Polymer Bulletin 59, 597–605 (2007)
DOI 10.1007/s00289-007-0803-8

Polymer Bulletin

Preparation of β-Cyclodextrin-Dextran Polymers and their Use as Supramolecular Carrier Systems for Naproxen

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Received: 4 March 2007 / Revised version: 1 June 2007 / Accepted: 17 June 2007
Published online: 30 June 2007 –

Summary

Dextran, previously activated by periodate oxidation, was grafted with mono-6-amino-6-deoxy-β-cyclodextrin, mono-6-ethylenediamino-6-deoxy-β-cyclodextrin, and mono-6-butylenediamino-6-deoxy-β-cyclodextrin by reductive alkylation in the presence of $NaBH_4$. The polymers were able to form inclusion complexes with Naproxen, increasing the solubility of the drug by 2.2-2.6 folds. The β-cyclodextrin-grafted dextrans were used as macromolecular carriers for Naproxen, improving the "in vivo" anti-inflammatory activity of the drug.

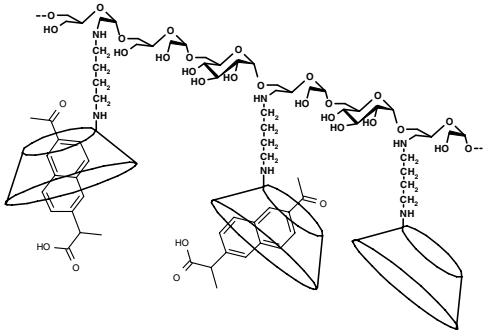
Keywords

Drug delivery systems, dextran, polysaccharides, supramolecular complexes, fluorescence, anti-inflammatory, cyclodextrin

Introduction

In the last years, different types of carriers have been used to improve the therapeutic effects and reduce toxicity of drugs. These carriers regulate the pharmacokinetic properties of the drug and are able to liberate them in a site-specific mode. Both synthetic and natural polymers have been reported as drug carriers [1, 2]. For such application the polymer must be biodegradable, biocompatible and contain moieties that can be chemically transformed. Besides that, the polymer and its degradation products must not be immunogenic and easily excreted [3]. Dextran, chitin and albumin are among the natural polymers submitted to clinical trials as drug carriers, while poly(vinyl maleic anhydride) copolymers (DIVEMA) and poly(styrene maleic anhydride) (SMA) are among the synthetic polymers mostly used [4,5]. Studies in this direction have received great attention nowadays.

Cyclodextrins and their derivatives have been widely used in the pharmaceutical industry in control-release systems [6]. β-cyclodextrin (βCD) and its derivatives have received the major attention in this sense due to its low prices. Its structural characteristics have made it an excellent host for the formation of inclusion complexes with diverse drugs [7]. The formation of inclusion complexes of a drug with βCD favors an increase in its water solubility, stability in biological fluids and attenuates or eliminates different types of side effects of the drug [8, 9].
Physical mixtures of βCD and its derivatives with hydrosoluble macromolecules such as poly(vinylpirrolidone) (PVP) and carboxymethylcellulose (CMC) can increase the water solubility of different drugs, a property that has found a wide number of applications [10]. Nevertheless, little effort has been made on the use of covalently bonded βCD to natural or synthetic polymers as carriers for low molecular weight drugs. In this sense, we have recently reported the preparation of βCD-polysaccharide conjugates and their interaction with Naproxen [11].
In the present work we describe the preparation of dextran with covalently bound β-cyclodextrin derivatives having different spacer arms. These functionalized polysaccharides were evaluated as supramolecular carriers for Naproxen (Scheme 1).



Scheme 1. Supramolecular complex between NAP and Dex-CDBN.

Naproxen ((*S*)-(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid, NAP) is a well-known commercial non-steroidal anti-inflammatory drug, but its pharmacological applications are currently limited by its deleterious effects on the gastrointestinal tract, low water solubility and short plasma half-life [12].

