Influence of the "Host−Guest" Interactions on the Mobility of Genistein/β-Cyclodextrin Inclusion Complex

Vincenza Crupi,[†] Domenico Majolino,*[,†] Alessandro Paciaroni,[‡] Rosanna Stancanelli,[§] and Valentina Venuti[†]

Department of Physics, University of Messina and Consorzio Nazionale Interuniversitario per le Scienze Fisiche della Materia, C.da Papardo, S.ta Sperone 31, P.O. Box 55, 98166 S. Agata, Messina, Italy, Department of Physics, University of Perugia and CNR−INFM CRS SOFT, Via A. Pascoli, 06123, Perugia, Italy, and Pharmaco-Chemical Department, University of Messina, Viale Annunziata, 98168, Messina, Italy

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Inclusion complexes of cyclodextrins with nonpolar drugs are a topic of current interest in pharmaceutical science, because they increase the aqueous solubility, chemical stability and bioavailability of poorly water-soluble drugs. By means of elastic incoherent neutron scattering (EINS) technique on the backscattering spectrometer IN13, we measured, in the 150−340 K temperature range, the mean square displacements (MSD) of hydrogen atoms derived from elastic spectra of inclusion complex of β-cyclodextrin (β-CyD) with genistein (Gen), a phytoestrogen of great interest for its antioxidant, anticarcinogenic and antiosteoporotic activities. The mobility of the complex has been compared with the single components and the physical mixture. The elastic intensity has been interpreted in terms of the double-well model in the whole temperature range. In the case of Gen, a mainly vibrational dynamics is revealed, while for the other samples, the elastic intensity becomes non-Gaussian above a temperature $T_d \cong 220-230$ K. This dynamical activation, which is represented by an Arrhenius trend, seems to be promoted by the crystallization water molecules. The dynamics of the Gen/β-CyD inclusion complex is restricted with respect to the physical mixture, due to the action of specific "host−guest" interactions upon complexation. Finally, the dynamical response of our systems to temperature is put in relationship with their thermal stability.

Introduction

The α-, β- and γ-cyclodextrins (CyDs) are naturally occurring, water-soluble, nonreducing macrocyclic sugar molecules connecting, respectively, six, seven and eight glucose units via α-(1,4)-linkages, obtained from the hydrolysis of starch by means of enzyme *Bacillus macerans* amylase.[1]

They have a toroidal shape containing a hydrophobic inner cavity and two hydrophilic rims, where primary and secondary hydroxyl groups are located. This unusual structure gives to CyDs the capability of including a wide variety of polar and apolar "guest" molecules into their hydrophobic "host" cavity, via noncovalent interactions, forming axial and equatorial type inclusion complexes, in both solution and the solid phase.[2,3]

Steric factors, extrusion of water from the inner cavity, relief of ring strains in the cyclodextrin ring, "host−guest" hydrophobic, H-bond and electrostatic interactions, induction and London dispersion forces have been shown to play a fundamental role in driving the cyclodextrin inclusion complexes formation.[4−6] Complexation with cyclodextrins has been reported in a number of studies to modify chemical, physical and biological properties of the guest molecules.[7,8] Relevant application areas include environmental protection, food industry, catalysis, separation technologies and textiles processes.[9−11] In pharmaceutical research, CyDs have been extensively used as complexing agents to improve solubility, stability and bioavailability of a variety of poorly soluble and labile drugs in formulation and delivery systems.[12,13] Furthermore, the chiral recognition ability of CyDs has been recently employed in many drugs, for separation of enantiomers with different pharmacological activities,[14,15] and for modeling the activity of many enzymes.[16,17]

To date, the thermodynamic stability of the drug/CyD systems has been studied by evaluation, in liquid phase, of the constant binding from UV−vis, circular dichroism, fluorescence, calorimetry, NMR, electron spin resonance (ESR), potentiometry, chromatography, capillary electrophoresis, etc.[18−21] The water solubility of a free drug, when not reported in literature, is generally determined from the phase-solubility diagram by means of electronic and emission spectroscopy.

