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Journal of Pharmaceutical and Biomedical Analysis 42 (2006) 126–131
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

Simultaneous effect of cyclodextrin complexation, pH, and hydrophilic polymers on naproxen solubilization

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Received 13 October 2005; received in revised form 10 November 2005; accepted 15 November 2005
Available online 6 January 2006

Abstract

The effect of pH variation on complexation and solubilization of naproxen (pK_a 4.2) with natural βCyclodextrin (βCyD) and various neutral, cationic and anionic βCyD-derivatives has been investigated. The combined effect of pH variation and hydrophilic polymer addition on CyD solubilizing and complexing efficiency has also been determined. Phase-solubility analysis in buffered aqueous solutions (pH from 1.1 to 6.5) was used to study the interaction of the drug with each CyD, in the presence or not of the water-soluble polymer. A clear influence of the substituent type was observed, the methylderivative being the most efficient agent; on the contrary, unexpectedly, no influence of the CyD charge in the interaction with the ionizable drug was detected. As expected, total drug solubility increased with increasing pH; however, the solubility increment with respect to drug alone obtained by CyD complexation progressively decreased, with a parallel reduction of the complex stability, attributed to the reduced affinity of charged drug for the hydrophobic CyD cavity. The addition of the polymer in part counterbalanced the destabilizing effect obtained with increasing pH, by improving the CyD complexation power towards naproxen. In particular, the presence of PVP allowed an increase of the complex stability constant with hydroxypropyl βCyD up to 60% with respect to the corresponding drug–CyD binary system. Therefore, the combined strategy of pH control and polymer addition to the CyD complexing medium can be successfully exploited to improve naproxen solubilization and reduce the amount of CyD needed. The construction of theoretical drug solubility curves as a function of pH for any given CyD and polymer concentration enables selection of the best experimental conditions for obtaining the desired drug solubility value.

Keywords: Naproxen; Cyclodextrins; Solubilization; pH Control; Hydrophilic polymers

1. Introduction

Inclusion complexation with cyclodextrins (CyDs) has been widely exploited to improve solubility and/or stability, of various drug molecules [1,2]. However, the efficiency of complexation is often not very high, and therefore, relatively large amounts of CyDs must be used to obtain the desired effect [3]. On the other hand, for a series of reasons (e.g. including relatively high cost, possible toxicity, problems of formulation bulk, etc.), pharmaceutical dosage forms should contain as small amounts of CyD as possible [3]. It is hence of great interest to find effective methods for enhancing CyD's complexing and solubilizing abilities, thus making it possible to considerably reduce their dose.

Recent works have suggested the possibility of taking advantage, in the case of weakly acid and basic drugs, of a combined effect of pH control and CyD complexation for improving their solubility [4–6]. However, this approach generally leads to a reduction of CyD complexing power, since in most cases the stability constant of the complex with the unionized form of the drug is much larger than with the ionized one [4,7,8]. On the other hand, the positive effect of addition of small amounts of suitable water-soluble polymers on both the complexing and solubilizing abilities of CyDs has also been demonstrated [9–11]. Therefore, it could be possible to obtain even larger solubilization enhancements by applying the two methods simultaneously [12].

We have previously shown that the aqueous solubility of naproxen, a non-steroidal, anti-inflammatory, very poorly water-soluble (25 mg/L at 25 °C) drug, can be improved by complexation with both native and chemically modified CyDs [13–15]. Furthermore, both complexing and solubilizing properties of

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doi:10.1016/j.jpba.2005.11.029

CyD towards naproxen can be significantly enhanced by the presence of water-soluble polymers [16,17].

Naproxen is a weak acid compound ($pK_a = 4.2$ [18]), whose solubility is strongly influenced by pH variations. Thus, in the present work, we thought it worthy of interest to extend our previous studies and investigate in-depth the combined effect of pH control and CyD complexation on naproxen solubilization. Both natural βCyD and a series of neutral, cationic and anionic βCyD-derivatives were tested, to evaluate the influence of the type of substituent. Furthermore, βCyD and hydroxypropyl βCyD were selected for investigating the combined effect of pH and hydrophilic polymers (such as sodium carboxymethylcellulose or polyvinylpyrrolidone) on their solubilizing and complexing efficiency towards the drug.

