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Interaction of naproxen with β-cyclodextrin in solution and in the solid state

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Summary

The behaviour of the inclusion complex of naproxen with β-cyclodextrin was studied by molecular modelling using interactive computer graphics, solubility diagrams at different temperatures and pH values, infrared spectroscopy and differential scanning calorimetry. The complex was prepared by freeze-drying, spray-drying and kneading. Three-dimensional modelling shows that naproxen lies totally enclosed in the interior of the β-cyclodextrin cavity. The energy calculated for the model indicates a high stability. The dependency of the stability in solution on pH and temperature, and the thermodynamic parameters calculated show the possible existence of intermolecular hydrogen bonds that are less stable with increasing pH. Infrared spectra show a shift to lower wavenumber in the carbonyl-stretching bands of naproxen for the products prepared by freeze-drying and spray-drying. This may be due to the formation of hydrogen bonds between the host and guest. Thermal analysis confirmed that freeze-drying and spray-drying led to complex formation. The kneading method, however, gives poor drug complexation; only one-third of the naproxen penetrates the cyclodextrin cavity, the rest remaining freely dispersed between uncomplexed host and inclusion compound.

Introduction

The cyclodextrins (CDs) are cyclic polysaccharides that can act as host molecules, forming inclusion complexes with many drugs of different structures, modifying their physical and chemical properties.

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An important step in the formation and study of inclusion complexes is to determine the structure and forces that contribute to their formation and stabilization. Nevertheless, there is a debate about the forces responsible for forming the complex and the interactions that stabilize the system (Uekama and Otagiri, 1987). The nature and contribution of the different forces that may take part in inclusion complexes are not altogether well known. Different authors emphasize Van der Waals interactions (Otagiri et al., 1976; Saenger, 1980; Jones et al., 1984), formation of hydrogen bonds (Cohen and Lach, 1963; Lach

and Cohen, 1963; Jones et al., 1984), hydrophobic interactions (Rosanske and Connors, 1980; Saenger, 1980; Jones et al., 1984), release of energy by substitution of polar water molecules for more apolar molecules (Otagiri et al., 1976; Saenger, 1980; Jones et al., 1984) and energy release from conformational changes (Jones et al., 1984), though the contribution of each one depends on the guest molecule.

In this paper, the principal interactions in solution and in the solid state that arise in the naproxen-β-cyclodextrin complex (NAP-β-CD) have been studied. Molecular modelling using interactive computer graphics techniques has been employed to discover the most likely orientation and stoichiometry. Calculations from solubility diagrams at different pH values and temperatures are used to give the thermodynamic parameters associated with the formation of the complex. Infrared spectroscopy and differential scanning calorimetry were used to characterize the complex in the solid state.

Materials and Methods

Materials

Naproxen (Sigma®) and β-cyclodextrin (Chinoin®) were obtained from the indicated sources. All the reagents used were analytical grade.

Molecular graphics

The SYBYL 5.10 program (1988) and an Evans and Sutherlan PS300 high-resolution graphics system, controlled by a VAX computer, were used for the molecular graphics study. The naproxen molecule was assembled by a subroutine, using naphthalene as a base molecule to which the required atoms were added.

The model of the complex was obtained using a manual docking, with the screen in ORTHOGONAL mode. The ensemble was subjected to a process of energy minimization using the OPTIMIZATION subroutine of the program SYBYL MAXIMIN-2.

Solubility studies

The method proposed by Higuchi and Connors (1965) was used. Cyclodextrin solutions of different concentrations were prepared at pH 1, 3, 5 and 7 and an excess of naproxen added to each. The samples were continuously stirred while remaining in a water bath at 25, 35 and 45 ± 0.5 °C for 6 days. Once equilibrium had been reached, the samples were removed and filtered through 0.45 μm nylon membranes (Millipore). The amount of drug dissolved was determined spectrophotometrically at 272 nm ($E_1^1 = 218$) (Shimadzu UV-240 spectrophotometer). Stability constants were calculated from the straight section of the solubility diagrams on the basis of a 1 : 1 stoichiometry.