When dealing with complexes in the solid phase, experimental studies are not so abundant, mainly because of the comparatively reduced number of techniques. Crystalline compounds have been identified by X-ray and neutron diffraction, since the guest inclusion gives rise to a new crystal lattice.[22−24] The effect of the inclusion process on the guest molecule, by evidencing the interactions that drive complexation between CyDs and drugs of every kind, has been the subject of Fourier transform Raman and infrared (FT-Raman and FT-IR, respectively) investigations.[25−28] In these studies, the "host−guest" interactions have been evidenced by monitoring, in the vibrational spectra, the changes in some guest molecule bands relative to those observed in the spectra of the physical mixtures (blends of drug and macrocycle without complexation) and complexes.

* To whom correspondence should be addressed. Tel: +390906765237. Fax: +39090395004. E-mail: majolino@unime.it.
[†] Department of Physics, University of Messina and CNISM.
[‡] University of Perugia and CNR−INFM CRS SOFT.
[§] Pharmaco-Chemical Department, University of Messina.

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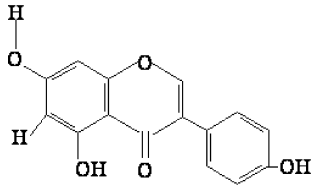

Figure 1. Chemical structure of Genistein (Gen, 4′,5,7-trihydroxyisoflavone).

Genistein (4′,5,7-trihydroxyisoflavone, Gen, see Figure 1), a phytoestrogen belonging to the isoflavone class of compounds, plays a relevant role in the treatment and prevention of various cancers and other diseases, such as cardiovascular and osteoporosis among others. It is a potent tyrosine kynase inhibitor and also possesses antioxidant activity.[29−32]

In vitro and animal studies proved that Gen activity is involved in the inhibition of cancerous cell growth. In addition, products containing Gen are now marketed as nutritional supplements. Nevertheless, Gen seems to have some disadvantages, which considerably limit its potential clinical utility. These include rapid *in vivo* metabolism and excretion, low serum level after oral administration, and insufficient targeting of cancer cells. In addition, poor solubility and slow dissolution in water engender problems in oral bioavailability of Gen. Complexation with CyD represents an effective strategy to improve the rate of absorption of Gen, achieving a rapid onset of activity.[33,34]

In a previous study,[26] we investigated, in pure water, the effect of β-CyD complexation on the UV absorption of Gen. A phase solubility study allowed us to evaluate the changes of isoflavone in the complexation state, suggesting that it forms complexes with a stoichiometry of 1:1. Then, FT-IR spectroscopy in attenuated total reflectance (ATR) geometry has been successfully used to put into evidence "host−guest" interactions at the molecular level in the solid phase.

A deeper understanding of the interactions between drug and macrocycle can be achieved by comparing the amplitude of picosecond time scale motions of the Gen/β-CyD inclusion complex with that of the physical mixture, once the dynamics of the single components has been clarified. Here we report the results of an elastic incoherent neutron scattering (EINS) investigation performed as a function of temperature. It is shown that "host−guest" interactions significantly lower the thermal fluctuations of the complex. This finding can be of key importance to optimize the performances of drugs for specific therapeutic effects, in view of the existence of a relationship between the molecular mobility on the picosecond time scale and thermal stability.

Experimental Methods

Samples. A powder of crystalline isoflavone genistein (Gen, 4′,5,7-trihydroxyisoflavone, $C_{15}H_{10}O_5$, FW ≈ 270.24) in native form was purchased from Sigma Aldrich Chemie (USA). Powder of fully hydrated crystalline β-cyclodextrin (β-CyD, FW ≈ 1135.0, mp ≈ 290−300 °C) was purchased from Fluka Chemie (Switzerland). They were employed without any further purification.