Phase-solubility studies were performed by adding excess drug to buffered (pH from 1.1 to 6.5) aqueous solutions containing increasing concentrations of βCyD and each βCyD-derivative in the presence or not of a fixed amount of polymer, in order to evaluate the role of the ionized species of naproxen in improving solubility by CyD complexation and to be able to select the most suitable conditions for optimizing drug solubilization. The results were evaluated in terms of complex stability constant and solubilizing efficiency.

2. Materials and methods

2.1. Materials

Naproxen ((*S*)-(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid, NAP) and β-cyclodextrin (βCyD) were from Sigma Chemical Co. (St. Louis, MO, USA). Trimethylammoniumpropyl-βCyD (TMA βCyD), hydroxypropyl-βCyD with an average molar substitution degree per anhydroglucose unit of 0.9 (HP βCyD), hydroxyethyl-βCyD with an average molar substitution degree per anhydroglucose unit of 1.0 (HE βCyD) and methyl-βCyD with an average substitution degree per anhydroglucose unit of 1.8 (Me βCyD) were kindly donated by Wacker-Chemie (Munich, D). Sulfobutylether-βCyD (SBE βCyD) (Captisol) was a gift from CyDex Inc. (Texas, USA). Polyvinylpyrrolidone K30 (PVP) and sodium carboxymethylcellulose (NaCMC) were from Sigma, St. Louis, MO, USA). All other materials and solvents were of analytical reagent grade.

2.2. Phase-solubility studies

Solubility measurements of NAP were carried out by adding an excess of drug (ensuring saturation) to 20 mL of chloride or phosphate buffer solution (pH varying from 1.1 to 6.5) of βCyD or βCyD-derivative in the 0–13 mM or 0–25 mM concentration range, respectively, in the presence or in the absence of 0.1% (w/v) of NaCMC or PVP, in a sealed glass container electromagnetically stirred (500 rpm) in a thermostated bath at 25 ± 0.5 °C) until equilibrium was achieved (3 days). An aliquot was then withdrawn and filtered (pore size 0.45 μm), and the NAP concentration was determined by a second derivative ultraviolet absorption method at 274 nm [13]. Each experiment was performed in triplicate (coefficient of variation C.V. <2%). Apparent 1:1 stability constants were calculated from the straight-line portion of the phase solubility diagrams, according to the following equation [19]:

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope})}$$

where S_0 is the solubility of the drug, in each examined buffer solution, in the absence of ligand.

Table 1
Apparent stability constants of equimolar complexes of naproxen (NAP) with the examined cyclodextrins (CyDs) at 25 °C in aqueous solutions at different pH values

CyD	$K_{1:1}$ (M^{-1})		
	pH 1.1	pH 4.0	pH 6.5
βCyD	3270	1885	210
HE βCyD	4360	2305	165
HP βCyD	4890	2605	230
Me βCyD	13880	7275	390
TMA βCyD	2640	1455	205
SBE βCyD	12190	5920	295

3. Results and discussion

Fig. 1 shows the solubility of NAP under different pH conditions in the presence of each examined CyD. Drug solubility increased linearly as a function of concentration of each examined CyD, showing in all cases typical A_L-type profiles, independent from pH. This was indicative of the occurrence of soluble complexes of 1:1 mol/mol stoichiometry [19] with both the neutral (pH 1.1) and the charged (pH 6.5) forms of NAP, differently from that observed for other drugs, such as levemopamil [6], but in agreement with that reported for other ones such as naringenin [20].

The effect of drug ionization on the stability of its complexes with the different CyDs is shown in Table 1, where the apparent stability constant values of the various complexes, calculated according to Higuchi–Connors [19] from the phase-solubility diagrams at the different pH values examined, are collected. At pH 1.1, when NAP ($pK_a = 4.2$) is practically completely in the unionized form, the complexes exhibited the greatest stability, since the drug in the uncharged state has more affinity for the hydrophobic cavity of CyD. A clear influence of the type of CyD substituent can be highlighted: Me βCyD give the most stable complex ($K_{1:1}$ 13880 M^{-1}), immediately followed by the negatively charged SBE βCyD. On the contrary, the positively charged TMA βCyD give the lowest stability constant value ($K_{1:1}$ 2640 M^{-1}), also in comparison with the natural βCyD, in spite of the lower solubility of this latter. As expected, the complex stability constants decreased with increasing pH, due to the progressive increase of the drug ionized form, which has less affinity for the inclusion in the apolar CyD cavity [4,7,8,21]. However, unexpectedly and differently from that reported by other authors [22–24], the positive charge of cationic CyD did not favour interactions with the anionic drug, nor did the neg-