Methods for preparing the complex

Kneading Stoichiometric quantities of naproxen and β-cyclodextrin were thoroughly mixed in a mortar and an amount equalling the weight of the drug/carrier mixture of a 50% ethanol/water mixture was added. The pestle was used to stir the mixture until a dense paste formed after the solvent had evaporated. The paste was dried in a vacuum oven and pulverized in a mortar.

Freeze-drying Stoichiometric quantities of naproxen and β-cyclodextrin were weighed and dissolved in deionized water. A little (0.5 ml/l) ammonium hydroxide (35%) was added to help the naproxen to dissolve. The solution, when transparent, was stirred for 24 h. It was then placed in a freezer at −12 °C until completely frozen (4–5 h). The frozen solution was then freeze-dried (Telstar freeze-dryer).

Spray-drying The procedure was similar to that described for freeze-drying. The solution was deionized water with a little ammonium hydroxide added to aid dissolution of the active principle. When the naproxen and the β-cyclodextrin had dissolved, the solution was atomized (Büchi 190 Mini Spray-dryer).

Infrared spectroscopy (IR)

The IR spectra were recorded on a Perkin Elmer 1330 IR spectrophotometer using KBr discs.

Differential scanning calorimetry (DSC)

A Perkin Elmer DSC4 apparatus was used, with heating at 10 °C/min over a range of 10

mcal/s and employing nitrogen as purging gas. Pulverized samples (5 mg weighed in a Perkin Elmer AD 4 autobalance) were placed in aluminium sample pans.

Results and Discussion

Studies in solution

Computer-aided molecular modelling is a new technique frequently used in studying the synthesis of new organic molecules, and as such has great importance in studies of inclusion complexes with cyclodextrins. The technique permits the most probable stoichiometry to be determined, and shows the adaptation of the drug to the cyclodextrin cavity, revealing the most probable position and orientation from an energetic point of view (Wouessidjewe et al., 1989; Cabral-Marques et al., 1990; Wiese et al., 1991).

Using the SYBYL program to model the 1:1 NAP-β-CD complex the final energy of the system can be calculated, taking into account the formation of Van der Waals interactions and deformations of the bonds and angles of the molecules.

Fig. 1 shows the NAP-β-CD complex after performing energy minimization. The naproxen molecule is totally included in the cyclodextrin cavity, with the methoxyl group orientated toward the narrowest part of the cavity formed by the primary hydroxyl groups and the carbonyl radical towards the widest part formed by secondary hydroxyl groups. The naproxen molecule is slightly inclined in the cavity due to steric effects derived from the molecular volumes of the methoxyl and carbonyl radicals. This slight inclination causes the carboxyl group to lie close to the primary β-cyclodextrin hydroxyl groups. The close proximity of the groups may favour the formation of hydrogen bonds between them, resulting in additional stability of the molecule.

The energy decrease after complex formation (−20 kcal/mol; SYBYL program) confirms the absence of repulsive Van der Waals interactions. The existence of a favourable energetic situation makes it possible to predict the formation of a stable 1:1 NAP-β-CD complex, although no information on the dynamic behaviour of the system can be obtained. Given that the study does not take hydrophilic-hydrophobic interactions into account, which are highly important in complex formation, nor the role of the solvent in the inclusion process, the energies obtained must not be considered from anything other than a purely qualitative point of view.

The solubility diagrams of the NAP-β-cyclodextrin system obtained at different pH values (Fig. 2) shows that as pH increases the equilibrium solubility of naproxen in the absence of the carrier increases as a result of the ionization of the drug. The corresponding stability constants,

