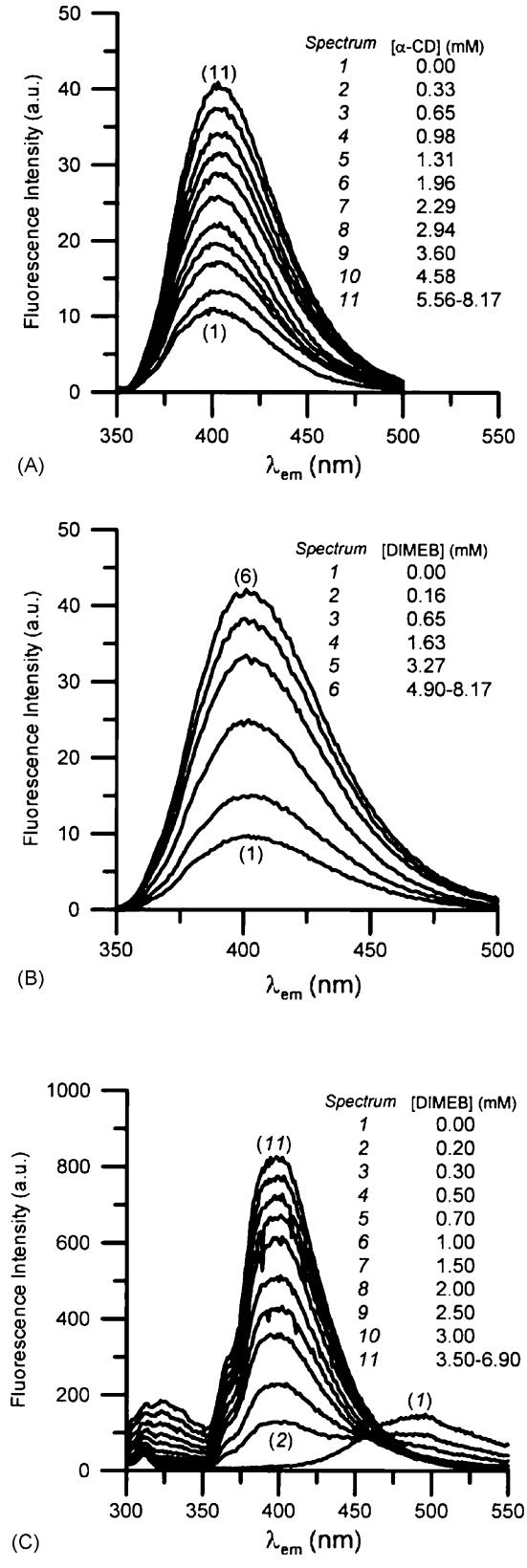

Fig. 2. Influence of (A) α-CyD concentration on the fluorescence emission spectra of 2.4×10^{-6} mol L^{-1} cinalukast solution (λ_{exc} = 336 nm), DIMEB concentration on the fluorescence emission spectra of (B) 2.4×10^{-6} mol L^{-1} cinalukast solution (λ_{exc} = 335 nm) and (C) 1.8×10^{-6} mol L^{-1} montelukast solution (λ_{exc} = 281 nm).