In particular, no hydration water molecules are entrapped by Gen, because of its hydrophobic character, whereas fully hydrated β-CyD crystallizes from aqueous solutions in the form β-CyD·12H_2O.[35] ∼7 water molecules are located within the β-CyD cavity, where hydrophobic interactions are dominant, as a cluster, disordered over 11 partially occupied sites. 5.4 molecules are distributed over eight sites located in the interstitial spaces among different β-CyD molecules, where hydrogen bonds with β-CyD macrocycles take place.[36]

Preparation of the Physical Mixture. Gen + β-CyD physical mixture was obtained by pulverizing in a glass mortar and carefully mixing an accurately weighed (1:1 molar ratio) amount of Gen and β-CyD, until the mixture was homogeneous. Both the crystalline structures of Gen and β-CyD, as well as the twelve H_2O molecules hydrating β-CyD, are maintained in the physical mixture.

Preparation of the Inclusion Complex. Inclusion complex with 1:1 Gen/β-CyD molar ratio was prepared by coprecipitation method, by shaking the appropriate amounts of drug and macrocycle in water at 60 °C. The water used throughout the study was deionized and double-distilled, then filtered through 0.22 μm Millipore GSWP filters (Bedford, MA). The mixture was stirred for 1 h, under controlled temperature (25.0 ± 0.01 °C) using Telesystem stirring bath thermostat 15.40 with a Telemodul 40 °C control unit which allowed an accuracy of 0.01 °C. The reaction mixture was cooled in the refrigerator (4 °C) for about 24 h, and the water was subsequently evaporated under vacuum (∼30 °C), obtaining a powder of Gen/β-CyD inclusion complex. The coprecipitated product consists of an amorphous solid dispersion or molecular encapsulation of the drug into the cyclodextrin cavity. In these operative conditions, thermogravimetric analysis indicates that the residual water in the Gen/β-CyD inclusion complex corresponds to an amount of twelve H_2O molecules, as in the case of β-CyD alone, as also verified by previously FTIR-ATR measurements.[26] Uncomplexed drug has been filtered, prior to solution evaporation, through Sartorius Minisart-SRP 15 PTFE 0.45 μm filters.

Incoherent Neutron Scattering. The aim of a neutron scattering experiment is to probe the functional dependence on E and $\mathbf{Q}$ of the dynamical structure factor $S(\mathbf{Q}, E)$, which represents the probability for an incident neutron to be scattered by the sample with energy and momentum transfer $E = \hbar\omega$ and $\hbar\mathbf{Q}$, respectively. $S(\mathbf{Q}, E)$ is the Fourier-transform of the time-correlation function of the density fluctuations in the system, that provides, through its $\mathbf{Q}$- and E-dependence, information on the characteristic times and geometry of molecular motions that give rise to the revealed signal.

In principle, then, the dynamical structure factor includes both coherent and incoherent contributions, which arise from, respectively, inter- or self-particle correlations of collective or individual atomic motions. However, for the systems under investigation, the dominant contribution to the revealed signal is due to the large amount of hydrogen atoms, characterized by a very large, almost exclusively incoherent neutron cross section (σ_{inc} = 79.90 barns, σ_{coh} = 1.76 barns), by far higher than the coherent or incoherent cross section of any other element.[37] Therefore, neutron scattering experiment will allow the study of the self-particle dynamics of hydrogen atoms which, in turn, can be considered as a probe of the thermal fluctuations of the whole molecule.[38−41]

In addition, the present samples being isotropic, the dynamical structure factor depends only on the modulus of the momentum transfer.