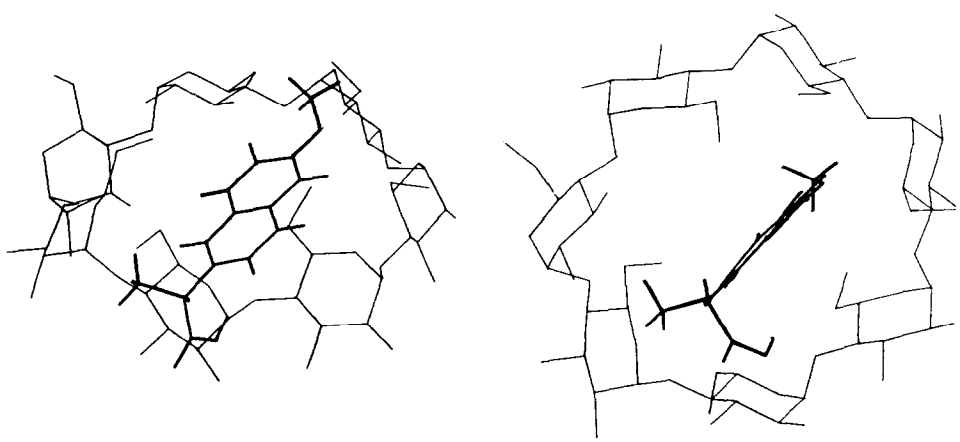

Fig. 1. Molecular graphics model of the naproxen-β-cyclodextrin complex.

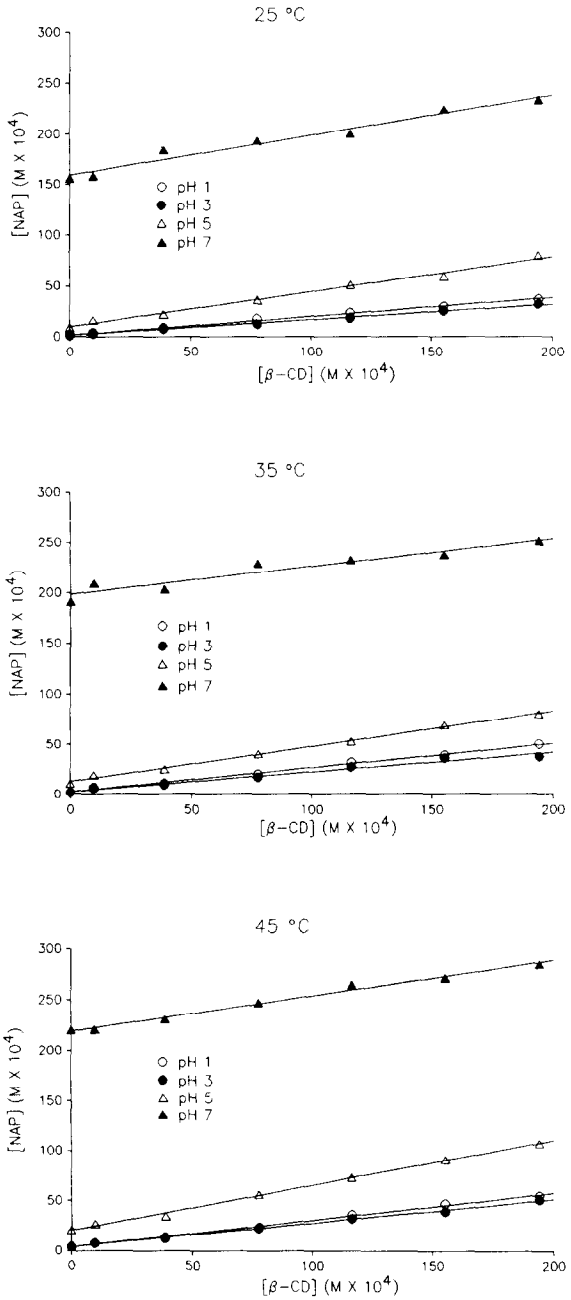

Fig. 2. Phase-solubility diagrams of naproxen-β-cyclodextrin system at various pH values and temperatures.

k_c (1 : 1), at each temperature and pH, calculated from the profiles are listed in Table 1.

To evaluate and select the terms and interactions contributing most to the stability of the complex in solution, a sequential multiple regression (Dixon, 1983) was applied, with the independent variables: pH and temperature, interaction between the two and the quadratic effect of these factors. The result of the analysis (Table 2) indicates a marked influence on the behaviour of the complex in solution for all the terms introduced. Moreover, the value obtained for the multiple correlation coefficient ($r_{\text{multiple}} = 0.9974$) indicates the high predictive value of the equation

$$k_c = -63.32t - 270.21\text{pH} + 0.4444t^2 - 8.82\text{pH}^2 + 4.79t \times \text{pH} + 2798 \quad (1)$$

The response surface plot corresponding to this equation (Fig. 3) shows that the k_c values decrease as pH increases, which is to say the fraction of ionized drug increases.

