African Journal of Pharmacy and Pharmacology Vol. 7(3), pp. 83-97, 22 January, 2013
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP12.710
ISSN 1996-0816 © 2013 Academic Journals

Full Length Research Paper

Relaxation versus diffusion on the diclofenac sodium release from matrix tablets containing hydroxypropylmethylcellulose and/or chitosan

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Accepted 23 July, 2012

Different formulations of diclofenac sodium (DS) containing hydroxypropylmethylcellulose (HPMC) and/or chitosan were prepared, with a view to appraise the effect of the said polymers on the drug release behaviour from matrix tablets prepared by the direct compression method. The tablets were tested for different assays, including swelling and release performance. Differential scanning calorimetry (DSC) and Raman spectroscopy were performed in order to estimate the compatibility between the matrix components (DS and excipients). From the DSC and Raman results, non-negligible drug:excipient interactions were detected, although, these were found not to constitute an incompatibility effect. The dissolution tests and the kinetic analysis data indicated that the rate and the mechanism of DS release from tablets are mainly controlled by the drug/polymer ratio. The release rate became slower for a high polymer content of HPMC. Moreover, the results demonstrated that chitosan could accelerate the drug release with lower amount in the formulation. The analysis of the drug release profile was performed in the light of distinct kinetic mathematical models. Release from formulations F2 and F3 occurs by an anomalous transport mechanism (coupling of diffusion/erosion mechanisms), with Kosmeyer-Peppas exponent (n) values of 0.626 and 0.706, respectively. The balance between diffusion and polymer erosion competing mechanisms of drug release were assessed by the Peppas-Sahlin model.

Key words: Diclofenac sodium (DS), drug release, hydroxypropylmethylcellulose (HPMC), chitosan, Raman spectroscopy, differential scanning calorimetry (DSC).

INTRODUCTION

Diclofenac sodium (DS) [2-[(2,6-Dichlorophenyl)amino] benzeneacetic acid monosodium salt] is a synthetic non-steroidal anti-inflammatory drug (NSAID) that has anti-inflammatory, analgesic, and antipyretic properties. It is used for the treatment of degenerative joint diseases such as rheumatoid arthritis, osteoarthritis, and ankylosing spondilitis (Adeyeye and Li, 1990).

DS is rapidly dissolved in intestinal fluid reaching its maximum blood concentration (C_{max}) within 30 min and is metabolised mainly by hepatic hydroxylation and subsequent conjugation. In healthy human volunteers, mean elimination half-life of the terminal phase was found to be 1.2 to 1.8 h (Fowler et al., 1983). Due to its rapid elimination, a controlled release dosage form, allowing the maintenance of the DS therapeutic level for a longer

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time period, improving the pharmacological activity, and reducing toxic effects would be very appreciated by the patients (Bravo et al., 2002).

Matrix type formulations appear to be a very attractive approach from both process development and scale up points of view. They consist of a system for delaying and controlling the release of a drug which is dissolved or dispersed in a support resisting to disintegration. One method to prepare controlled-release formulations is the incorporation of the active principle in a matrix containing a hydrophilic, rate-controlling polymer (Li et al., 2005; Mourão et al., 2010). Recently, studies have shown the effect of polymer blends on release profiles of DS from matrices and the results evidenced a dependence of the drug release on the kind of polymer and also on its proportion in the formulation (Samani et al., 2003; Mourão et al., 2010).

Hydroxypropylmethylcellulose (HPMC) is the most important hydrophilic polymer used for the preparation of oral controlled release drug systems, due to its non-toxic nature, its capacity to incorporate active principles of varying characteristics, its non-pH dependence, its swelling properties which have a considerable effect on the release kinetics of the incorporated drug (Ghimire et al., 2010). Water penetration, polymer swelling, drug dissolution, drug diffusion, and matrix erosion from these dosage forms are controlled by the hydration of HPMC, which forms a gel barrier through which the drug is able to diffuse (Velasco et al., 1999; Siepmann et al., 2002; Vueba et al., 2005). The influence of the DS:HPMC ratio, particle size of the drug and the polymer, and the compression force, on the drug release process from HPMC matrices was evaluated by Hiremath and Saha (2008), showing that the rate and the drug release mechanism are mainly controlled by the drug:HPMC ratio. Tablets prepared using HPMC on contact with aqueous fluids gets hydrated to form a viscous gel layer through which drug will be released by diffusion and/or by erosion of the matrix (Katzhendler et al., 2000). Moreover, studies carried out before (Kim and Fassihi, 1997), has demonstrated that the hydration-gelation contributes to the development of swelling/erosion boundaries and consequently to constant drug release. Combination of these of two polymers facilitates rapid formation of necessary boundaries (that is, gel layer and solid core boundaries) to control overall mass transfer processes.

Chitosan [Poly-(1-4)-2-Amino-2-deoxy-β-D-Glucan], in turn, is a linear cationic polysaccharide obtained by N-deacetylation of chitin, a naturally-occurring structural polysaccharide abundant in crab and shrimp shells. It has recently attracted great attention in the pharmaceutical and biomedical fields due to its favourable biological properties such as biocompatibility, inertness, versatility, and biodegradability. The conjugation of chitosan to various medicinal agents is also facilitated by its nature as an amino sugar polymer. Moreover, chitosan has antacid and antiulcer activities, which may prevent or weaken drug-induced irritation in the stomach. All these interesting properties render this natural polymer an ideal candidate for controlled drug release formulations (Majeti and Ravi, 2000; George and Abraham, 2006).

The aim of this study is to evaluate the effect of both a cellulose ether polymer, HPMC K15M, and a non-cellulose semi-synthetic polymer, chitosan, on the release behaviour of the DS active principle from a matrix tablet system, using distinct formulations in order to understand how they rule this process.

MATERIALS AND METHODS

Drug: DS (BP grade), Capsifar, Oeiras, Portugal. Polymers: hydroxypropylmethylcellulose, Methocel® (HPMC K15M), England and chitosan (90.5% deacetylation degree), Exquim S.A, Barcelona, Spain. Diluent: lactose monohydrate (LAC), Granulac® 200, Meggle, Wasserburg, Germany. Magnesium stearate was used as lubricant (Analytical grade).

Pre-formulation studies

Differential scanning calorimetry (DSC)

DSC measurements were performed using a Shimadzu DSC-50 with a thermal analyser (Shimadzu TA-50, Tokyo, Japan). About 3 mg of either drug or excipient, or 6 mg of the drug/excipient 1:1 (w/w) mixture were analysed, in sealed aluminium pans under nitrogen flow (20 ml/min), at a heating rate of 10°C min^{-1}, from 25 to 350°C. An empty sealed pan was used as reference. The equipment was calibrated with indium (99.98%, m.p.156.65°C, Aldrich®, Milwaukee, USA).

Raman spectroscopy

The Raman spectra were obtained on a triple monochromator Jobin-Yvon T64000 Raman system (focal distance, 0.640 m; aperture, f/7.5) equipped with holographic gratings of 1800 grooves/mm. The premonochromator stage was used in the subtractive mode. The detection system was a liquid nitrogen cooled non-intensified 1024 × 256 pixel (1") Charge Coupled Device (CCD). A Coherent (model Innova 300-05) Ar^+ laser was used as the light source, the output of which, at 514.5 nm, was adjusted to provide 50 mW at the sample position. A 90° geometry between the incident radiation and the collecting system was employed. The entrance slit was set to 100 µm. 5 scans with integration times of 60 s for pure DS and DS:HPMC K15M mixture and 3 s for DS:LAC mixture, were used in all the experiments. Samples were sealed in Kimax glass capillary tubes of 0.8 mm inner diameter. Under the aforementioned conditions, the error in wavenumbers was estimated to be within 1 cm^{-1}.

Preparation of the matrix tablets

The distinct formulations of the matrix tablets analysed in this study are provided in Table 1. Matrix tablets were produced by varying both the polymer and diluent content, for a fixed amount of drug, 100 mg. DS, polymer or polymer mixture, and diluent were passed through a 100 mesh sieve and were thoroughly mixed in a plastic bag for 15 min. Magnesium stearate (lubricant) was also sieved

(500 mesh), added to the previous mixture, and blended for an extra 5 min. All matrices (total mass of 276 mg) were prepared by direct compression in an automatic hydraulic press (Specac Press, England), using flat 10 mm diameter punches and a compaction pressure of 624 MPa, as described by Vueba et al. (2004).

DS quantification in the matrix tablets

Five randomly chosen tablets of each formulation were thinly minced in a mortar. 41.4 mg of the resulting powder was solubilised in phosphate buffer (pH 6.8), up to a final volume of 500 ml. Several aliquots were then filtered and assayed by UV spectrometry at 275 nm (Shimadzu UV-1603 spectrometer). The determination was carried out as described in USP 34 (2011), the results reported being the average of 3 independent measurements.