In our experiment, the neutron-scattered intensity has been recorded within a narrow energy interval of 2 μeV centered at the nominal value of the elastic peak ($E \approx 0$). This channel corresponds to the energy window that we took as representative of the elastic peak and should not be confused with the energy resolution, that usually assumes a larger value. In this operative condition, the intensity of elastically scattered neutrons $S(Q, E$

≈ 0), as a function of both Q and T, in the incoherent approximation,[37] can be written as

$$S(Q, E \approx 0) \propto e^{-\langle u^2\rangle_G Q^2}[A_0(Q)\,\delta(E)] \otimes R(Q, E) \quad (1)$$

In the above equation, the Gaussian term is the so-called Debye−Waller factor, which describes the Q-dependence of the elastic intensity due to the vibrational atomic mean-square displacement MSD $\langle u^2\rangle_G$. The contribution of internal motions to the elastic peak is described by the expression within the square brackets. Its energy dependence is obviously given by a delta function $\delta(E)$, whereas the modulation as a function of Q is provided by the elastic incoherent structure factor (EISF) $A_0(Q)$, that is the space-Fourier transform of the scatterers' distributions taken at infinite time, averaged over all the possible initial positions. Its Q-dependence furnishes information on the geometry and type of motions of the scatterers. A reasonable model to describe the observed dynamics is the so-called double-well jump model,[42] extensively applied to picosecond proton dynamics in several kinds of disordered systems.[43,44] It provides an oversimplified description of the energy landscape, taking into account the deviation of the elastic intensity from the Gaussian behavior. In spite of this approximation, one can obtain, as explained in the following, a quantitative estimate of the mean square displacement MSD. In this framework, the protons are considered dynamically equivalent, and their motions are schematized as jumps between two distinct sites with a free energy difference ΔG. The corresponding EISF is written as

$$A_0(Q) = 1 - 2p_1p_2\left(1 - \frac{\sin(Qd)}{Qd}\right) \quad (2)$$

where p_1 and $p_2 = (1 - p_1)$ are the occupation probabilities of the ground and the excited state, and d is the spatial distance between the two potential wells.

Again, in the fitting procedure, the T-dependence of $\langle u^2\rangle_G$ has been described by an Einstein model of independent oscillators:[38]

$$\langle u^2\rangle_G = \frac{\hbar\omega}{2K}\coth\left(\frac{\hbar\omega}{2k_BT}\right) - \langle u^2\rangle_0 \quad (3)$$

in which K and ω represent, respectively, the average force field constant and the average frequency of the set of the oscillators; accordingly, the relationship $\langle u^2\rangle_0 = \hbar\omega/2K$ provides the zero-point mean square displacements. Actually, because the measured elastic intensity has been normalized with respect to the lowest temperature (as indicated below), the zero-point mean square displacements have been subtracted in the right side of eq 3.

To provide a quantitative measure of the average hydrogen mobility, the total MSD $\langle u^2\rangle_{tot}$ has been derived from eq 1, with $A_0(Q)$ given by eq 2 and $\langle u^2\rangle_G$ given by eq 3, via the relationship[44]

$$\langle u^2\rangle_{tot} = -\left[\frac{d\ln S(Q, E \approx 0)}{d(Q^2)}\right]_{Q=0} = \langle u^2\rangle_G + \frac{1}{3}p_1p_2d^2 = \langle u^2\rangle_G + \langle u^2\rangle_c \quad (4)$$

In this equation, $\langle u^2\rangle_G$ is the aforementioned Gaussian vibrational MSD term, whereas $p_1p_2d^2/3 = \langle u^2\rangle_c$ represents the conformational contribution to the total MSD[45] and describes the proton mobility due to jumping between the two energetic sites.

The internal motions are convoluted with the experimental resolution function $R(Q, E)$. Considering the narrow energy interval we explored, this function can be approximated by a Q-independent Gaussian function $R(E) = \exp(-\pi E^2/4\sigma^2)/2\sigma$, where the half-width at half-maximum (HWHM) is $\Gamma_R = 2\sigma\ln 2/\sqrt{\pi}$.