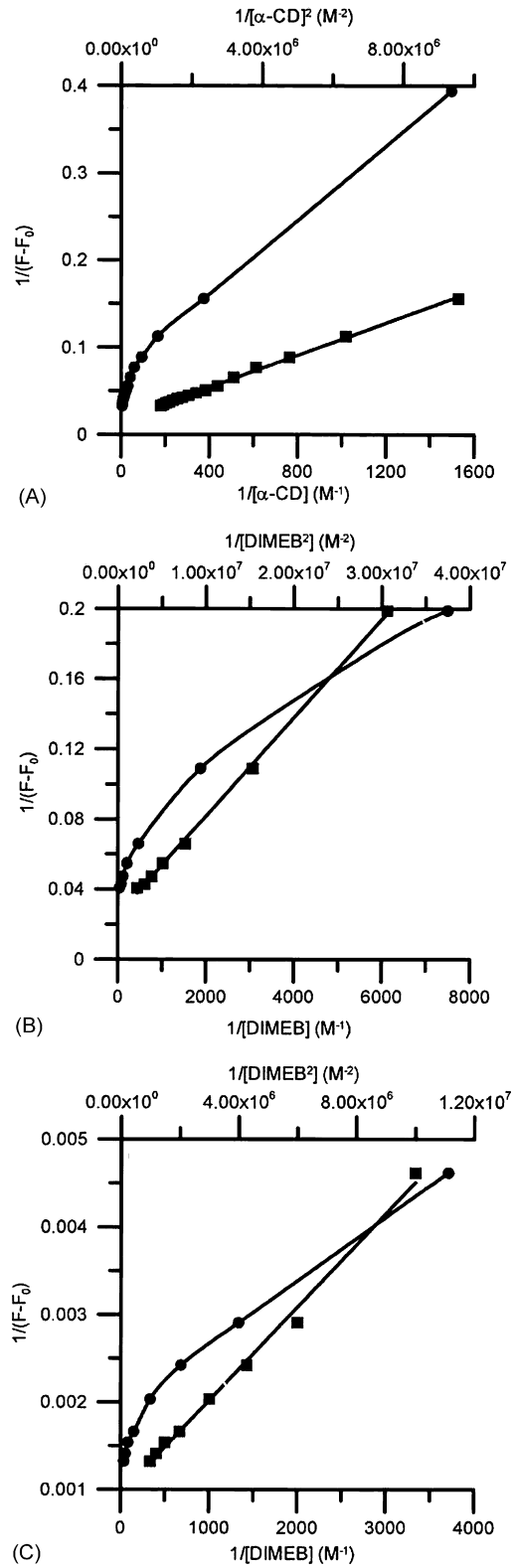

Fig. 3. Double-reciprocal Benesi-Hildebrand plots for (A) cinalukast in presence of α-CyD and (B) DIMEB, and for (C) montelukast in presence of DIMEB, in aqueous solution. A linear relationship when the data are plotted under the assumption of a 1:1 stoichiometry (■) and a downward concave curvature when the data are plotted under the assumption of a 1:2 stoichiometry (●). (A) [Cinalukast] = 2.4×10^{-6} M, $\lambda_{exc/em}$ = 336/403 nm, pH 6.0; (B) [Cinalukast] = 2.4×10^{-6} M, $\lambda_{exc/em}$ = 335/402 nm, pH 4.7; (C) [Montelukast] = 1.8×10^{-6} M, $\lambda_{exc/em}$ = 281/396 nm, pH 4.7.

Scatchard method, the association constant is given by the slope of the straight line (($F - F_0$)/[CyD]$_0$ versus ($F - F_0$)) and the values obtained for cinalukast–α-CyD, cinalukast–DIMEB and montelukast–DIMEB are 188, 997 and 959 M^{-1}, respectively.

By the Benesi–Hildebrand's method, the association constant is determined by dividing the intercept by the slope of the straight line obtained in the double-reciprocal plot. The values obtained for cinalukast–α-CyD, cinalukast–DIMEB and montelukast–DIMEB are 188, 918 and 898 M^{-1}, respectively.

However, the linear transformations used in these graphical methods do not properly weigh the data [25]. The double reciprocal plots tend to place more emphasis on lower concentration values in comparison to higher ones. As a result, the value of the slope is very dependent upon the ordinate value corresponding to the point having the smaller CyD concentration. Therefore, a more adequate estimation can be made by using non-linear least-squares regression analysis (NLR). The formation constant calculated from the linear methods may be used, however, as a parameter estimate in the NLR method. Experimental data can be directly fitted according to the equation:

$$F = F_0 + \frac{(F_\infty - F_0)\, K_1\, [\mathrm{CyD}]_0}{1 + K_1\, [\mathrm{CyD}]_0} \quad (1)$$

The NLR analysis of the data has been performed by an iterative Marquardt-type process, and values of 245, 1152 and 969 M^{-1} have been obtained for cinalukast–α-CyD, cinalukast–DIMEB and montelukast–DIMEB. Cinalukast complex with α-CyD is less robust than with DIMEB.