Characterization of the tablets

Mass uniformity of the tablets

A total of 30 tablets of each formulation were evaluated for their weight, using an analytical balance (KERN 770). The results were expressed as mean values of 30 independent determinations, according to USP 34 (2011).

Tablet thickness

The thickness of the matrix tablets was determined using a micrometer (Roche, Switzerland) and the results were expressed as mean values of 10 individual tablets of each formulation.

Hardness determination

The hardness of the tablets was determined using a tablet hardness tester (Erweka TBH28, Erweka GmbH, Germany) and the results were expressed as the mean of 10 determinations.

Mechanical tensile strength

The tensile strength (T) of the tablets was assessed on a tablet hardness tester (Erweka TBH28, Erweka GmbH, Germany), for 10 tablets of each formulation, from the force required to fracture them by diametral compression, according to the following equation:

$$T = \frac{2P}{\pi D t} \quad (1)$$

where P represents the applied load, and D and t are the diameter and thickness of the tablet, respectively (Fell and Newton, 1970).

Friability

Twenty tablets were weighed and placed into a friability tester (Erweka TA20, Erweka GmbH, Germany). The tablets were subject to 25 rpm for 4 min and were then re-weighted to obtain the friability, by determining the weight before and after the test. This process was repeated for all formulations and the percentage friability was calculated using the following equation:

$$F = \frac{W_1 - W_2}{W_1} \times 100 \quad (2)$$

where F represents the percentage weight loss, and W_1 and W_2 are the initial and final tablets weights, respectively.

Swelling studies

Swelling studies were carried out for all formulations tested. Three metallic baskets were weighed with a tablet from each formulation and were placed in 1000 ml of phosphate buffer pH = 6.8 at 37.0 ± 0.5°C. At hourly intervals, the baskets were taken out from the vessel, gently wiped with a tissue to remove surface water, re-weighted and placed back into the vessel as quickly as possible. The mean weights were determined for each formulation, and the swelling degree (S) was calculated according to the relationship (Efentakis et al., 1997):

$$S = \frac{W_s - W_d}{W_d} \times 100 \quad (3)$$

where W_d and W_s are the dry and swollen matrix weights, respectively. The swelling degree was the mean of 3 independent assays.

Drug release analysis

Dissolution studies were performed according to the USP 34 paddle method (2011). The dissolution medium was phosphate buffer (pH = 6.8, 1000 ml) at 37.0 ± 0.5°C, and a stirring speed of 100 rpm was used. Six different tablets were tested in six dissolution vessels (Vankel VK-7000 dissolution testing station, in-line with a closed flow through system using a peristaltic pump, connected to a Shimadzu UV-1603 spectrophotometer). The progress of the dissolution process was monitored by determining the amount of DS spectrometrically, at 275 nm, for samples withdrawn and filtered every 5 min, for a total of 1200 min. The corresponding drug-release profiles were represented by plots of the cumulative percentage of drug release (calculated from the total amount of DS contained in each matrix) versus time.

Kinetic mechanism

Several mathematical models can be used to describe the kinetic behaviour of the drug release mechanism from matrix tablets; the most suited one being that which best fits the experimental results. The choice of a specific model for a particular data set depends on the shape of the plot obtained, as well as on the underlying mechanism. The kinetics of DS release from hydrophilic cellulose matrix tablets was determined by finding the best fit of the dissolution data (amount of drug released versus time) to distinct models: zero-order (Equation 4), first-order (Equation 5), and Higuchi (Equation 6) (Higuchi, 1961, 1963).

$$M_t = M_0 + k_0 t \quad (4)$$

where M_t is the amount of drug released at time t, M_0 is the amount of drug in the solution at t = 0 (usually, $M_0 = 0$), and k_0 is the zero-order release constant.

Table 1. Composition of the distinct formulations of DS.

Component	Formulations (mg)			
	F1	F2	F3	F4
DS	100	100	100	100
HPMC K15M	50	100	85	68
Chitosan	–	–	–	17
LAC	125	75	90	90
Magnesium stearate	1	1	1	1

$$M_t = M_\infty(1-e^{-k_1 t}) \quad (5)$$

M_∞ being the total amount of drug in the matrix and k_1 the first-order kinetic constant.

$$M_t = k_H\, t^{1/2} \quad (6)$$

k_H representing the Higuchi rate constant.

Moreover, to better characterise the drug release behaviour for the polymeric systems under study, and particularly to gain some insight on the corresponding mechanism, the Korsmeyer-Peppas (Equation 7) semi-empirical model was applied (Korsmeyer et al., 1983).

$$\frac{M_t}{M_\infty} = k_{KP}\, t^n \quad (7)$$

M_t/M_∞ representing the fraction of drug released at time t, k a constant comprising the structural and geometric characteristics of the tablet, and n, the release exponent, being a parameter which depends on the release mechanism and is used to characterise it (Peppas, 1985). For cylindrical tablets (Ritger and Peppas, 1987), in particular, $n \leq 0.45$ corresponds to a Fickian diffusion release (case I diffusional), $0.45 < n \leq 0.89$ to an anomalous (non-Fickian) transport, $n = 0.89$ to a zero-order (case II) release kinetics, and $n > 0.89$ to a super case II transport.

The direct fitting of the drug release data to the nonlinear equations mentioned earlier is usually avoided through linear transformation of the data, followed by a linear regression analysis. However, this method may not be mathematically accurate, since it uses transformed values (logarithms) instead of the original data (Lu et al., 1996). Consequently, a direct nonlinear fitting of the experimental results was performed in this work, for each of the mathematical models considered (through minimisation of the sum of the squared residuals). Only the points comprised in the interval $0.1 < M_t/M_\infty < 0.6$ were used.

Additionally, to calculate the relative contribution of diffusional and relaxational mechanisms on the drug release, the Peppas-Sahlin heuristic model was applied (Peppas and Sahlin, 1989):

$$\frac{M_t}{M_\infty} = k_F\, t^m + k_R\, t^{2m} \quad (8)$$

The first term on the right-hand side of Equation 8 refers to the Fickian diffusional contribution, while the second term represents the case II erosional contribution. The coefficient m is purely Fickian diffusional exponent, that depends on the geometrical shape of the releasing device through its aspect ratio, which, for the flat-faced, disc-shaped used tablets, was calculated to be 3.8 (diameter/thickness). Thus, according to the figure presented by Peppas and Sahlin (1989), the m value is about 0.447. The percentage of drug release through a Fickian mechanism (F) was calculated by Equation 9, whereas the ratio of relaxational (R) over Fickian mechanism was obtained according to Equation 10 (Peppas and Sahlin, 1989):

$$F = \frac{1}{1+(k_R/k_F)\,t^m} \quad (9)$$

$$\frac{R}{F} = \frac{k_R}{k_F}\, t^m \quad (10)$$

Mean dissolution time

To further characterise the drug release, the mean dissolution time (MDT) was calculated according to the following equation:

$$MDT = \frac{\sum_{j=1}^{n} \hat{t}_j\, \Delta M_j}{\sum_{j=1}^{n} \Delta M_j} \quad (11)$$

where j is the sample number, n is the number of dissolution sample times, $\hat{t}_j$ is the time at midpoint between t_j and t_{j-1}, and ΔM_j is the additional amount of drug dissolved between t_j and t_{j-1}.

Dissolution efficiency

The dissolution efficiency (DE) is defined as the relationship between the area under the curve (AUC) of dissolved percentage, as a time function, at an observed time and the area of a rectangle corresponding to 100% dissolution at the same time, according to the following equation (Khan and Rhodes, 1972; Khan, 1975):

$$DE = \frac{\int_0^t y \times dt}{y_{100} \times t} \times 100 \quad (12)$$

where y is the percentage of drug dissolved at time t.