Neutron Scattering Experiment. Incoherent elastic neutron scattering scans were performed by using the high-energy resolution, wide momentum transfer backscattering spectrometer IN13, at the Institut Laue-Langevin in Grenoble (France). An energy resolution of $\Gamma_R = 4.5\ \mu$eV (half-width at half-maximum, HWHM) and an average wave-vector transfer resolution of ~0.2 Å^{-1}, corresponding to an incident neutron wavelength of 2.23 Å, were achieved in the Q range 0.2−4.6 Å^{-1}. The energy resolution and exchanged momentum range will correspond to time and space windows determined, respectively, by $1/\Gamma_R$ and $2\pi/Q$. In our case, the instrumental resolution corresponds to a time window for accessible motion faster than ~150 ps, in a spatial region with dimensions from ~30 to ~1 Å. As sample holder, a standard, indium sealed, flat aluminum cell, with variable internal spacing was used. In particular, a thickness of 0.2 mm was used for Gen, Gen + β-CyD physical mixture and Gen/β-CyD inclusion complex, whereas a thickness of 0.5 mm was used for β-CyD. For each measurement, the sample holder was placed at an angle of 135° with respect to the incident beam direction. The temperatures explored ranged from 150 to 340 K, with a precision of ±1 K and an acquisition time of ~3 h for Gen, ~1 h for β-CyD, ~2 h for Gen + β-CyD physical mixture and Gen/β-CyD inclusion complex. The acquired data were corrected in order to take into account the incident flux, cell scattering, self-shielding, and detector responses. Then, the elastic intensity of each sample relative to a given temperature was normalized with respect to the corresponding lowest measured temperature. A transmission of ~96% was obtained for Gen, of ~87% for β-CyD, of ~94% for Gen + β-CyD physical mixture and Gen/β-CyD inclusion complex. An estimate of the multiple scattering confirms that it is below 10% on the elastic peak and it affects approximately in the same way all the analyzed samples, so multiple scattering contributions have been neglected.

Results and Discussion

A model-independent qualitative measure of the molecular mobility of the systems we investigated can be obtained by looking at the departure from unity of their elastic intensities.[46] In Figure 2 we report as a function of the temperature the elastic intensities integrated over the Q range from 0.2 Å^{-1} up to 3.5 Å^{-1} for all the analyzed samples.

First of all, we observe that above a certain temperature the elastic intensity of β-CyD departs from that of Gen becoming lower and lower with increasing T, indicating a more and more enhanced mobility of the macrocycle with respect to the pure drug. This is a reasonable result, taking into account the presence of a certain amount of hydration water in β-CyD but not in Gen. These H_2O molecules, as reported for a variety of systems,[47−49] should promote the activation of additional degrees of freedom over the harmonic behavior. Again, we notice that the EINS spectrum of the physical mixture turns out to be a weighted addition of pure Gen and β-CyD. This result, already put into evidence by FTIR-ATR spectroscopy, is indicative of

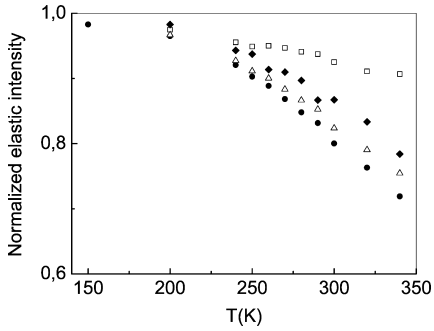

Figure 2. Normalized incoherent elastic intensities versus T integrated over the Q range from 0.2 Å^{-1} up to 3.5 Å^{-1} for all the analyzed samples: Gen (□), β-CyD (●), Gen + β-CyD physical mixture (Δ), Gen/β-CyD inclusion complex (◆).