This influence of pH on the stability of the complex indicates that ionized drug interacts much more weakly with β-cyclodextrin as against the unionized drug. The interaction is practically zero at the pH at which total ionization of the guest molecule occurs. The significance that the

TABLE 1

Stability constants (k_c) at different temperatures and pH values

pH	% Ionized	Temperature (°C)		
		25	35	45
1	0.06	1379	975	778
3	5.60	950	741	639
5	86.31	487	430	387
7	99.84	27	26	24

TABLE 2

Results of multiple regression (BMDP P2R) for the stability constant parameter, k_c (M^{-1})

Variable in model	Variable not in model	Partial r	F
pH	–	−0.9321	66.21
t	–	0.9688	98.73
$t \times$pH	–	0.9927	180.27
pH^2	–	0.9960	218.07
t^2	–	0.9974	226.56

quadratic effects and interactions have in the system is possibly because these factors directly influence drug ionization; the influence is never linear or independent. There is a marked decrease in the stability of the complex when the pH varies from 1 to 3. In principle, these pH variations do not strongly modify the initial solubility of the drug; however, the solubilizing capacity of cyclodextrin at equal concentrations is less at pH 3. The structure obtained for the unionized drug by molecular modelling could be the basis of this. As mentioned, the position of the naproxen molecule within β-cyclodextrin assists formation of hydrogen bonds with the cyclodextrin, which help to stabilize the system. At pH 3, the naproxen molecule starts to ionize, causing the destabilization of the hydrogen bonds and making the molecule more hydrophilic, as a result of which hydrophobic interactions decrease, adding to the repulsive interactions.

Determining the temperature dependence of the stability constant of the complex, we have calculated the thermodynamic parameters associated with the complex formation, ΔH^0, ΔS^0 and ΔG^0, according to Eqns 2 and 3:

$$\ln k_c = -\frac{\Delta H^0}{R} \cdot \frac{1}{T} + \frac{\Delta S^0}{R} \qquad (2)$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \qquad (3)$$

As can be seen (Table 3), the enthalpy of formation of the inclusion compound is negative at all pH values, indicating that complex formation is

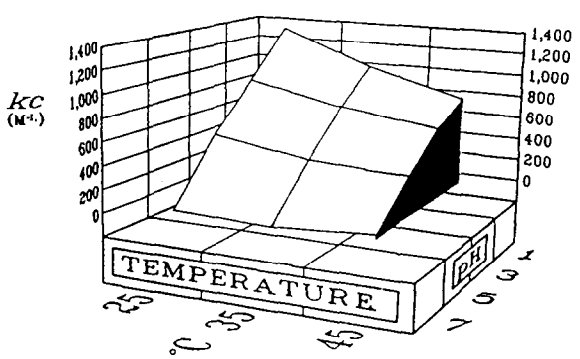

Fig. 3. Stability response surface of naproxen-β-cyclodextrin system.

TABLE 3

Thermodynamic parameters for the inclusion compound NAP-β-CD in aqueous solution at different pH values

pH	ΔG^0 (cal/mol)	ΔH^0 (cal/mol)	ΔS^0 (cal/K per mol)	r
1	−4270.6	−5403	−3.8	0.9947
3	−4054.4	−3816	0.8	0.9936
5	−3657.1	−2173	4.98	0.9940
7	−859.6	−466	1.32	0.8892

exothermic. Hence, there is a release of energy which favours formation of the complex. The value of this parameter, however, increases with pH, indicating a decrease in stability as the drug ionizes. The high negative enthalpy values suggest that hydrogen bonds between host and guest participate in the formation of the complex, in addition to the typical interactions that operate in most of these systems (Van der Waals forces, interactions between dipoles and energy release by substitution of the water molecules contained by cyclodextrins in the cavity). Taking into account the entropy variation found in the different media tested, complex formation at pH 1 involves a negative entropy, showing the order of the system increases. The ΔS^0 value may indicate a difference between the hydrogen bonds before and after complex formation. Hence, the entropy observed at pH 1 would point to there being a greater number of hydrogen bonds in the complex than in free molecules. Nevertheless, increasing the pH increases the entropy to positive values, showing ionization of the complex destroys the hydrogen bonds between host and guest. Lastly, there is a decrease at pH 7 that might be due to the low correlation coefficient obtained and the formation of a hydrate layer on the ionized drug, leading to increased order in the system.