3.7. Proposed structures of the inclusion complexes

With the purpose of further characterizing the studied inclusion complexes, semiempirical MO calculations using the AM1 method were performed. This semiempirical method is commonly used to study geometric and thermodynamic properties of organic molecules, especially when hydrogen bonding occurs [26]. Several initial modes of inclusion were proved and optimized by energy minimization. In the case of cinalukast, the complexes structures leading to the minimum heat of formation shows the thiazol ring conjugated with benzene included in the CyD, getting the benzene ring outside of the CyD cavity in the cinalukast–α-CyD complex and inside in cinalukast–DIMEB. The quinoline radical is located inside of the DIMEB cavity in montelukast–DIMEB. These facts are not surprising, since the most probable mode of binding in the CyD inclusion complexes involves the insertion of the less polar part of the molecule into the cavity, while the more polar groups are exposed to the bulk solvent outside the opening of the cavity. The inclusion of the aromatic zone of the three molecules in the CyD cavities gives rise to enhanced fluorescence signals, what is in agreement with experimental evidences. The optimized structures of the complexes, obtained by energy optimization, are displayed in Figs. 4 and 5.

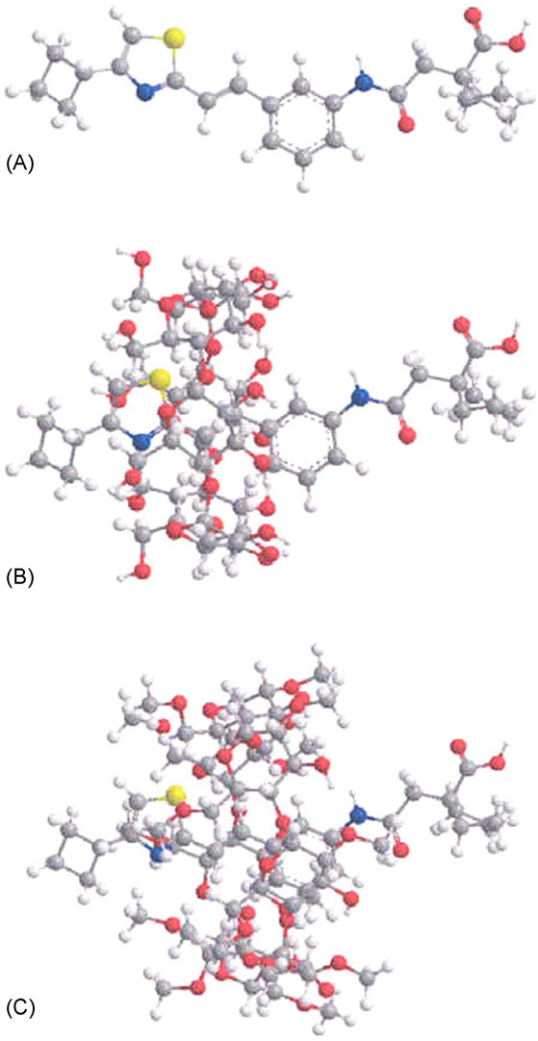

Fig. 4. Optimized structures of (A) cinalukast, (B) cinalukast–α-CyD and (C) cinalukast–DIMEB.

3.8. Analytical parameters

The spectrofluorimetric determination of cinalukast and montelukast, in both, the absence and the presence of CyD, involves the construction of the corresponding calibration curves. In Table 2, the analytical and statistical parameters of the determinations procedures, are summarized. As can be appreciated, the calibration sensitivity in presence of CyD, significantly improves with respect to those without CyD, while the limit of detection is quite similar in presence or absence of CyD. The repeatability of the methods have been analyzed by measuring eleven identical solutions of each analyte and of each complex in similar conditions as employed in the construction of calibration curves. The relative error percentage upon the average of the concentration found of each analyte is inferior to 3% in all the cases.