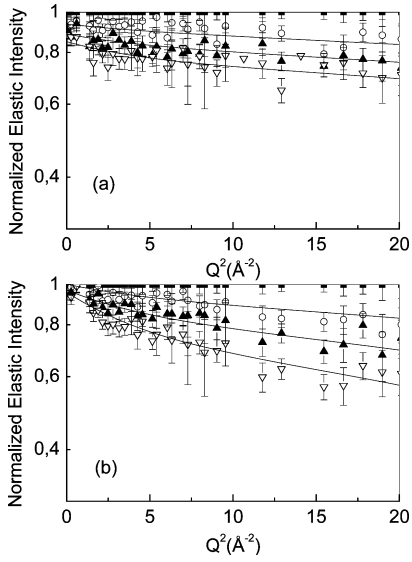

Figure 3. Normalized incoherent elastic intensities versus Q^2 of Gen (a) and Gen/β-CyD inclusion complex (b) at four different temperatures: 200 K (■), 260 K (○), 300 (▲), and 340 K (▽). Solid lines are fits according to eq 1 with $A_0(Q)$ given by eq 2 and $\langle u^2\rangle_G$ given by eq 3. See text for details. The absence of data at ∼10 Å^{-2} and ∼14 Å^{-2} is due to noisy behavior of detectors 12 and 15 of IN13 spectrometer during the experiment.

the absence of any kind of "host−guest" interactions.[26] On the contrary, the comparison of the elastic intensities of Gen + β-CyD physical mixture and Gen/β-CyD inclusion complex reveals that the latter is definitely less mobile above 200 K. This difference cannot be ascribed to a different hydration degree of these two systems, since, as indicated in the Experimental Methods section, they are characterized by the same amount of residual water. A quantitative description of the activated dynamics will be given below in terms of the MSD, as derived through the double-well model.

The measured elastic intensity as a function of Q^2 for pure drug and inclusion complex is reported in Figure 3(a) and Figure 3(b), at different temperatures. We remark that the expected trend to 1 of the elastic intensity when $Q \rightarrow 0$ is not always observed. This is due to multiple scattering contributions, not taken into account in the data analysis, that become more relevant when T increases. We expect that this slightly Q-dependent multiple scattering contribution will not significantly affect the results.

A comparative plot of the elastic intensity behavior at low (T = 240 K) and high (T = 340 K) temperature for all the analyzed samples is shown in Figures 4(a) and 5(b), respectively.

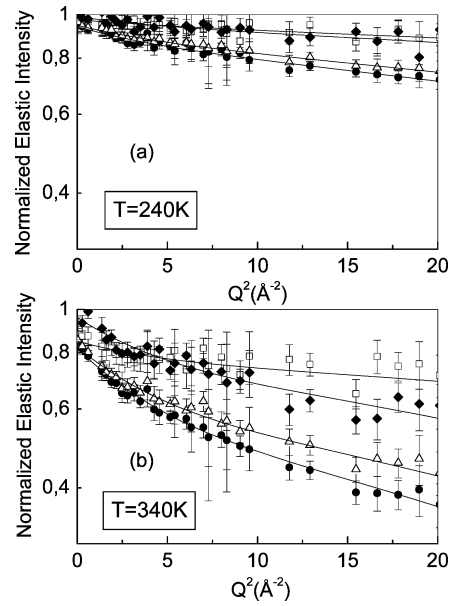

Figure 4. Normalized incoherent elastic intensities versus Q^2 at T = 240 K (a) and T = 340 K (b) for all the analyzed samples: Gen (□), β-CyD (●), Gen + β-CyD physical mixture (Δ), Gen/β-CyD inclusion complex (◆). Solid lines are fits according to eq 1, with $A_0(Q)$ given by eq 2 and $\langle u^2\rangle_G$ given by eq 3. See text for details.