Statistics

In order to assess statistical significance among the data, one way analysis of variance (ANOVA) was used to test variation in tablets formulations containing different polymer (HPMC K15M and Chitosan) at different % *w/w* and in the same dissolution media. ANOVA was utilized as well as to test differences in the physical characterization of the matrix tablets. The difference between variants was considered significant for $P < 0.05$, followed by Bonferroni comparison t-test. Statistical analysis was done using

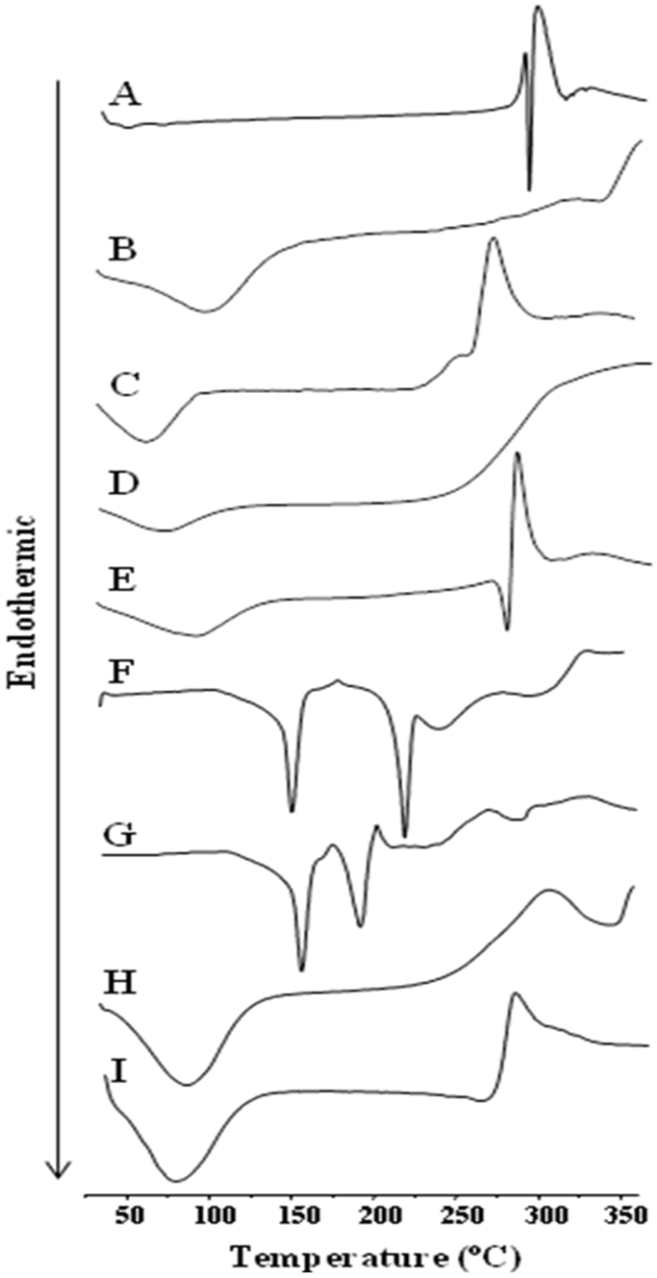

Figure 1. DSC curves of DS (A), HPMC K15M (B), DS:HPMC K15M (C), Chitosan (D), DS:Chitosan (E), LAC (F), DS:LAC (G), Chitosan:HPMC K15M (H), and DS:Chitosan:HPMC K15M (I) in 1:1 (C, E,G, and H) or 1:1:1 (I) (w/w) in the cases of physical mixtures.

Sigma Stat® for Windows (version 2.03, SPSS Inc).

RESULTS AND DISCUSSION

DSC

Pre-formulation studies are an important step in the selection of excipients for formulations of dosage forms. In fact, some physical or chemical incompatibilities between the drug and the excipients may occur being reflected on: thermal events variation, such as the appearance or disappearance of an endothermic signal; changes in the peak shape and variations in T_{onset} or T_{endset} derived the interactions in the simple mode from DSC curves. In order to investigate the possible interactions between DS and distinct polymers, polymer mixture and/or diluent, DSC was carried out for this purpose (Figure 1). The 1:1 (w/w) ratio was chosen, because it maximises the likelihood of observing any interactions (Mura et al., 1995). The DSC curve of DS was typical of a crystalline anhydrous substance as shown in Figure 1A. The peak temperatures as well as DS enthalpy values are collected in Table 2.

A large broad endothermic effect, over the temperature range 60 to 140°C, was observed for HPMC K15M (Figure 1B), upon evaporation of adsorbed water (Ford, 1999). The DSC curve of chitosan, in turn, was typical of amorphous hydrated compounds, showing a broad endothermic effect ranging between 50 and 100°C (Figure 1D) due to a dehydration process. The exothermic effect observed around 300°C was probably due to the oxidative decomposition or to the glass transition of the sample (Khalid et al., 2002). LAC thermogram, displayed two sharp endothermic peaks, at both 147 and 219°C (Figure 1F). The physical mixture 1:1 (w/w) of both polymers (HPMC K15M/chitosan) did not produce significant modifications on the thermal curve in comparison with that of either HPMC K15M or chitosan (Figure 1H). The combination of the drug with both polymers, DS/HPMC K5M (Figure 1C) and DS/chitosan (Figure 1E), demonstrated an interaction between the components with the drug dispersion in the polymer. A progressive reduction in peak size and a considerable downward shift of the drug peak temperature, causes a decrease of the melting endothermic onset and a reduction of the melting enthalpy (Table 2), suggesting a probable eutectic formation that is actually possible between active drugs and amorphous hydrated polymers (Zalac et al., 1999; Mahendran et al., 2001). The miscibility between the components, in both cases, seems to occur in a large extension.

On the other hand, when LAC was combined with the drug in a 1:1 (*w:w*) ratio, a significant downward shift of the drug melting peak and also a downward shift of the excipient melting peak were detected, coupled to a broadening effect (Figure 1G). These observations reflect the existence of solid-solid interactions between the two components, demonstrating the mixture of the drug with the diluent in accordance with findings previously reported by other authors (Verma and Garg, 2004; Sipos et al., 2008). Moreover, solid-solid interactions between LAC and ketoprofen were already reported (Batista de Carvalho et al., 2006).

The DSC assay of the drug:HPMC K15M:chitosan 1:1:1 (*w/w*) mixture (Figure 1I) presented a decrease in

Table 2. Peak temperature and enthalpy values of DS sodium in various drug-polymer mixtures and drug-LAC mixture.

Component	Drug:Excipient (w/w)	T_{peak} (ºC)	T_{onset} (ºC)	T_{endset} (ºC)	ΔH_f corr [a] (J/g)
DS	–	293.72	283.16	313.44	184.77
DS:HPMC K15M	1:1	273.03	262.23	284.70	96.88
DS:Chitosan	1:1	288.00	278.48	295.12	143.03
DS:LAC	1:1	191.71	179.67	196.99	47.12
DS:HPMC K15M:Chitosan	1:1:1	270.96	260.53	285.72	161.63

[a] ΔH_f corr = ΔH_f obs/% DS in sample × 100 (Vueba et al., 2005a).

both the onset and the drug melting temperatures (Table 2).

In general, the thermograms of solid state drug/excipient mixtures allow the detection of interactions between the components. However, some authors recognise that the occurrence of physical or chemical interactions does not necessarily indicate an incompatibility (Vueba at al., 2005a). Additionally, they agree that a change observed in the DSC curves is an unambiguous proof of interaction between drug and excipients (Balestrieri et al., 1996).

Raman spectroscopy

Raman spectroscopy has been widely applied to evaluate drug-excipient compatibility in pre-formulation studies (Marques et al., 2002; Vueba et al., 2006, 2008; Santos et al., 2012), and was presently used to detect solid-state interactions among DS and the excipients considered in the tested formulations. A 1:1 *(w:w)* drug:excipient ratio was chosen, as this is known to take advantage of the probability of occurrence intermolecular interactions.

The detailed assignment of the DS Raman bands has been previously proposed by Iliescu et al. (2004). The spectrum presents intense and well-defined features, most of them directly correlated to specific groups within the molecule, thus, allowing an objective identification of those involved in intermolecular interactions.

Figures 2a and 3a comprise the Raman spectra of the DS:HPMC K15M and DS:LAC physical mixtures, respectively, after one week of mixture preparation. The mixtures containing chitosan exhibited a very strong fluorescence background (Figure not shown) making it impossible to obtain its Raman spectrum with the available Ar^+ laser.

In order to detect changes in the DS molecule upon mixing with HPMC K15M or LAC, the spectra of the excipients (Figures 2b and 3b) were subtracted from those of the physical mixtures, yielding the “DS changed” spectra (Figures 2c and 3c). The similarity between the latter and the spectrum of pure DS (Figures 2d and 3d) is quite remarkable. However, careful inspection allows the detection of some subtle differences, highlighted in Figure 4 obtained through subtraction of the DS spectrum (Figures 2d or 3d) from the “DS changed” spectra (Figures 2c and 3c). The new signals at 267 and 360 cm^{-1} (a shoulder of the 367 cm^{-1} band), could be assigned to O-C-O^- deformation modes of the DS interacting with the excipients, either HPMC K15M (Figure 4a) or LAC (Figure 4b). The difference signals at 1049 cm^{-1} (Figures 4c and 4d) and around 1600 cm^{-1} (Figures 4e and f), mainly assigned to changes in the O-C-O^- stretching modes intensities, corroborate this interpretation. No other spectral changes were observed. In particular, no evidences of either DS conformational equilibrium modification or polymorphism were detected.

The observed changes in the DS Raman spectra were thus related with the interaction between the diclofenac COO^- functional group and the excipients. However, these spectroscopic results support the absence of significant intermolecular close contacts that could eventually lead to an incompatibility between the drug and the different formulation components.