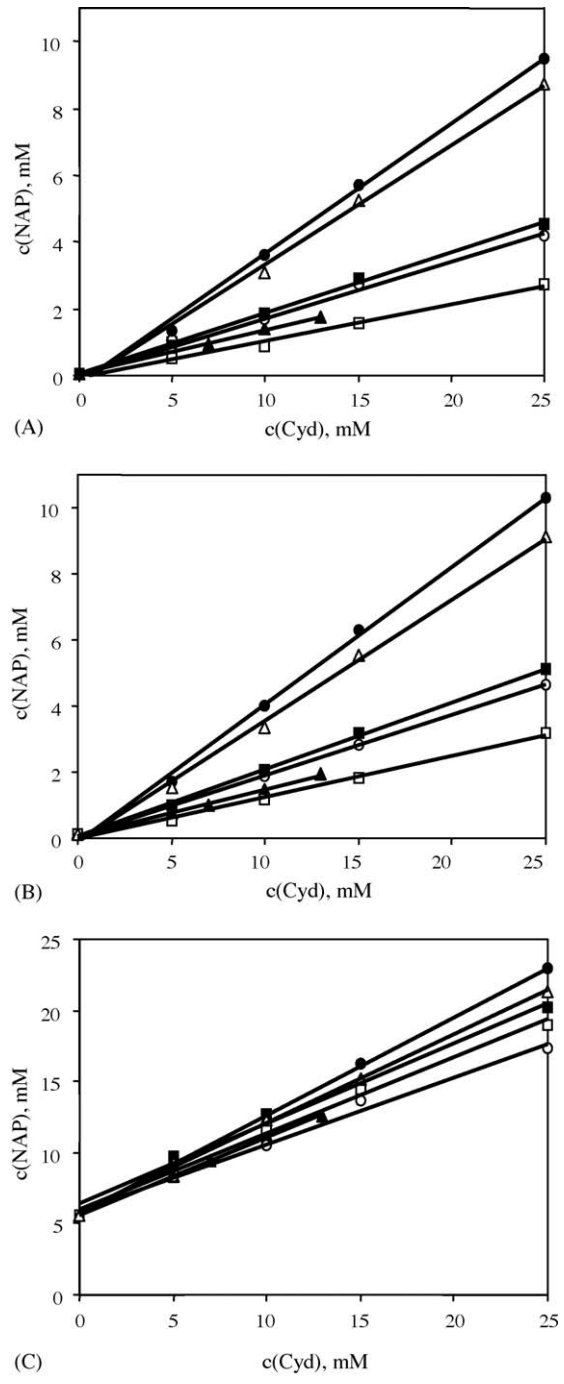

Fig. 1. Naproxen (NAP) phase-solubility diagrams at 25 °C in buffered solutions at pH 1.1 (A), pH 4.0 (B) or pH 6.5 (C), in the presence of Me βCyD (●), SBE βCyD (△), HP βCyD (■), HE βCyD (○), TMA βCyD (□), and βCyD (▲).

ative charge of the anionic one hamper them. In fact, also at pH 4.0 (where about 40% of drug is in the ionized form) the same order of CyD complexing ability towards the drug (i.e. Me βCyD > SBE βCyD > HP βCyD ≈ HE βCyD > βCyD > TMA βCyD) already found at pH 1.1 was maintained, and an almost constant 50% reduction of the stability constant values of the different complexes was observed independently from the type of CyD. The results related to the ionic CyDs were in agreement with those previously observed in unbuffered (pH ≈ 5.0) aqueous solutions [15] in the presence of about 85% of the dissociated anionic form. A further very strong reduction of the complex stability constant was observed at pH 6.5, where the drug is almost completely (99.5%) in the ionized form. However, also at this pH value, the stability constant of the complex with anionic SBE βCyD (292 M^{-1}) was higher than that with the cationic TMA βCyD (204 M^{-1}). Such results further confirmed the prevailing role of the type of substituent and the negligible effect of the CyD charge towards the complexation with the ionisable drug, independent of the different ratios of unionized–ionized forms. This was true for both the possible inhibition foreseeable in the case of the negatively charged CyD, or the possible electrostatic interaction prophesized in the case of the positively charged one. The lowest stability constant of the complex with the TMA-derivative has evidently to be attributed to serious steric hindrance effects, which hampered the inclusion of the guest molecules, thus reducing the potential attractive effects due to the CyD positive charge [15].