Studies in the solid state

The solid inclusion complex prepared by freeze-drying, spray-drying and kneading was a whitish powder, freeze-drying giving a more flaky product. Spray-drying resulted in low final product yields (50–60%) compared to the other methods (almost 100%).

The solid state was studied with IR spectroscopy and DSC.

The IR spectra of the freeze-dried and spray-dried products are shown in Fig. 4, demonstrating displacement and reduction in intensity in the stretching band corresponding to the naproxen carbonyl radical (1721 cm^{-1}).

Initially, formation of an ammonium salt with naproxen, resulting from the ammonium hydroxide used in the two processes, could be suspected.

Fig. 4. IR absorption bands in the 1500–2000 cm^{-1} region: (a) naproxen; (b) β-CD; (c) kneaded-mixture inclusion complex; (d) spray-dried inclusion complex; (e) freeze-dried inclusion complex.

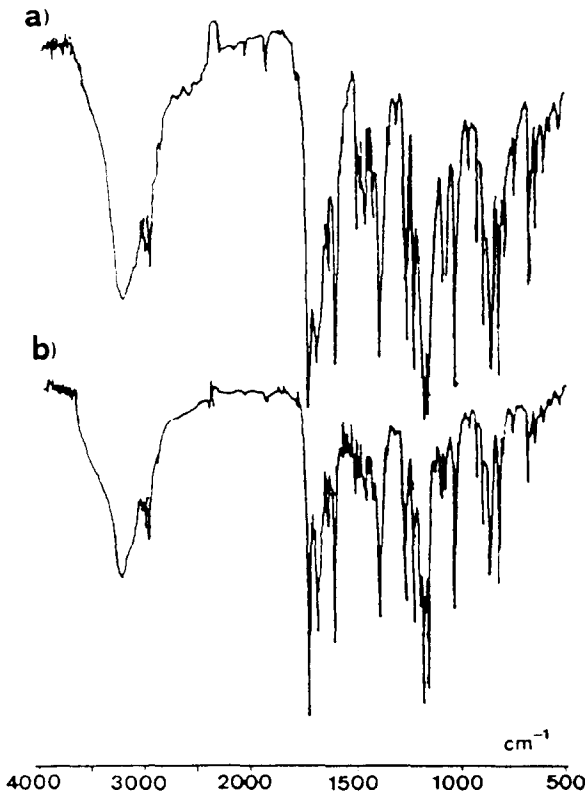

Fig. 5. IR spectra of: (a) naproxen; (b) freeze-dried naproxen.

Formation of a salt would shift the peak corresponding to the carbonyl group to longer wavenumber and strongly diminish the broad band (around 3000 cm^{-1}) corresponding to the -OH radicals of the acid group; nevertheless, the spectra corresponding to naproxen and freeze-dried naproxen (Fig. 5) show no differences between the bands for one or the other product which suggests the difference is a consequence of the inclusion of naproxen within the cyclodextrin cavity.

Stable hydrogen bonds forming between naproxen and β-cyclodextrin are a possible reason for the spectral shift. When the carbonyl group is joined to a hydroxylic compound by hydrogen bonds, the stretching band is displaced to lower wavenumber due to a weakening of the carbonyl radical double bond (Couley, 1979). The reduction in intensity might be due in part to the vibrational restrictions imposed on the guest molecule in the cyclodextrin cavity. Lin and Kao

(1989) found that spray-dried inclusion complexes prepared using several drugs and β-cyclodextrin also behaved like this. These authors observed shifts to lower wavenumber for the C = O of the amine group of acetaminophen (from 1568 to 1555 cm^{-1}), the carbonyl group of indomethacin (from 1716 to 1666 cm^{-1}) and the hydroxyl group of pyroxicam (1180 to 1154 cm^{-1}).

The infrared spectrum corresponding to the inclusion complex prepared by kneading shows no band displacements, the naproxen and cyclodextrin peaks being mixed. After this method of preparation, the naproxen might not be entirely complexed, such that nonincluded naproxen molecules are left between cyclodextrin molecules and the complex.