Assay of DS in matrix tablets

As summarised in Table 3, evaluation of the hydrophilic matrix tablets containing DS showed that the drug content of all formulations ranged from 95.0 to 97.1% of the defined, which evidences a content uniformity. The differences in the mean values among the treatment groups are not large enough to exclude the possibility of arising from random sampling variability and the observed difference were not statistically significant ($F = 1.45$; $P = 0.27$). These values conform to USP 34 (2011) norms concerning DS delayed release tablets, which require an even amount of drug in all formulations (from 90.0 to 110.0% of the labelled amount).

Characterisation of the tablets

Table 4 shows several parameters, namely, mean values, standard deviation (SD), relative standard deviation (RSD), median, minimum and maximum values of the formulations of DS matrix tablets concerning to

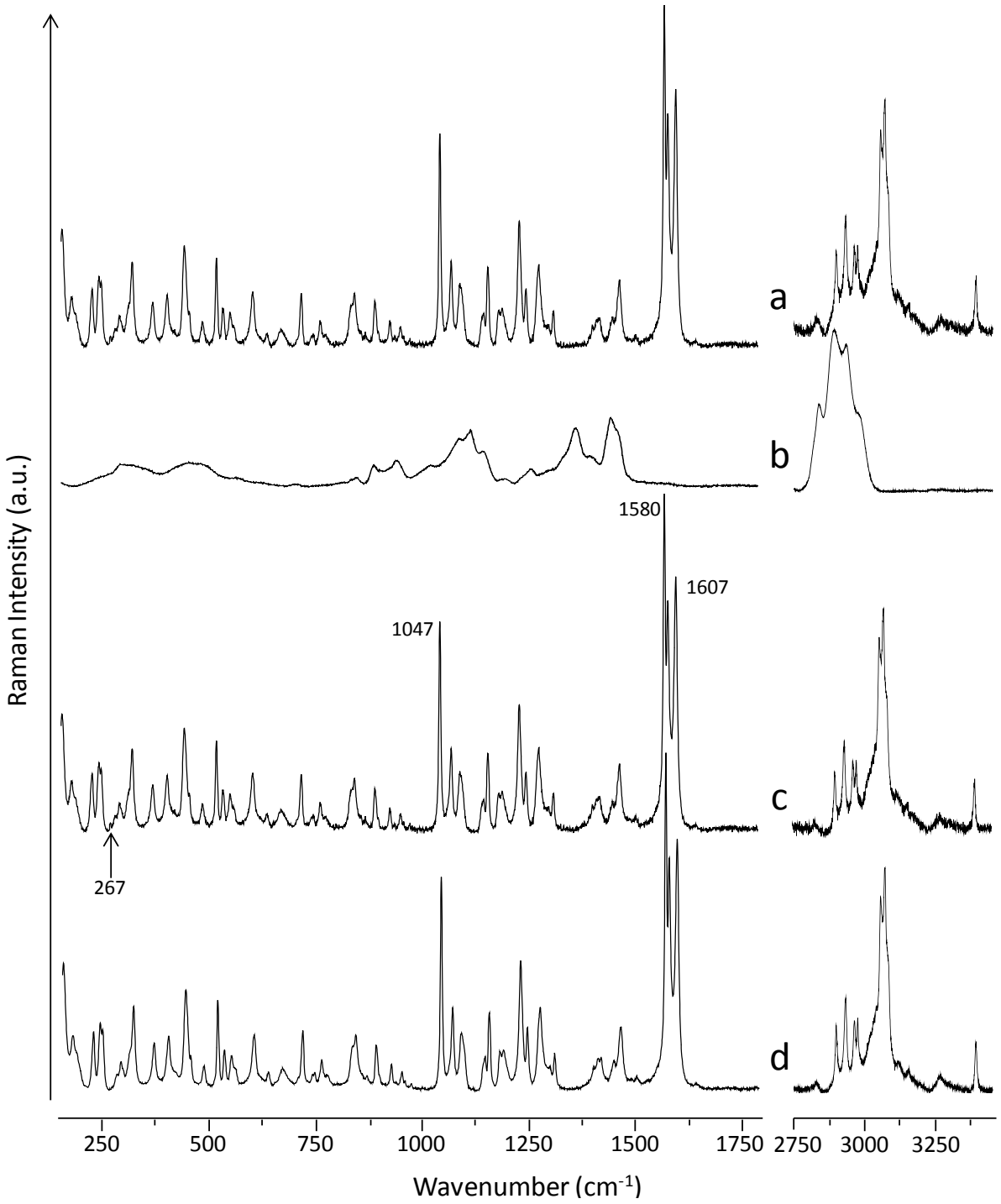

Figure 2. Raman spectra, in the 150 to 1800 and 2750 to 3450 cm^{-1} regions, of DS:HPMC K15M physical mixture (a), pure HPMC K15M (b), the result of subtraction (c), and pure DS (d).

uniformity of mass (e.g. homogeneity). Comparing formulations F1 and F4, it was possible to verify a uniformity of tablets since SD was less than 1.0; whereas, RSD was found to be lesser to 0.4%. The variance (data not shown) of all formulations was lower than 0.1 indicating a homogenous distribution of the drug in the matrix tablets. The maximum value for formulations F1 to F4 was 277.70 mg and the minimum value was 273.80 mg. USP 34 (2011) norms require an average weight of 250 mg or more, and therefore, not more than two tablets are permitted to deviate from the mean weight by more than 5% and none more than 10%. These four

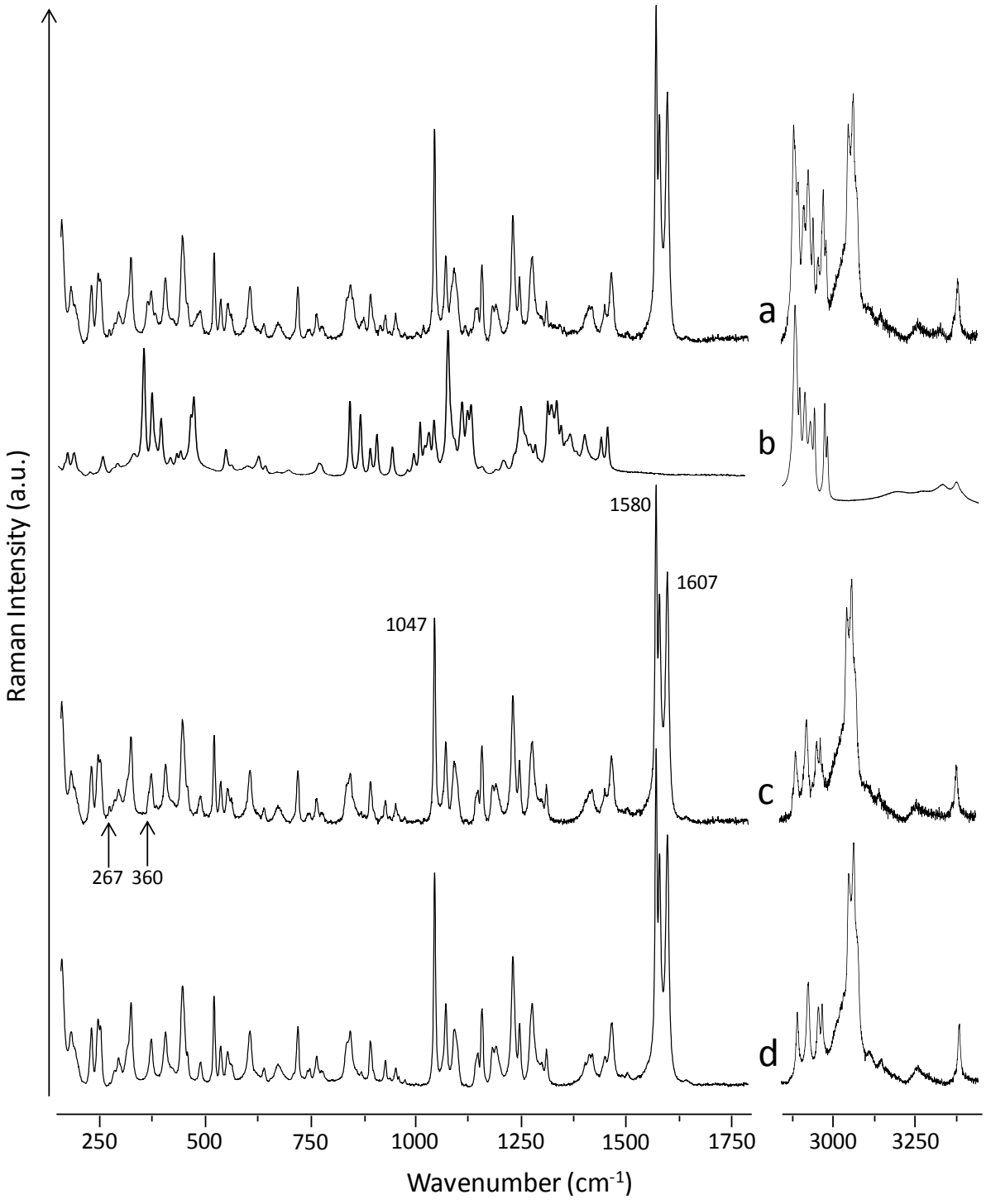

Figure 3. Raman spectra, in the 150 to 1800 and 2750 to 3450 cm^{-1} regions, of DS:LAC physical mixture (a), pure LAC (b), the result of subtraction (c), and pure DS (d).

formulations respected these criteria.