Materials and Methods

Materials

Dextran was purchased from Amersham-Pharmacia (Sweden) and its molecular weight (7×10^4) was determined by HPLC-GPC. Naproxen, sodium borohydride, sodium meta-periodate and other chemicals were purchased from Merck (Germany). βCD was received from Amaizo (USA). Male albino Wistar rats were purchased from the National Centre of Laboratory Animal Production (CENPALAB, Cuba).

Synthesis of β-CD-amino-derivatives

The βCD-amino-derivatives used in this study, namely: mono-6-amino-6-deoxy-β-cyclodextrin ($CDNH_2$), mono-6-ethylenediamino-6-deoxy-β-cyclodextrin (CDEN)

and mono-6-buthylenediamino-6-deoxy-β-cyclodextrin (CDBN) were prepared by treating mono-6-*O*-tosyl-β-cyclodextrin [13] either with concentrated ammonia [14] or the corresponding freshly distilled diamine [15]. The products were purified by Sephadex C-25 (NH_4^+ form) chromatography and the identity and purity of the obtained products was verified by TLC, ^{1}H- and ^{13}C-NMR and FABMS.
CDNH$_2$: ^{1}H NMR(D_2O) δ 2.75 (dd, 1H, H-6`), 2.98(dd, 1H, H-6`), 3.40 (t, 1H, H-4`), 3.47–4.05 (m, 39H, H-2, H-3, H-4, H-5, H-5`, H-6), 5.05 (m, 7H, H-1). ^{13}C NMR (D_2O) δ 36.2 (CH_3), 52.8 (C-6`), 61.5 (C-6), 71.5 (C-5`), 72.9, 73.21 (C-3, C-5), 74.2 (C-2), 82.4 (C-4), 85.2 (C-4`), 103.0 (C-1). FABMS *m/z* 1169.3 $(M + H_2O + H)^+$.
CDEN: ^{1}H NMR (D_2O) δ 2.7–2.9 (m, 3H, H-6`, NCH2), 3.10 (dd, 1H, H-6`), 3.21 (t, 2H, NCH2), 3.45 (t, 1H, H-4`), 3.5–4.1 (m, 39H, H-2, H-3, H-4, H-5, H-5`, H-6), 5.1 (m, 7H, H-1). 13C NMR (D2O) δ 27.6 (CH2NH2), 41.1 (CH2NH), 52.6 (C-6_), 61.2 (C-6), 71.4 (C-5), 72.9, 73.0 (C-3, C-5), 74.3 (C-2), 82.2 (C-4), 85.5 (C-4`), 103.0 (C-1). FABMS *m/z* 1177.2 $(M + H)^+$.
CDBN: ^{1}H NMR (D_2O) δ 2.3–2.5 (m, 4H, CH_2) 2.7–2.9 (m, 3H, H-6`, $NCH_2$), 3.11 (dd, 1H, H-6`), 3.23 (t, 2H, CH2), 3.47 (t, 1H, H-4`), 3.5–4.1 (m, 39H, H-2, H-3, H-4, H-5, H-5, H-6), 5.1 (m, 7H, H-1). $^{13}$C NMR ($D_2O$) $\delta$ 21.5, 23.4 ($CH_2$), 26.1 ($CH_2NH_2$), 40.0 ($CH_2NH$), 52.7 (C-6`), 61.0 (C-6), 71.8 (C-5`),72.5–74.0 (C-2, C-3, C-5), 82.8 (C-4), 86.7 (C-4`),103.1 (C-1). FABMS *m/z* 1205.2 $(M + H)^+$.

Synthesis of dextran dialdehyde

2 g of $NaIO_4$ were added to a 60 mL aqueous solution of dextran (2 g). The reaction mixture was stirred in dark at 4ºC for 1 h, after which 2 mL of ethyleneglycol were added and the mixing was continued for another hour. The solution was further exhaustively dialysed against distilled H_2O and kept at -20^0C.