3.9. Cinalukast determination in human serum

The proposed method for the determination of cinalukast, based on the formation of an inclusion complex with DIMEB

Table 2
Analytical and statistical parameters for the determination of cinalukast and montelukast, as free analytes and of its complexes with CyDs

	Cinalukast			Montelukast	
	50% ethanol	α-CyD	DIMEB	70% ethanol	DIMEB
Linear range (ng mL^{-1})	42–250	33–148	33–164	18–360	90–540
Slope	0.93 ± 0.01	1.58 ± 0.02	1.36 ± 0.02	0.208 ± 0.002	1.27 ± 0.01
Intercept	7.8 ± 1.4	3.5 ± 2.4	28.4 ± 1.9	1.2 ± 0.3	32.4 ± 2.0
Correlation coefficient (r)	0.999	0.998	0.998	0.999	0.999
R.S.D.[a] (%) (concentration level)	1.4 (200 ng mL^{-1})	2.1 (65 ng mL^{-1})	1.1 (98 ng mL^{-1})	3.0 (200 ng mL^{-1})	2.1 (270 ng mL^{-1})
LOD[b] (ng mL^{-1})	9.2	8.83	7.95	11	9.48

[a] Relative standard deviation for 11 individual replicates, $n = 11$.
[b] Limit of detection according to Clayton et al. [27] ($\alpha = \beta = 0.05$).

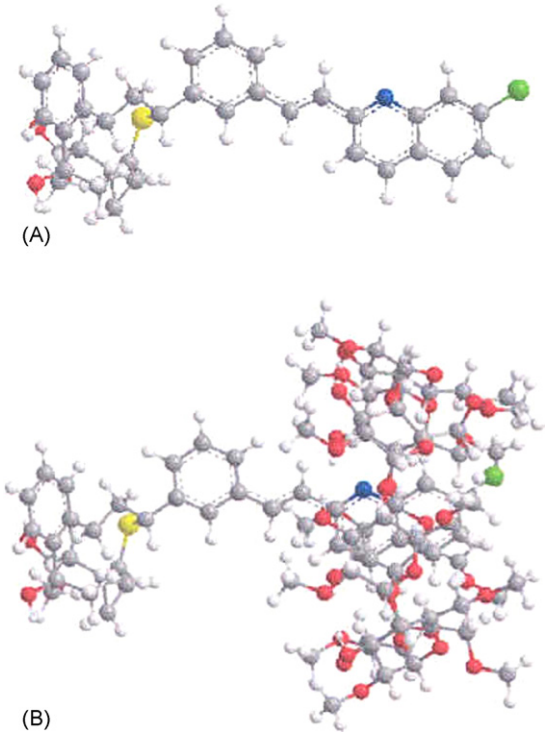

Fig. 5. Optimized structures of (A) montelukast and (B) montelukast-DIMEB.

was applied to the determination of this compound in spiked human serum. In order to examining the possible matrix effect of serum, a standard addition of cinalukast on a blank human serum pool was performed by adding increasing volumes of cinalukast stock solution on serum and manipulating it as described in Section 2. The emission intensity of treated blank serum at 402 nm is negligible compared with the analyte signal included in the CyD cavity. Slopes comparison test was applied to calibration and standard addition graphs and it reveals that does not exist matrix effect, and therefore cinalukast may be directly analyzed in serum, by measuring its emission intensity at 402 nm. Recoveries obtained at five different concentration levels are shown in Table 3.

Table 3
Recoveries for cinalukast determination in human serum

[Cinalukast]$_{added}$[a] (μg mL^{-1})	[Cinalukast]$_{found}$ (μg mL^{-1})	Recovery (%)
1.00	1.01	101
1.50	1.50	100
2.00	2.05	103
2.50	2.36	95
3.00	2.95	98

[a] Amount added in original human serum pool.

4. Conclusion

Results reported here support the formation of 1:1 inclusion complexes between cinalukast and α-CyD or DIMEB and between montelukast and DIMEB. Cinalukast complexes are highly fluorescent in neutral and basic media however montelukast complex is preferably formed in acidic medium. Cinalukast complex with α-CyD is less robust than with DIMEB. In the three complexes the inclusion of the aromatic zone in the cyclodextrin cavity give rise to enhanced of the fluorescence. For the first time a fluorimetric method to determine cinalukast in human serum is reported in this paper. Previous proteins precipitation with acetonitrile the proposed method appears selective for cinalukast. The average recovery in the range 1–3 μg mL^{-1} of cinalukast in human serum was 99.4%.

Acknowledgments

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