The combined effect of pH variation and CyD complexation on NAP solubility is shown in Table 2. As can be seen, the total drug solubility progressively increased with increasing pH. However, even though the highest drug solubility values were obtained at pH 6.5, where the drug is almost completely in the anionic form, the relative increments of solubility with respect to drug alone obtained by CyD complexation at this same pH, were the lowest, in accordance with the concomitant strong reduction of the CyD complex stability (see Table 1).

It must be considered that in such a combined approach of complexation and pH control for an ionisable drug such as NAP, four species are in equilibrium, i.e. the free unionized drug (HNAP), the free ionized one (NAP^-) and the corresponding HNAP–CyD and NAP^-–CyD complexes. Therefore, the total concentration of the drug in solution is determined by the sum of the concentrations of these four species:

$$[NAP_{tot}]=[HNAP]+[NAP^-]+[HNAP–CyD] + [NAP^-–CyD]$$

Ling et al. [5] showed that such an equation can be expanded into:

$$[NAP_{tot}] = [HNAP] + [HNAP] \times 10^{pH-pK_a} + \frac{K_u[HNAP]}{1 + K_u[HNAP]}[CyD_{tot}] + \left\{\frac{K_i[HNAP] \times 10^{pH-pK_a}}{1 + K_i[HNAP] \times 10^{pH-pK_a}}\right\}[CyD_{tot}]$$

where K_u and K_i are the stability constant values of the complexes with the unionized and ionized forms of the drug, respectively, and $[CyD_{tot}]$ is the total amount of CyD used.

Hence, the total drug solubility is dependent on its intrinsic solubility, the difference between pH and its pK_a and the complex stability constants of the unionized and ionized forms of the drug. It is evident from this equation that the pH of the solution plays an important role in determining the relative contributions of the complexed as well as the free ionized species toward the total drug solubility [5].

Knowing the intrinsic solubility of the drug in the completely unionized form (which can be considered constant, indepen-

Table 2
Equilibrium solubility of naproxen (NAP) at 25 °C in aqueous solutions at different pH values, in the presence or not of 25 mM CyD (or 13 mM βCyD, i.e. its saturation solubility), and relative solubility increment (R.I.) with respect to the drug alone

CyD	pH 1.1		pH 4.0		pH 6.5	
	NAP solubility (mM)	R.I.	NAP solubility (mM)	R.I.	NAP solubility (mM)	R.I.
–	0.046	–	0.098	–	5.6	–
βCyD	1.74	37.8	1.93	19.7	12.9	2.3
HE βCyD	4.18	90.8	4.65	47.5	19.3	3.4
HP βCyD	4.52	97.8	5.13	52.3	22.7	4.0
Me βCyD	9.50	206	10.3	105	24.9	4.5
TMA βCyD	2.73	59.3	3.15	32.1	21.0	3.7
SBE βCyD	8.75	190	9.10	92.9	23.8	4.2

dent from pH), and determining the stability constant of the drug–CyD complex at pH values at which the drug is almost totally in the ionized and unionized forms, respectively, it should be possible to apply this equation to calculate the total drug solubility obtainable by the combined CyD complexation-pH control approach for each intermediate pH value, in the presence of a given CyD concentration.

Table 3 shows the calculated and experimentally determined values of total NAP solubility at pH 4.0 in the presence of two different concentrations of each CyD. The good agreement between experimental and theoretical values supports the validity of the above proposed model equation [5] to describe the effect of the combined approach of CyD complexation and pH control on drug solubility; it can therefore be used to predict the theoretical total drug concentration obtainable as a function of pH for any given ligand concentration, and then to choose the most suitable combination to achieve the desired drug solubility.

It must be underlined, however, that the improvement of NAP solubility obtained by increasing pH is to the detriment of the stability of the CyD complex and then of the CyD solubilizing efficiency. Therefore, it seemed worthy of interest to investigate the possible synergistic effect of pH adjustment and polymer addition to the CyD complexation medium, by taking into account the positive influence previously observed on NAP complexation and solubilization in the presence of a small amount (0.1–0.25%, w/v) of hydrophilic polymers [16,17]. βCyD and its hydroxypropyl-derivative were then selected as model CyDs, and the influence on NAP solubility of the presence of PVP or NaCMC, chosen as the most effective polymers [16], was evaluated by phase-solubility studies.