Preparation of CD-grafted dextrans

2 g of dextran dialdehyde in 30 mL of water were reacted with 4 g of the corresponding βCD-amino-derivatives and $NaBH_4$ was added in order to achieve a final concentration of 0.2 mol/L. The reaction mixture was stirred for 4 h. The modified polymer solutions were further dialyzed against distilled H_2O and finally lyophilized. The three products are represented as Dex-CDNH$_2$, Dex-CDEN and Dex-CDBN corresponding to the interaction of dextran dialdehyde with CDNH$_2$, CDEN and CDBN, respectively. The molecular weights of the polymers were determined by analytical GPC-HPLC on TESEK Hema-bio columns 40, 100, 300 and 1000 (4 × 30 cm) calibrated with dextran standards. The degree of substitution was estimated by ^{1}H-NMR spectrometry using a Bruker DRX-500 apparatus as described bellow.

Determination of the stability constants

The formation of the host-guest complexes between NAP and the CD-grafted dextrans were evaluated in phosphate saline solution at 37 ºC by fluorescence spectroscopy on a Perkin Elmer LS 50B apparatus. The stability constants (K_{st}) were determined from the fluorescence variations using the following modified Benesi-Hildebrand equation (double reciprocal plot) [16]:

$$\frac{1}{(F - F_o)} = \frac{1}{(K_{st}kQ[NAP]_o[CD]_o)} + \frac{1}{(kQ[NAP]_o)}$$

where F and F_o represent the fluorescence signals of NAP in the presence and absence of CD forms, $[NAP]_o$ and $[CD]_o$ represent the initial concentration of the drug and CD derivative, k is an instrumental constant, K_{st} is the stability constant of the complex, and Q is the quantum yield for the complex. The values of K_{st} were thus obtained from the slope and intercept of the $1/(F\text{-}F_0)$ *vs* 1/[CD] plot.

Solubility studies

Solubility measurements of NAP were carried out by adding an excess of drug (30 mg) to 1 mL of solution 15 mg/mL in β-cyclodextrin forms in 10 mM phosphate buffer pH 7.4 (also containing 120 mM NaCl and 2.7 mM KCl). The solution of grafted polymers and βCD were sealed in glass containers and magnetically stirred at constant temperature (37.0 ºC ± 0.3) until equilibrium was achieved (1 day). An aliquot was withdrawn and filtered (pore size 0.45 μm) and the NAP concentration was determined at 330 nm using a calibration curve. Control experiments showed that the presence of polymer in the solution did not interfere with the NAP determination. The results presented are mean values of at least three determinations.

Carrageenan-induced paw oedema test

This test was carried out as described by Boughon-Smith et al. [17] Male Wistar rats (250 – 300 g, 10 animals per group corresponding to each drug formulation) were injected in the planar aponeurosis of the right hind paw with 100 μL of 1% carrageenan suspended in 0.9% NaCl. Naproxen formulations (4 mg NAP/kg, 20 mg polymer/kg), dissolved in phosphate buffer saline, pH 7.4, were administered intraperitoneally after 1 h of the carrageenan injection. Physical mixtures of CD and the native polysaccharide were also tested in order to evaluate any effect not mediated by the covalent nature of the host carriers. Paw volume was measured by plethysmometry 4 h after sample administration. Oedema was determined by subtracting the volume of the control paw (only saline injected, without NAP or any sugar compound) from that of the treated paw. The inhibition of oedema was calculated in percent with reference to the negative control group.

Statistical Methods

Microcal Origin 7.0 software (Microcal Software, Inc., MA, USA) was used for all statistical analyses. The data were analysed by ANOVA, and the mean values were compared using Tukey's test. Differences were considered to be significant at $P < 0.05$.

Results and Discussion

Activation of the hydroxyl groups of β-cyclodextrin by treatment with an electrophilic reagent is the most common method employed to attach a substituent on the βCD core. For this reason, the well known mono-6-*O*-tosyl derivative of βCD was first prepared and subsequently treated with ammonia or the corresponding diamine to form the required βCD amines ($CDNH_2$, CDEN and CDBN) through a nucleophilic substitution reaction in good yields. The aminated CD derivatives were purified by cation exchange chromatography over CM-Sephadex C-25 and gave satisfactory NMR and mass spectra.