The hardness of the different formulations studied was within the range of 187.7 to 215.4 N ($F = 76.920$; $P < 0.001$), corresponding to obvious variations in the tablet tensile strength from 4.70 to 5.18 MPa ($F = 14.187$; $P < 0.001$), demonstrating a good solidity. The thickness of the tablets (Table 3) was found within the range of 2.55 to 2.65 mm ($F = 46.889$; $P < 0.001$), statistically different which may be influenced by the properties of each polymer. A lower percentage of friability is synonymous of

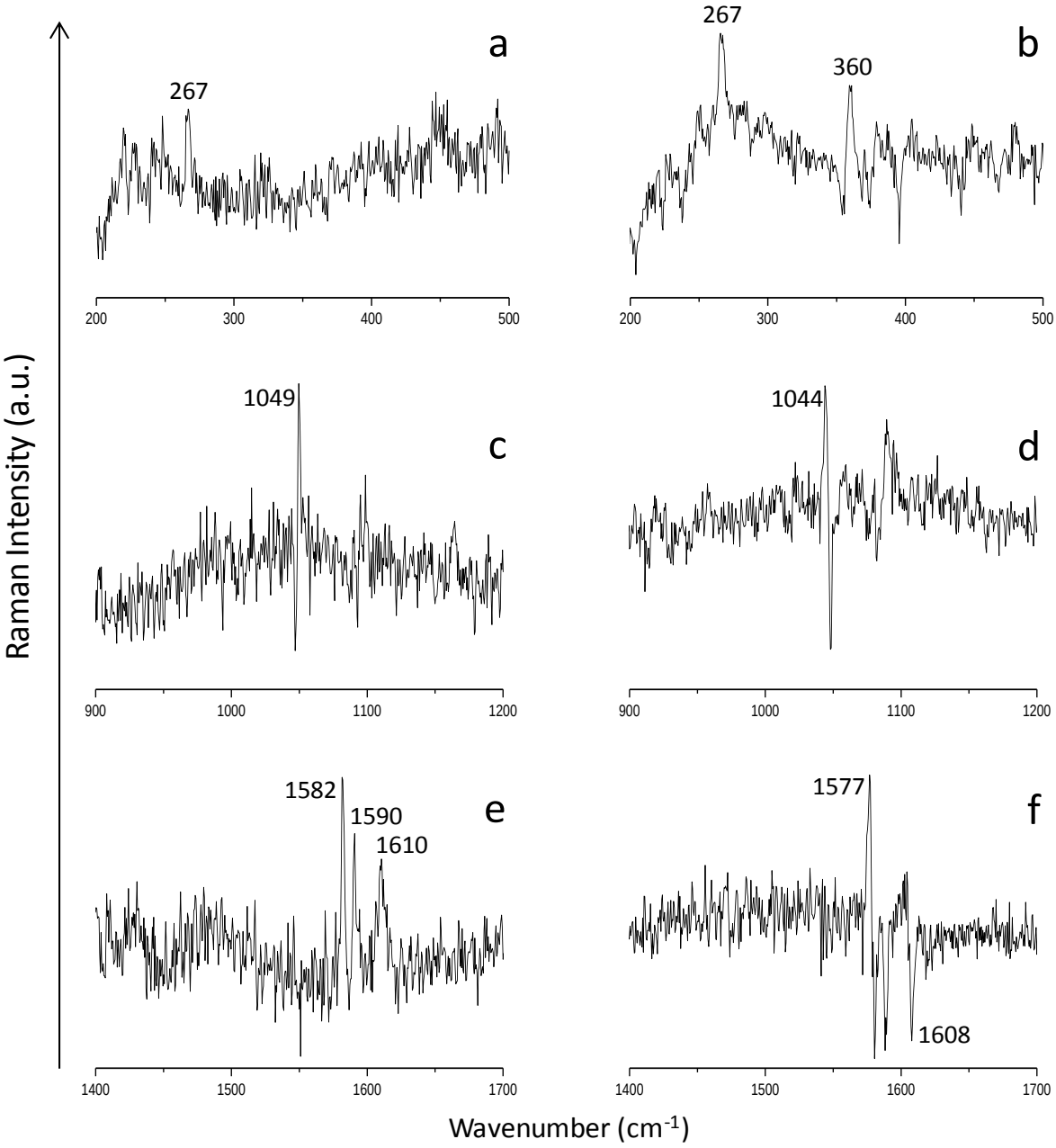

Figure 4. Different regions of the difference Raman spectra between the "DS changed" spectra and DS spectrum (see text) for: the HPMC K15M system (a), (c), (e); and LAC system (b), (d), and (f).

tablets compactness. Hence, these four formulations presented very similar compactibility since the results showed friability values in the interval of 0.10 to 0.23%, indicating that all formulations lie within the USP 34 (2011) limits.

Swelling studies

Hydrophilic polymer substances are well known to play a significant role in the swelling process of matrix tablets (Nerurkar et al., 2005). The swelling rate, and thus the formation of a continuous gel layer, was found to be strongly dependent on both polymer hydration speed and viscosity grade (Roy and Rohera, 2002). Moreover, the polymer hydration must be fast enough to allow the formation of the gel layer before the contents of the matrix tablet; in particular, the carried drug can dissolve. In this work, swelling studies were performed in order to assess the effect of the distinct formulations on the swelling process. When a matrix is immersed in a dissolution medium, wetting occurs, first at the surface

Table 3. Physical characterisation of DS matrix tablets.

Formulation	Drug content (mg)	Hardness (N)	Tensile strength (MPa)	Thickness (mm)	Friability (%)
	n=5	n=10	n=10	n=10	n=20
F1	95.04±0.44	187.70±4.59	4.694±0.223	2.55±0.01	0.21
F2	96.08±0.62	215.40±4.42	5.182±0.020	2.65±0.01	0.10
F3	97.11±1.31	202.40±3.80	4.946±0.183	2.61±0.02	0.15
F4	96.31±2.78	208.60±4.06	5.100±0.215	2.62±0.03	0.23

[a]n is the number of measurements.

Table 4. Descriptive statistics parameters of the formulations of DS tablets relating to the uniformity of mass (n = 30).

Formulation	Average (mg)	SD (mg)	RSD (%)	Median (mg)	Minimum (mg)	Maximum (mg)
F1	275.11	0.85	0.31	274.80	273.80	277.20
F2	275.69	0.93	0.34	275.50	274.20	277.60
F3	274.85	0.50	0.18	274.80	274.10	276.50
F4	275.92	0.66	0.24	275.90	274.70	277.70

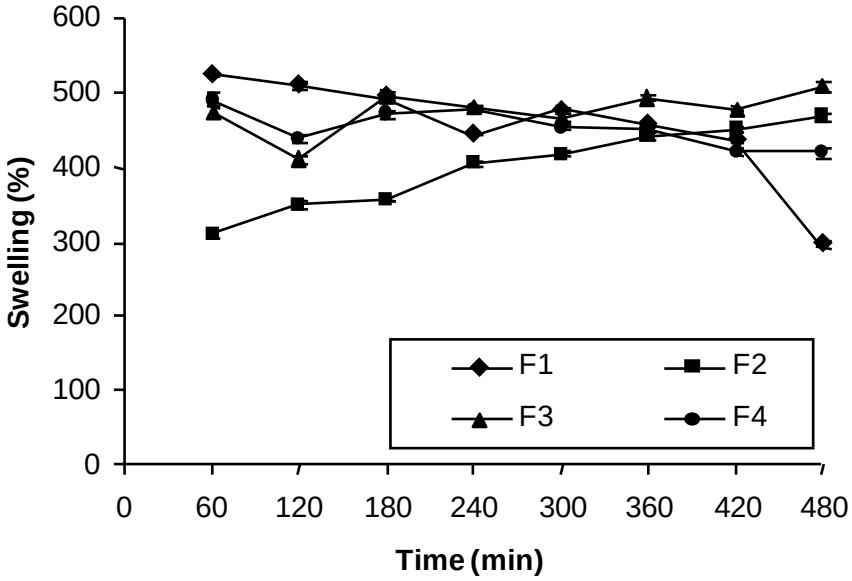

Figure 5. Graphical representation of the water uptake versus time of DS matrix tablets formulations containing different concentrations of HPMC K15M and binary mixtures of HPMC K15M with chitosan (Table 1).

and then progressing into the matrix (Sriamornsak et al., 2007). The results of the swelling studies are gathered in Figure 5. Formulations F1, F3, and F4 attained their maximum hydration degree (between 400 and 500%) during the first hour. However, while F3 and F4 roughly retain this hydration level; for F1, a progressive decrease was observed over the next 7 h. This may be explained by the low amount of the polymer and the higher quantity of LAC on the F1 formulation (Table 1). These results are in accordance with previous reported studies on the HPMC swelling behaviour (Borguist et al., 2006), explained by the presence of the substituent groups which, through interaction with water molecules, lead to an increased swelling. Formulation F2, in turn, due to the higher quantity of HPMC K15M and low amount of LAC, displayed a slower water uptake, but it attains the same level hydration after at 6 h. The polymer surface swells to form a continuous gel layer and the matrix size progressively increases (Gao et al., 1996).