Table 3
Calculated and experimental equilibrium solubility values of naproxen (NAP) at 25 °C in aqueous solutions at pH 4.0, in the presence of 10 or 25 mM CyD

CyD	NAP solubility (mM) [CyD] = 10 mM		NAP solubility (mM) [CyD] = 25 mM	
	Experimental	Calculated	Experimental	Calculated
βCyD	1.46	1.51	1.93[a]	1.94[a]
HE βCyD	1.89	1.85	4.65	4.48
HP βCyD	2.10	2.06	5.08	4.99
Me βCyD	3.97	4.19	10.3	10.3
TMA βCyD	1.17	1.28	3.15	3.06
SBE βCyD	3.35	3.80	9.10	9.45

[a] [βCyD] = 13 mM, saturation solubility.

Phase-solubility diagrams obtained in the presence of 0.1% (w/v) of polymer at the different pH values showed in all cases a constant linear increase of the drug solubility, maintaining the A_L-type features [19]. The presence of both types of polymers enabled an appreciable increase of the slopes of the solubility curves, at all examined pH values, as is shown, for example, in Fig. 2 for the systems with HP βCyD and PVP. No further significant improvements were observed by performing the same experiments in the presence of higher amounts (up to 0.25% (w/v)) of polymer, according to previous studies [16,17].

The stability constants of the complexes in the presence of the polymers are presented in Table 4. When comparing these data with the corresponding ones in the absence of polymer, it appears evident that both NaCMC and PVP had a favourable effect on the CyD complexing ability, reducing the destabilizing effect due to the pH increase. For example, in the case of the complex with HP βCyD in the presence of PVP, an about 60% increase of the stability constant values at both pH 4.0 and at pH 6.5 was observed, and an about 25% increase in the corresponding cases with βCyD. Moreover, the stability constant value at pH 4.0 of the HP βCyD complex in the presence of PVP was only

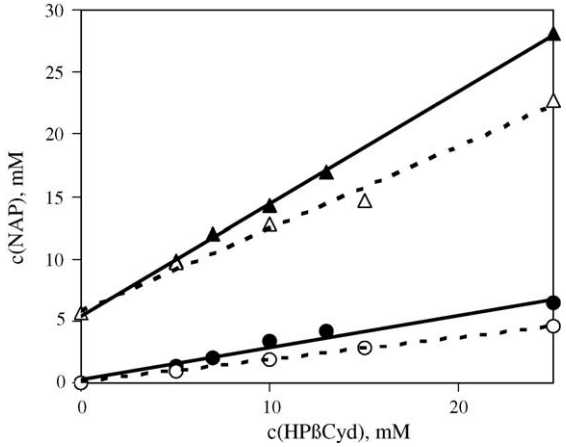

Fig. 2. Phase-solubility curves of naproxen (NAP) at 25 °C in buffered solutions at pH 1.1 (●, ○) or pH 6.5 (▲, △), with increasing concentrations of HP βCyD in the presence (closed symbols) or in the absence (open symbols) of 0.1% (w/v) PVP.

Table 4
Effect of polymers (0.1%, w/v) on the apparent stability constants of naproxen (NAP) complexes with βCyD and HP βCyd in aqueous solutions at different pH values at 25 °C

CyD	pH 1.1		pH 4.0		pH 6.5	
	$K_{1:1}$ (M^{-1})	K_t/K_b[a]	$K_{1:1}$ (M^{-1})	K_t/K_b[a]	$K_{1:1}$ (M^{-1})	K_t/K_b[a]
βCyd	3270	–	1885	–	210	–
βCyd + NaCMC	3930	1.20	2292	1.22	230	1.10
βCyd + PVP	4110	1.26	2352	1.25	260	1.24
HP βCyd	4890	–	2605	–	230	–
HP βCyd + NaCMC	6340	1.30	3620	1.39	322	1.39
HP βCyd + PVP	7025	1.44	4125	1.58	368	1.59

[a] Ratio between the stability constant values of ternary (K_t) and binary (K_b) complexes.

of about 15% lower than that obtained at pH 1.1 in the absence of polymer.

The favourable effect of polymers on CyD complexing efficiency, also reflected in a higher solubilizing efficiency, as can be seen in Fig. 3, where the relative NAP solubility increases obtained at the different pH values with binary and ternary systems are compared.