The aminated derivatives were then linked to dextran pre-activated by oxidation with $NaIO_4$ through a reductive alkylation reaction. As a result, three CD-grafted dextrans: Dex-$CDNH_2$, Dex-CDEN and Dex-CDBN that present high molar contents in βCD were obtained (Table 1).

Table 1. βCD content in the CD-grafted polymers

CD-grafted polymers	mol β-CD / mol polymer
Dex-$CDNH_2$	135
Dex-CDEN	98
Dex-CDBN	92

Figure 1 shows the 500 MHz ^{1}H-NMR spectra of dextran and its CDBN derivative in D_2O. In the spectrum of dextran, only one signal is observed in the anomeric proton region (4.9–5.1 ppm) while in Dex-CDBN a new peak corresponding to the anomeric protons of CD appears at 5.05 ppm. In addition, the resonances associated to the central methylene groups of the butylenediamine bridge are evident at higher field (1.4-1.7 ppm). In spite of the polymeric nature of these materials and the relative signal broadening observed, we were able to evaluate the average CD content in the three derivatives from their proton spectra by calculating the integration ratio between the signals at 2.5–3.2 ppm (corresponding to the CH_2 groups of the alkyl spacer and the substituted C-6 of the CD residue) and those of the anomeric protons of the carrier (4.9–5.3 ppm), and considering the number of protons involved in both groups of signals. Alternatively, the CD content was also confirmed by the ratio between the signals corresponding to the anomeric protons. On the other hand, the molecular weight of these modified carriers was determined by analytical GPC as $(2.3 \pm 0.4) \times 10^5$, $(1.9 \pm 0.2)\ 10^5$ and $(1.8 \pm 0.4) \times 10^5$ for Dex-CDBN, Dex-$CDNH_2$ and Dex-CDEN, respectively.

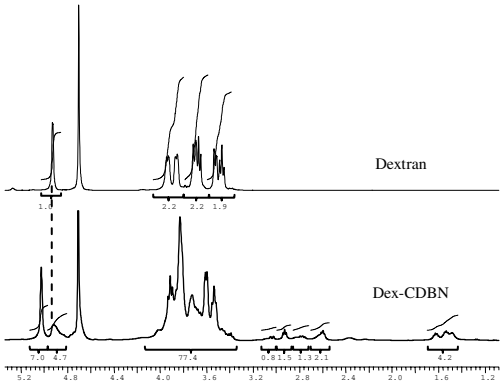


Figure 1. ^{1}H-NMR spectrum of Dextran and Dex-CDBN in D_2O (20 mg/ml).

Naproxen is a non–steroidal anti-inflammatory drug that exhibits fluorescence. In aqueous solution, a decrease in quantum yield has been detected for NAP and other

naphthalene derivatives when solubilized in protic solvents [18,19]. The reason of this behavior has been attributed to solute–solvent intermolecular hydrogen bond formation. The effects of Dex-CDBN concentration on the emission spectrum of Naproxen is shown in Figure 2. A pronounced increase in the peak emission intensity is observed as Dex-CDBN concentration increases. These changes clearly indicate the inclusion of the drug within the apolar CD cavity. The formation of the inclusion complex produced a hyperchromic effect on the absorption and emission spectra of NAP. It is well known that the enhancement of the luminescent processes of luminophores partially or completely encapsulated in the CD cavity is a result of the better protection from quenching and other processes that occur in the bulk solvent [20,21]. Similar behaviors in the fluorescence spectral characteristics have been reported for other related non-steroidal anti-inflammatory drugs after interaction with CD derivatives [22].