The similarity observed for the swelling behaviour of F3

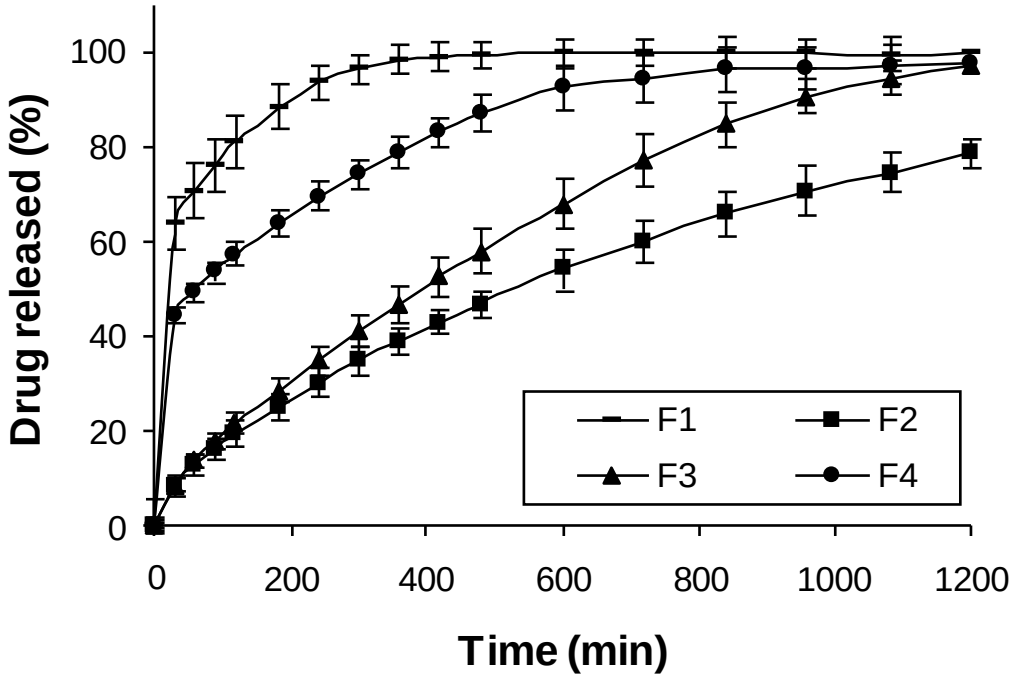

Figure 6. Dissolution profiles of DS matrix tablets formulations containing different concentrations of HPMC K15M and binary mixtures of HPMC K15M with chitosan (Table 1).

Table 5. Experimental results according to the dissolution parameters of DS matrix tablets.

Formulation	$t_{50\%}$ (h)	DE (%)	MDT (h)[a]	AUC	[b]P (%) 20 h
F2	8.86	50.27 ± 1.67	6.26 ± 0.13	1005.48	78.54 ± 0.02
F3	6.55	62.77 ± 1.48	4.53 ± 0.07	1255.38	97.06 ± 3.65
F4	1.07	83.92 ± 1.62	1.05 ± 0.05	1678.36	97.96 ± 5.03

[a]Mean ± SD (6 measurements). [b]P = percentage of DS dissolved at 20 h.

and F4 is quite interesting. In fact, the two formulations differ in the partial substitution of HPMC K15M (present in F3) by chitosan, keeping the LAC amount constant. The results now obtained may be an indication that the swelling characteristics of HPMC K15M and chitosan are comparable.

Drug release analysis

The DS release profiles from matrix tablets are as shown in Figure 6. In the case of the F1 formulation, more than 80% of the drug was released in about 3 h, due to the lower amount of polymer which leads to tablet disintegration (Abdelbary and Tadros, 2008). The slowest drug release, in turn, is observed for F2 (Figure 5), due to its highest polymer content. The gel layer viscosity increased, which results in a higher resistance to both dissolution and erosion (Borguist et al., 2006).

Concerning formulations F3 and F4, the presence of chitosan was found to have a marked effect on the drug release profile (Figure 6). In particular, the release of the drug from chitosan containing formulation (F4) was quite rapid in the first hour, but became slow after that, which may be attributed to the lower percentage of chitosan in the formulation (Akbuga, 1993). The MDT calculated values (Table 5) corroborated these observations. In effect, this parameter may be used to characterize both the drug release process and the retarding efficacy of a polymer; a higher value of MDT indicates a higher drug retarding ability of the polymer and *vice-versa*. On the other hand, DE is a dissolution parameter widely used as a significant index of drug dissolution performance. Actually, differences were detected between the calculated dissolution parameters (Table 5).

Previous work reported that Fickian diffusion through a hydrated chitosan-containing matrix is not the only mechanism that accounting for the release, as tablets with a low concentration of chitosan showed significant disintegration characteristics (Savaser et al., 2005). In fact, this quick drug release was attributed to the rapid chitosan dissolution, even in the presence of an interaction with a negatively charged (acidic) drug, such as DS (Puttipipatkhachorn et al., 2001). Burst release is

Table 6. Results of fitting the DS release data for F2 and F3 formulations to different kinetic equations*.

Formulation	Zero order		First order		Higuchi		Korsmeyer–Peppas			Peppas–Sahlin		
	k_0 (% min^{-1})	R^2	k_1 (min^{-1})	R^2	k_H (% $min^{-1/2}$)	R^2	k_{KP} (min^{-n})	*n*	R^2	k_F ($min^{-0.447}$)	k_R ($min^{-0.894}$)	R^2
F2	0.09501 (0.00115)	0.8285	0.00135 (0.00001)	0.9667	2.11984 (0.01033)	0.9717	0.00981 (0.00004)	0.6259 (0.0006)	0.9999	0.01647 (0.00012)	0.00082 (0.00001)	0.9993
F3	0.13036 (0.00143)	0.9123	0.00181 (0.00001)	0.9895	2.41834 (0.02222)	0.9385	0.00735 (0.00004)	0.7062 (0.0010)	0.9999	0.01262 (0.00007)	0.00152 (0.00001)	0.9999

*Values in parenthesis mean standard deviation; R^2 is the coefficient of determination.

often observed prior to or during development of a diffusion barrier capable of controlling the penetration of the dissolution medium and the drug diffusion process (Huang and Brazel, 2001).

This behaviour of low chitosan content matrices, combined with the HPMC retarding release ability, may be used to design formulations with a more rapid initial release, so that the drug reaches a suitable plasma level, followed by a slower release to maintain the desired (therapeutic, non-toxic) level.

Kinetic mechanism

DS (pK_a = 4.0) has a very poor solubility in water and aqueous acidic conditions, which gradually increases when the pH is raised above 6, and becomes freely soluble at pH = 7 (Liu et al., 1995). During this study, the experimental conditions were established for a pH = 6.8 phosphate buffer solution, used as the dissolution medium. Thus, both diffusion and erosion could contribute to the drug release process from the matrix tablets. In fact, in polymeric swellable hydrophilic matrices similar to the ones considered, water-soluble drugs are released mainly by diffusion across the gel layer, whilst barely water-soluble drugs are predominantly released by attrition mechanisms (Vazquez et al., 1992). Even if some processes could be characterised as either purely diffusional or purely erosion-controlled, several others could only be rationalised as being due to a coupling of both (Katzhendler et al., 2000). The use of Korsmeyer-Peppas (Equation 7), and particularly the interpretation of the release exponent values (n), allows the getting of an insight into the balance between these mechanisms (Costa and Sousa Lobo, 2001).

However, both F1 and F4 formulations must be discarded from this kind of analysis due to their very fast initial drug release, *ca.* 50 and 40% of the drug was released during the first 10 min, respectively (Figure 6).

For the F2 and F3 formulations, n was found to be equal to 0.626 and 0.706, respectively (Table 6), indicating an anomalous (non-Fickian) mechanism transport, best described by a coupling of diffusion and macromolecular relaxation processes. The difference between these two values confirms the previously mentioned dependence of the DS release mechanism on the HPMC K15M concentration.

To assess the relative contribution of Fickian diffusion and polymer relaxation (erosion) mechanisms, over the first 60% of the drug release, for formulations F2 and F3, the values of the Fickian constant, k_F, and the relaxational constant, k_R, were calculated according Equation 8 (Table 6). The estimated contributions of the two mechanisms are presented graphically in Figure 7.