The equation model proposed by Ling et al. [5] was also applied to such ternary systems, by substituting the K_u and K_i values of binary systems (NAP–CyD) with those obtained with ternary systems (NAP–CyD–polymer). A satisfying correspondence between predicted and experimental total drug solubility values was obtained. For example, the calculated values at pH 4.0 were 6.7 and 6.2 mM, respectively, in the presence of 25 mM HP βCyD and 0.1% (w/v) PVP or NaCMC, whereas, the corresponding experimental values were 7.1 and 6.6 mM, respectively. Fig. 4 shows the theoretical and the experimental drug solubility values as a function of pH in the presence of 25 mM HP βCyD and 0.1% (w/v) PVP. The good relationship between calculated and experimental data demonstrates the suitability of the above equation model to describe the combined effect of CyD complexation, pH control and polymer addition on the total drug solubility.

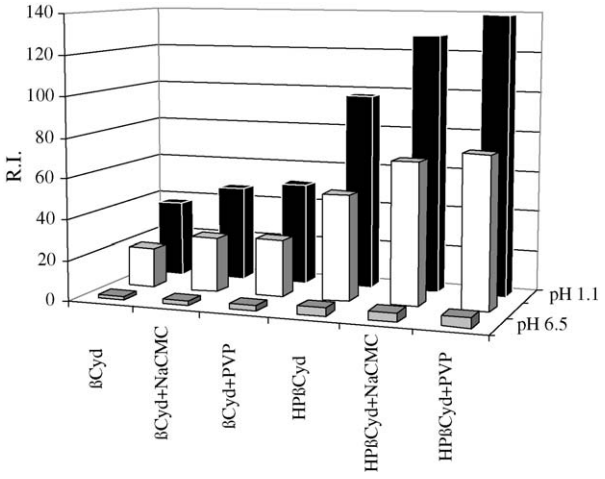

Fig. 3. Relative solubility increase (R.I.) of naproxen in buffered solutions at 25 °C containing 25 mM HP βCyD and 13 mM βCyD (saturation solubility) alone or in the presence of 0.1% (w/v) PVP.

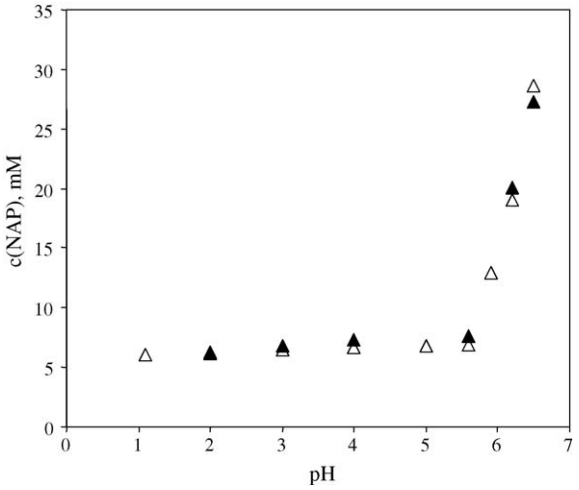

Fig. 4. Calculated (△) and experimental (▲) equilibrium solubility values of naproxen (NAP) at 25 °C in the presence of 25 mM HP βCyD and 0.1% (w/v) PVP.

4. Conclusion

Our study has shown that the integrated approach of pH adjustment and water soluble polymer addition can be successfully used for improving the CyD solubilizing power towards an ionisable drug such as NAP, thus allowing a smaller quantity of CyD to solubilize a given amount of drug, offering clear economic and technologic advantages as well.

The addition of the polymer was particularly effective, since it in part counterbalanced the destabilizing effect due to the increased drug ionization obtained with increasing pH. This phenomenon appeared related to the stronger complexation capacity of CyD towards NAP in the presence of polymer, as demonstrated by the improvement in the apparent stability constants of the NAP–CyD complexes. This was particularly pronounced for the NAP–HP βCyD–PVP ternary system, which showed an increase of the complex stability constant ranging from 1.4 to 1.6 times with respect to the corresponding binary system at the different pH values.

Therefore, the combined strategy of pH control and polymer addition to the CyD complexing medium can lead to achievement of the best results in terms of both complexation and solubilization efficiency towards NAP. The construction of the-

oretical NAP solubility curves as a function of pH for any given CyD and polymer concentration enables the selection of the best experimental conditions to obtain the desired drug solubility value.

Acknowledgment

Financial support from MIUR is gratefully acknowledged.

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