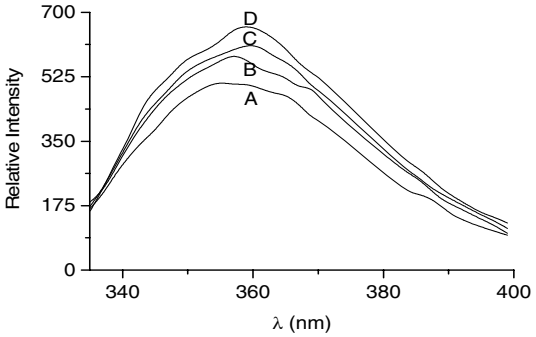


Figure 2. Fluorescence emission spectra of Naproxen in the absence (A) and the presence of Dex-CDBN polymer at equivalent concentrations of CD of 0.1 mM (B), 0.3 mM (C) and 0.7 mM (D).

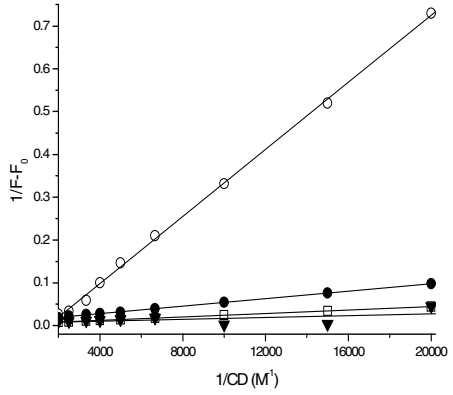


Figure 3. Double reciprocal (Benesi-Hildenbrand) plot of NAP in the presence of βCD (○), Dex-CDNH2 (●), Dex-CDEN (□), Dex-CDBN (▼) at 360 nm.

Figure 3 shows the Benesi-Hildenbrand plots corresponding to all formulations of NAP with the different CD forms. As can be seen, the 1/ΔF vs 1/[CD] plot is linear in the concentration range studied and is an indication of the existence of a complex with 1:1 host:guest composition. This result is in accordance with the results reported by other authors. Brown et al. have studied the inclusion behavior of Naproxen with four

different cyclodextrins and they have observed that the most adequate geometrical fitting occurs between NAP and βCD. Besides, the examination of the Corey-Pauling-Koltun space–filling molecular models confirms that the internal diameter of the βCD cavity match perfectly with the size of the naphthalene moiety [23]. In addition, computer-aided molecular modeling studies of the NAP-βCD complex and negative ion fast atom bombardment measurements have confirmed the existence of a 1:1 inclusion complex of NAP-βCD [24, 25].

The stability constants, K_{st}, for the complexes between NAP and βCD, Dex-CDNH$_2$, Dex-CDEN and Dex-CDBN were determined from the double reciprocal plots [26]. In this regard, K_{st} for NAP:CD, NAP: Dex-CDNH$_2$, NAP: Dex-CDEN and NAP: Dex-CDBN complexes were estimated as 1830, 2600, 2500 and 2200 M^{-1}, respectively. It is noticeable that CD forms more stable inclusion complexes with NAP after covalent attachment to neutral polysaccharides (K_{st} (NAP:CD) =1830 M^{-1}). The addition of macromolecular carrier resulted in a stability constant increase that varied from a minimum of 20 % to a maximum of 42% depending of the CD-grafted polymers.

Figure 4 depicts the effect of the CD-grafted polymers, at 20mg/kg doses, on the anti-inflammatory properties of NAP.

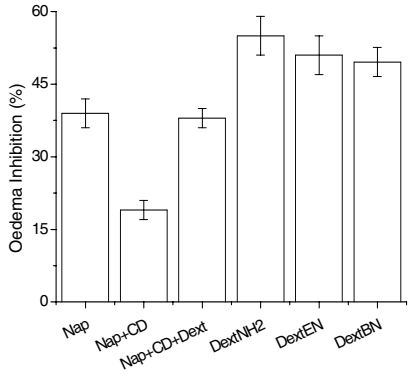


Figure 4. Anti-inflammatory activity of NAP formulations in the carrageenan-induced paw oedema test. Doses: NAP = 4 mg/kg, polymers = 20 mg/kg.