The release rate constant values (Table 6) permits the conclusion that for both the F2 and F3 formulations, diffusion was the primary release mechanism and polymer relaxation was the secondary one.

In the case of the F2 formulation, the Fickian mechanism predominates during the first 14 h of the release process (Figure 7a). Such behaviour is very well explained, since the HPMC polymer concentration in the formulation is around 36%. Consequently, an increase of the viscous gel layer around the matrix creates a longer path length for diffusion (Sujja-areevath et al., 1998).

For the F3 formulation, in turn, the decrease of hydrophilic polymer amount to *ca.* 31%, coupled to an equalizer increase of LAC, causes a drastic change in the diffusion/erosion mechanisms balance (Figure 7b). In fact, for this formulation, the diffusion dominates only during the first 2 h of the dissolution time, while polymer relaxation rapidly increases and hence becomes

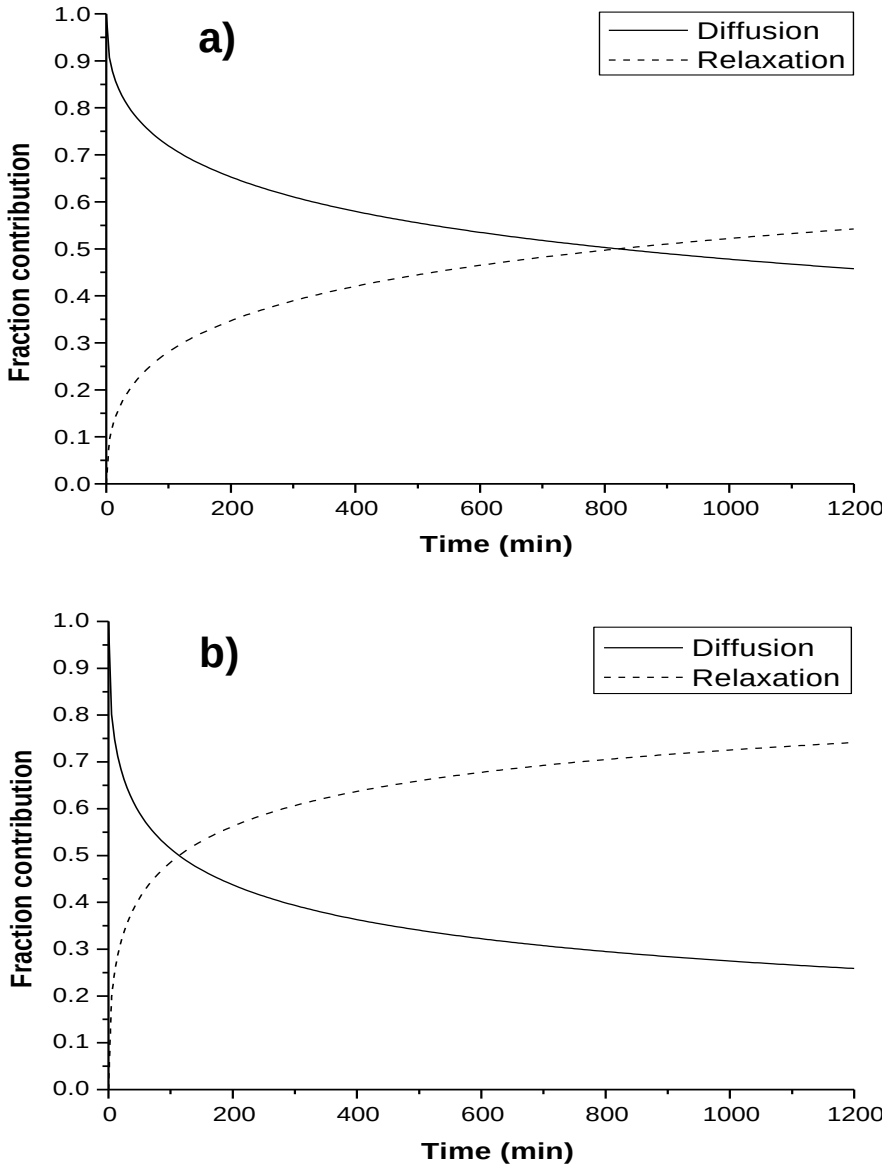

Figure 7. Fraction contribution of the Fickian diffusion and the erosion mechanisms (a) for formulation F2 (k_F = 0.01647 $min^{-0.447}$ and k_R = 0.00082 $min^{-0.894}$) and (b) for formulation F3 (k_F = 0.01262 $min^{-0.447}$ and k_R = 0.00152 $min^{-0.894}$).

predominant for the rest of the process. The contribution of polymer relaxation to the drug release mechanism was found to increase as the HPMC K15M concentration decreased as evidenced by the magnitude of the corresponding k_R values (Table 6). This behaviour confirms the previously mentioned dependence of the DS release mechanism on the polymer concentration.

Furthermore, these results are consistent with the Korsmeyer-Peppas exponent calculated values indicating an anomalous transport, but in which the Fickian contribution is greater for F2 formulation, corresponding to a smaller value of n (0.626) when compared with that obtained for F3 (n = 0.706).

Conclusions

Matrix formulations containing DS and different concentrations of HPMC K15M or HPMC K15M/chitosan were assessed for their drug content, weight uniformity, hardness, thickness, tensile strength, friability, porosity, swelling, and drug release performance. From the DSC thermograms of the mixtures tested, it was possible to detect some drug:excipient interactions. Raman spectroscopy data allowed the conclusion that these interactions occur mainly between the diclofenac COO^- functional group and the polymers. However, no intermolecular close contacts, which could eventually

lead to an incompatibility between the drug and the different formulation components, were detected. Hence, the selected excipients are suitable for the preparation of tablet formulations.

Regarding the DS release from matrix formulations, the results presently obtained indicate that low concentrations of HPMC K15M do not control the release of the drug. The release mechanisms of DS from formulations F2, F3, and F4 were evaluated using, among others, the Korsmeyer-Peppas kinetic model. Only for F2 and F3, could a clear fitting be obtained, reflecting an anomalous transport mechanism. A decrease in polymer concentration was found to lead to a marked change in the drug release characteristics, that is, in the diffusion/erosion balance, assessed by the Peppas-Sahlin model.

For the chitosan containing formulation (F4), a burst release is detected prior to the drug diffusion through the matrix. This behaviour of low chitosan content matrices, combined with the HPMC K15M retarding release ability, may be used to design formulations with a more rapid initial release, so that the drug reaches a suitable plasma level, followed by a slower release to maintain the desired therapeutic level.

ACKNOWLEDGEMENTS

This work was financially supported by a grant (PROGRAMA CIÊNCIA GLOBAL-SFRH/BPD/51473/2011) from FCT (Fundação para a Ciência e a Tecnologia, Portugal) and Instituto Superior de Ciências da Saúde da Universidade Agostinho Neto, Angola.

REFERENCES

Abdelbary GA, Tadros MI (2008). Design and in vitro/in vivo evaluation of novel nicorandil extended release matrix tablets based on hydrophilic interpolymer complexes and a hydrophobic waxy polymer. Eur. J. Pharm. Biopharm. 69:1019-1028.

Adeyeye CM, Li PK (1990). Diclofenac sodium. In: Florey K. (eds.) Analytical Profiles of Drug Substances. Vol. 19. New York: Academic Press pp. 123-141.

Akbuga J (1993). Use of chitosonium malate as a matrix in sustained-release tablets. Int. J. Pharm. 89:19-24.

Balestrieri F, Magri AD, Magri AL, Narini D, Sacchini A (1996). Application of differential scanning calorimetry to the study of drug excipient compatibility. Thermochemica Acta 285:337-345.

Batista de Carvalho LAE, Marques MPM, Tomkinson J (2006). Drug–Excipient interactions in ketoprofen: a vibrational spectroscopy study. Biopolymers 82:420-424.

Borguist P, Korner A, Larsson A (2006). A model for the drug release from a polymeric matrix tablets-effect of swelling and dissolution. J. Control. Rel. 113:216-225.

Bravo SA, Lamas MC, Salomón CJ (2002). In-vitro studies of diclofenac sodium controlled-release from biopolymeric hydrophilic matrices. J. Pharm. Sci. 5:213-219.

Costa P, Sousa Lobo MJ (2001). Modeling and comparison of dissolution profiles. Eur. J. Pharm Sci. 13(2):123-133.

Efentakis M, Vlachou M, Choulis NH (1997). Effects of excipients on swelling and drug release from compressed matrices. Drug Dev. Ind. Pharm. 23(3):107-112.

Fell JT, Newton JM (1970). Determination of tablet strength by diametral-compression test. J. Pharm. Sci. 59:688-691.

Ford JL (1999). Thermal analysis of hydroypropylmethylcellulose and methylcellulose: powders, gels and matrix tablets. Int. J. Pharm. 179:209-228.