The thermal curves obtained by DSC shown in Fig. 6 confirm the IR data. The endothermic melting peaks of naproxen (156 °C) and the peak in the cyclodextrin DSC curve at 100 °C corresponding to the evaporation of water from the cyclodextrin cavity completely disappear in the complexes prepared by spray-drying and freeze-drying.

The DSC trace of the product prepared by kneading still shows the melting peak of naproxen and the water endotherm, however. This confirms the idea that this method does not give complete encapsulation of naproxen.

The DSC curves obtained also reflect the conditions during synthesis. An example of this is the appearance of the acute melting peak in the freeze-dried naproxen, showing that the product formed is in a crystalline form. This is because the freezing prior to freeze-drying is performed slowly (over 4–5 h for complete sample freezing). The low solubility of naproxen in water as the temperature of the solution falls and freezing starts leads to precipitation in the form of fine crystals. Crystallisation prior to freeze-drying could be prevented by subjecting the mother liquor to a more rapid freezing step.

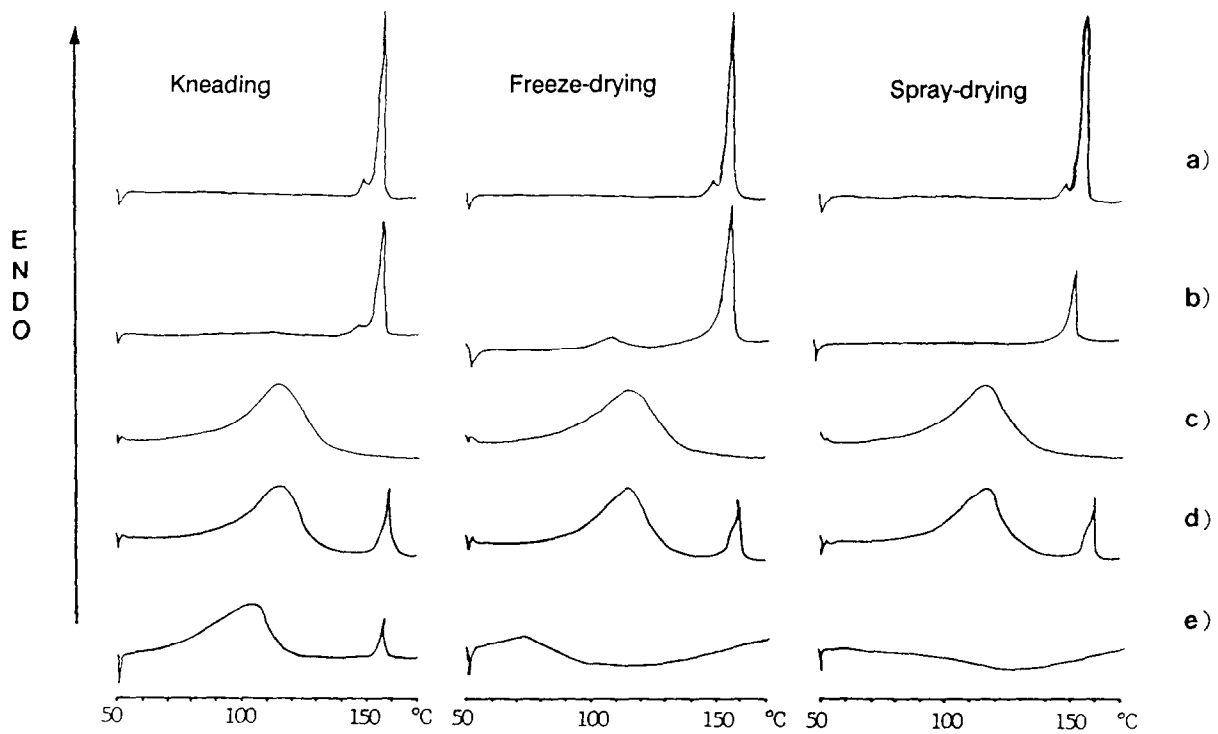

Fig. 6. Differential scanning calorimetry curves of: (a) pure drug; (b) treated drug; (c) β-cyclodextrin; (d) physical mixture; (e) inclusion compound.