Table 2. Solubility of NAP formulation in pH 7.4 buffer at 37ºC.

Formulation	Solubility (mg/ml)
NAP	2.0 ± 0.1
NAP:CD	2.6 ± 0.3
NAP: Dex-CDNH$_2$	5.1 ± 0.1
NAP: Dex-CDEN	4.64 ± 0.03
NAP: Dex-CDBN	4.4 ± 0.2

Interestingly, the pharmacological activity of the drug was not improved by the addition of βCD and its physical mixtures with the native polymer. On the contrary, an increase in the anti-inflammatory activity of NAP was observed in the presence of the CD-grafted polysaccharides. This difference should be attributed to the inclusion ability and of the novel carrier prepared, which is expressed in the observed increase

in the water solubility of NAP mixed with CD-grafted dextrans (Table 2). In fact, solubility of the drug was almost two-fold higher than for the unmodified βCD. On the other hand, larger spacer arms between the CD moieties and dextran could favour the inclusion process of NAP by reducing steric effects of the polymeric chains. βCD can be also degraded by enzymes (glycosidases) of the colonic microflora, allowing the release of NAP at this site [27].

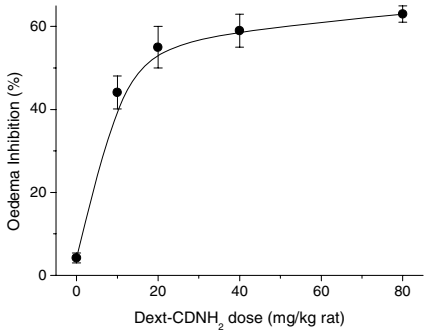


Figure 5. Anti-inflammatory activity of NAP formulations in the carrageenan-induced paw oedema test *vs* concentration of Dex-$CDNH_2$. Doses: NAP = 4 mg/ kg.

The dose effect of Dex-$CDNH_2$ on the anti-inflammatory activity of NAP is shown in Figure 5. The highest effect was obtained for a dose of 40 mg/kg, with a 59% reduction of inflammation. The high cyclodextrin content in this polymeric structure assists the supramolecular association of the drug and macromolecular carrier expressed in the highest increase in the NAP solubility (Table 2). The combination of these two factors improves the therapeutic effect of the drug.
Interestingly, the anti-inflammatory activity of NAP decayed in the presence of βCD while practically recovered its value when physically mixed with βCD and dextran. On the contrary, the CD-grafted dextrans increased the anti-inflammatory activity of NAP. Certain proportionality can be observed between the increase in anti-inflammatory activity of NAP in the presence of a CD-grafted dextran and the increase in its water solubility for each carrier.

Conclusions

Dextran was branched with monoaminated βCD derivatives, yielding water-soluble polymers able to form inclusion complexes with Naproxen. The use of these CD-grafted polysaccharides as carrier systems for the drug improved its anti-inflammatory properties “in vivo”. This effect should be mainly attributed to an increase in the drug solubility. In addition, it was also proved that larger spacer arms between the βCDs and the polymeric backbone increased the ability of the carrier for complexing Naproxen. Attending to our results, we suggest the application of these βCD-modified polysaccharides as carrier systems for anti-inflammatory drugs that can be complexed with βCDs.

Acknowledgements. This work was supported by the International Foundation for Science, Stockholm, Sweden, and the Organisation for the Prohibition of Chemical Weapons, The Hague, The Netherlands, through a grant to R. Villalonga (Grant F/3004-2). A fellowship from AECI (Spain), granted to H.L. Ramírez, is also aknowledged.