Fowler PD, Shadforth MF, Crook PR, John VA (1983). Plasma and synovial fluid concentration of diclofenac sodium and its major hydroxylated metabolites during long-term treatment of rheumatoid arthritis. Eur. J. Clin. Pharmacol. 25:389-394.

Gao P, Skoug JW, Nixon PR, Ju TR, Stemm NL, Sung KC (1996). Swelling of hydroxypropyl methylcellulose matrix tablets. 2. Mechanistic study of the influence of formulation variables on matrix performance and drug release. J. Pharm. Sci. 85 (7):732-740.

George M, Abraham TE (2006). Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan - a review. J. Control. Rel. 114:1-14.

Ghimire M, Hodges LA, O'Mahony JBB, McInnes FJ, Mullen AB, Stevens HNE (2010). In-vitro and in-vivo erosion profiles of hydroxypropylmethylcellulose (HPMC) matrix tablets. J. Control. Rel. 147 (1):70-75.

Higuchi T (1961). Rate of release of medicaments from ointment bases containing drugs in suspension. J. Pharm. Sci. 50:874-875.

Higuchi T (1963). Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J. Pharm. Sci. 52:1145-1149.

Hiremath PS, Saha RN (2008). Oral controlled release formulations of rifampicin. Part II: Effect of formulation variables and process parameters on in vitro release. Drug Deliv. 15(3):159-168.

Huang X, Brazel CS (2001). On the importance and mechanisms of burst release in matrix-controlled drug delivery systems. J. Control. Rel. 73:121-136

Iliescu T, Baia M, Kiefer W (2004). FT-Raman, surface-enhanced Raman spectroscopy and theoretical investigations of diclofenac. Chem. Phys. 298:167-174.

Katzhendler I, Mader K, Friedman M (2000). Structure and hydration properties of hydroxypropyl methylcellulose matrices containing naproxen and naproxen sodium. Int. J. Pharm. 200:161-179.

Khalid MN, Agnely F, Yagoubi N, Grossiord JL, Couarraze G (2002). Water state characterization, swelling behaviour, thermal and mechanical properties of chitosan based networks. Eur. J. Pharm. Sci. 15:425-432.

Khan KA (1975). The concept of dissolution efficiency. J. Pharm. Pharmacol. 27:48-49.

Khan KA, Rhodes CT (1972). Effect of compaction pressure on the dissolution efficiency of some direct compression systems. Pharm. Acta Helv. 47:594-607.

Kim H, Fassihi R (1997). Application of a binary polymer system in drug release rate modulation. 1. Characterization of release mechanism. J. Pharm. Sci. 86:316-322.

Korsmeyer RW, Gurny R, Doelker EM, Buri P, Peppas NA (1983). Mechanism of solute release from porous hydrophilic polymers. Int. J. Pharm. 15: 25-35.

Li LL, Martini GL, Ford LJ, Roberts M (2005). The use of hypromellose in oral drug delivery. J. Pharm. Pharmacol. 57:533-546.

Lu DR, Abu-Izza K, Chen W (1996). Optima: a windows-based program for computer-aided optimisation of controlled-release dosage forms. Pharm. Dev. Technol. 1(4):405-414.

Mahendran KH, Nagaraj S, Sridharan R, Gnanasekaran T (2001). Differential scanning calorimetric studies on the phase diagram of the binary LiCl–CaCl system. J. Alloys and Compounds. 325(1-2): 78-83.

Majeti NV, Ravi K (2000). A review of chitin and chitosan applications. Reactive and Functional Polymers, 46: 1–27.

Marques MPM, Oliveira PJ, Moreno AJM, Batista de Carvalho LAE (2002). Study of Carvedilol by Combined Raman Spectroscopy and Ab Initio MO Calculations. J. Raman Spectrosc. 33 (10):778-783.

Mourão S, Silva C, Bresolina TMB, Serrab CHR, Porta V (2010). Dissolution parameters for sodium diclofenac-containing hypromellose matrix tablet. Int. J. Pharm. 386:201-207.

Mura P, Manderioli A, Bramati G, Furlanetto S, Pinzauti S (1995). Utilization of differential scanning calorimetry as a screening technique to determine the compatibility of ketoprofen with excipients.

Int. J. Pharm. 119 (1):71-79.
Nerurkar J, Jun HW, Price JC, Park MO (2005). Controlled-releasematrixtablets of ibuprofen using celluloseethers and carrageenans: effect of formulation factors on dissolution rates. Eur. J. Pharm. Biopharm. 61:56-68.
Peppas N, Sahlin J (1989). A simple equation for the description of solute release. III. Coupling of diffusion and relaxation. Int. J. Pharm. 57:169-172.
Peppas NA (1985). Analysis of Fickian and non-Fickian drug release from polymers. Pharm. Acta Helv. 60(4):110-111.
Puttipipatkhachorn S, Nunthanid J, Yamamoto K, Peck GE (2001). Drug physical state and drug–polymer interaction on drug release from chitosan matrix films. J. Control. Rel. 75:143-153.
Ritger PL, Peppas NA (1987). A simple equation for description of solute release. II. Fickian and anomalous release from swellable devices. J. Control. Rel., 5: 37-42.
Roy DS, Rohera BD (2002). Comparativeevaluation of rate of hydration and matrix erosion of HEC and HPC and study of drug release from their matrices. Eur. J. Pharm. Sci. 16 (3):95-220.
Samani SM, Montaseri H, Kazemi A (2003). The effect of polymer blends on release profiles of diclofenac sodium from matrices. Eur. J. Pharm. Biopharm. 55:351-355.
Santos JV, Pina MET, Marques, MPM, Batista de Carvalho LAE (2012). New sustained release of Zidovudine Matrix tablets − cytotoxicity toward Caco-2 ells. Drug Dev. Ind. Pharm., Online. DOI: 10.3109/03639045.2012.669129.
Savaser A, Ozkan Y, Ismer A (2005). Preparation and in vitro evaluation of sustained release tablet formulations of diclofenac sodium. IL Farmaco. 60:171-179.
Siepmann J, Streubel A, Peppas NA (2002). Understanding and Predicting Drug Delivery from Hydrophilic Matrix tablets using the “sequential layer” model. Pharm. Res. 19:306-314.
Sipos P, Szucs M, Szabo A, Eros I, Szabo-Revesz P (2008). An assessment of the interactions between diclofenac sodium and ammonio methacrylate copolymer using thermal analysis and Raman spectroscopy. J. Pharm. Biol. Anal. 46:288-294.
Sriamornsak P, Thirawonga N Weerapola Y, Nunthanida J, Sungthongjeenc JNS (2007). Swelling and erosion of pectin matrix tablets and their impact on drug release behavior. Eur. J. Pharm. Biopharm. 67(1):211-219.
United States Pharmacopeia (2011). The National Formulary 29. 34/ USP Convention, Rockville, USA.
Vazquez MJ, Perez-Marcos B, Gomes-Amoza JL, Martinez-Pacheco R, Souto C, Concheiro A (1992). Influence of technological variables on release of drugs from hydrophilic matrices. Drug Dev. Ind. Pharm. 18:1355-1375.
Verma RK, Garg S (2004). Compatibility studies between isosorbide mononitrate and selected excipients used in the development of extended release formulations. J. Pharm. Biol. Anal. 35:449-458.
Vueba ML, Batista de Carvalho LAE, Veiga F, Sousa JJ, Pina ME (2004). Influence of cellulose ethers polymers on ketoprofen release from hydrophilic matrix tablets. Eur. J. Pharm. Biopharm. 58: 51-59.
Vueba, ML, Veiga, F, Sousa, JJ, Pina, ME (2005a). Compatibility Studies Between Ibuprofen or Ketoprofen with Cellulose Ether Polymer Mixtures Using Thermal Analysis. Drug Dev. Ind. Pharm. 31:943-949.
Vueba, ML, Batista de Carvalho, LAE, Veiga, F, Sousa, JJ, Pina, ME (2005). Role of cellulose ether polymers on ibuprofen release from matrix tablets. Drug Dev. Ind. Pharm. 31:653-665.
Vueba ML, Pina ME, Veiga F, Sousa JJ, Batista de Carvalho LAE (2006). Conformational study of ketoprofen by ab initio calculations and Raman spectroscopy. Int. J. Pharm. 307 (1):56-65.
Vueba ML, Pina ME, Batista de Carvalho LAE (2008). Conformational Stability of Ibuprofen Assessed by DFT Calculations and Optical Vibrational Spectroscopy. J. Pharm. Sci. 97 (2):834-848.
Zalac S, Zahirul M, Khan I, Gabelica V, Tudja M, Mestrovi E, Romih M (1999). Paracetamol–Propyphenazone Interaction and Formulation Difficulties Associated with Eutectic Formation in Combination Solid Dosage Forms. Chem. Pharm. Bull. 47 (3):302-307.