To calculate the solid-state stoichiometry, we have used a technique proposed by Giordano et al. (1989) based on carrying out quantitative thermal studies of mixtures containing excess drug with respect to their real stoichiometry.

Let n_a and n_b respectively be the initial number of moles of guest and host molecules in the mixture, and R the stoichiometric ratio of guest/host (in moles); then the moles of guest molecules remaining uncomplexed after the process (n_a^*) will be:

$$n_a^* = n_a - n_b R \tag{4}$$

where $n_b R$ represents the moles of drug interacting with the cyclodextrin. The total fraction of guest added to the mixture (TMFG) will be:

$$\text{TMFG} = n_a/(n_a + n_b) \tag{5}$$

Hence,

$$n_a^* = \text{TMFG}(n_a + n_b) - n_b R \tag{6}$$

The fraction of free drug uncomplexed after the process (FMFG) can be written as,

$$\text{FMFG} = n_a^*/(n_a + n_b)$$

$$= \text{TMFG} - (1 - \text{TMFG})R \tag{7}$$

which is the same as,

$$\text{FMFG} = \text{TMFG}(1 + R) - R \tag{8}$$

Consequently, plotting FMFG vs TMFG will give a straight line of slope $1 + R$ and ordinate at the origin, $-R$. Fig. 7 shows the results obtained. The continuous lines represent the theoretical curves calculated from Eqn 8 for different values of R and the dashed lines the quantities obtained by experimentation. It is seen that a 1:1 stoichiometry is obtained for the freeze-dried and spray-dried complexes, whereas the profile obtained for the kneaded complex fits the curve corresponding to a 1:3 system. Considering the chemical structure of naproxen, it seems impossible for a molecule to be simultaneously included in three molecules of cyclodextrin. We consider these results to represent the low yield of complexation in the method, more than the real stoichiometry of the complex. This supposition is borne out by the peak that continues to appear at temperatures slightly above 100 °C and corresponds to release of the water molecules from within the cyclodextrin cavity, indicating the existence of free uncomplexed carrier.

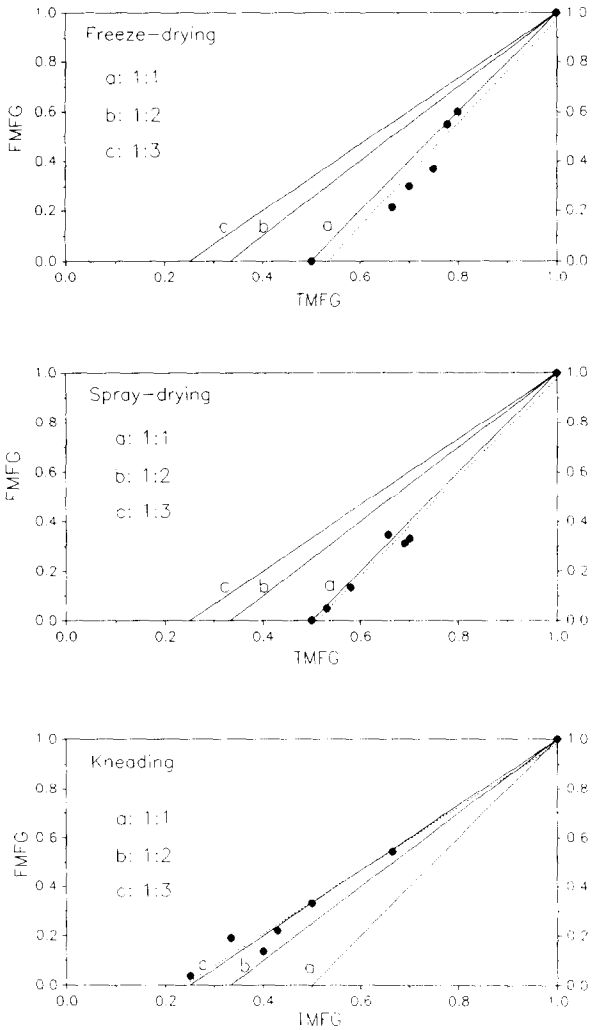

Fig. 7. Theoretical curves calculated from Eqn 8 at different R values together with FMFG values obtained from melting enthalpies at 156 °C.

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