References

1. Kumar, V.; Banker, G. S. *Target oriented drug delivery systems. In: Banker,G., Rhodes, C.T.* (Eds) Modern Pharmaceutics, Marcel Dekker, New York, (1996); p. 611
2. Hoffman, A. S. in *A Commentary on the advantages and limitations of synthetic polymer biomolecule conjugates*. Biorelated Polymers and Gels. Academic Press, New York, (1998)
3. Zhu, L.; Kumar, V.; Banker, G. S. (2001) Int. J. Pharm. 223: 35
4. Duncan, R. (1999) Anti-Cancer Drugs.3: 175
5. Putman, D.; Kopecek, J. (1995) Adv. Polym. Sci. 122 : 55
6. Kamada, M.; Hirayama, F.; Udo, K.; Yano, H.; Arima, H.; Uekama, K. (2002) J. Control. Release 82 : 407
7. Loftsson, Th.; Olafsdottir, J. B.; Fridriksdóttir, H.; Jonsdottir, S. (1993) Eur. J. Pharm. Sci. 1: 95
8. Másson, M.; Loftsson, Th.; Jonsdottir, S.; Fridriksdóttir, H.; Petersen, D. (1998) Int. J. Pharm. 164: 45
9. Dollo, G.; Corre Le, P.; Chevane, F.; Verge, R. (1996) Int. J. Pharm.131: 219
10. Loftsson, Th.; Fridriksdóttir, H.; Siguroardottir, M. A.; Ueda, H. (1994) Int. J. Pharm. 110 : 169
11. Ramírez, H. L.; Valdivia, A.; Cao, R.; Torres-Labandeira, J. J.; Fragoso, A.; Villalonga, R. (2006) Bioorg. Med. Chem. Lett. 16 : 1499
12. Valero, M.; Carrillo, C.; Rodriguez, L. (2003) Int. J. Pharm. 265: 141
13. Zhong, N.; Byun, H. S.; Bittman R. (1998) Tetrahedron Lett.. 38: 2919
14. Fernández, M.; Fragoso, A.; Cao, R.; Villalonga, R. (2003) J. Mol. Catal. B: Enzym. 21: 133
15. Schneider, H. J.; Xiao, F. (1992) J. Chem. Soc. Perkin Trans. 2: 387
16. Zhang, G.; Shuang, Sh.; Dong, Ch.; Pan, J. (2003) Spect. Acta., Part. A 59: 2935
17. Boughton-Smith, N. K.; Deakin, A. M.; Follenfant, R. L.; Whittle, B. J.; Garland, L. G. Br. (1993) J. Pharmacol. 110: 896
18. Velásquez, M. M.; Valero, M.; Rodríguez, L. J.; Costa, S. M. B.; Santos, M. A. J. (1995) Photochem. Photobiol. B: Biol. 29: 23
19. Chakrabarti, S. K.; Ware, W. R. (1971) J. Chem. Phys. 55: 5494
20. Valero, M.; Pérez-Revuelta, B.; Rodríguez, L. (2003) Int. J. Pharm. 253: 97
21. Oh, I.; Lee, M.; Lee, Y.; Shin, S.; Park, I. (1998) Int. J. Pharm., 175: 215
22. Valero, M.; Costa, M.B.S.; Ascenso, R. J.; Velázquez, M.; Rodríguez, J L. (1999) J. Incl. Phenom. Macrocycl. Chem. 35: 663
23. Brown, E.; Coates, J. H.; Easton, J.C.; Lincoln, S.F.; Luo, Y. (1991)Aust. J. Chem. 44: 855
24. Otero-Espinar, F. J.; Anguiano-Igea, S.; Garcia-Gonzalez, N.; Vila-Jato, J.L.; Blanco-Mendez, J. (1992) Int. J. Pharm. 79: 149
25. Wang, J.; Warner, I. M. (1993). Microchem, J., 48, 229
26. Bettinetti , P. G.; Mura, P.; Liguori, A.; Bramanti, G. (1989) Il Farmaco 44:195
27. Kamada, M.; Hirayama, F.; Udo, K.; Yano, H.; Arima, H.; Uekama, K. (2002) J. Control Rel. 82: 407