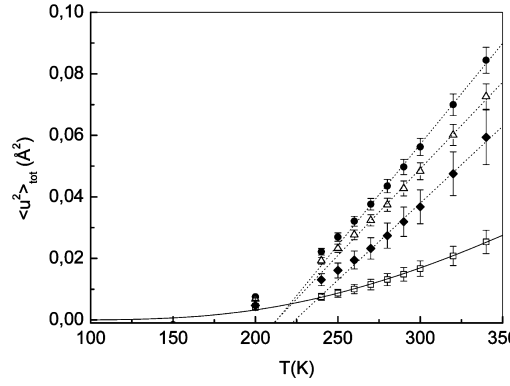

Figure 5. Total mean square displacements versus T for all the analyzed samples: Gen (□), β-CyD (●), Gen + β-CyD physical mixture (Δ), Gen/β-CyD inclusion complex (◆). Solid line represents the harmonic trend of Gen according to eq 3. Dashed lines are linear fits to the high-temperature data.

As can be seen, at low T the measured intensity of Gen and Gen/β-CyD inclusion complex can be satisfactorily approximated with a Gaussian trend, indicative of a mainly vibrational (harmonic-like) internal dynamics. On the contrary, the signal of β-CyD and Gen + β-CyD physical mixture slightly departs from the Gaussian behavior, as emphasized by the logarithmic scale. By increasing the temperature, the deviation from the Gaussian behavior is revealed for all the analyzed samples, and is more marked for the physical mixture and even more for the pure macrocycle. This non-Gaussian trend is reminiscent of that observed in glass-forming systems and biomolecules.[39,41,43,44] By analogy with these systems, we may suppose it originates from the activation of some additional anharmonic dynamics, thanks to the available thermal energy, involving double-well jumps over the energy barrier of different conformational substates, with an estimate of the mean spatial extent over which thermal fluctuations take place that is given by the distance between the two potential wells d, as reported in eq 2.

Then, the experimental spectra have been fitted according to eq 1, with $A_0(Q)$ given by eq 2 and $\langle u^2\rangle_G$ given by eq 3, and

reproduced in a quite satisfactory way (as shown in Figures 3 and 4). The value of the jump distance d is found to be 1.5 ± 0.2 Å for all the analyzed samples, independent of the temperature.

As previously specified, quantitative information on the average proton mobility, that can be compared with the results obtained by molecular dynamics (MD) simulations or other experimental techniques,[39,50] is provided from the behavior of $\langle u^2\rangle_{tot}$ vs T, as obtained from eq 4. The total MSD are shown in Figure 5 for all the analyzed samples.

In the case of Gen, those data reveal a harmonic trend which can be described by eq 3, thus indicating that drug protons mainly undergo vibrational motions in a quadratic potential well.[51] Different is the case of the pure macrocycle, physical mixture and inclusion complex, where a clear departure from the harmonic behavior can be observed at around $T_d \cong 220-230$ K. This phenomenon is reminiscent of the so-called dynamical transition which takes place in several disordered materials.[43,44] The values of T_d have been estimated by the intercept between the harmonic trend of Gen and the straight lines describing $\langle u^2\rangle_{tot}$ at higher temperatures. There is of course a certain degree of arbitrariness in estimating T_d, as the dynamical transition appears as a continuous variation of $\langle u^2\rangle_{tot}$ as a function of the temperature. However, we remark that the behavior of T_d for the different samples is consistent with the departure of their elastic intensities with respect to the harmonic behavior of pure Gen (Figure 2). In addition, the so estimated T_d values are in agreement with those calculated from the deviation of the total MSD with respect to the $\langle u^2\rangle_G$ as obtained from the Einstein model of independent oscillators (eq 3). The revealed anharmonic internal dynamics can be related to the relaxation of the hydrogen bonding network formed by the hydroxyl groups (OH groups of Gen, primary and secondary OH groups of β-CyD) and crystallization residual water molecules. These water molecules, which correspond to a hydration degree of around 0.2 g of water/g of β-CyD, may drive the dynamical transition through the onset of new translational degrees of freedom, as it happens in the case of hydrated protein powders.[39] This kind of conformational transition from glassy to flexible state does not take place in the case of Gen, which does not contain plasticizing water molecules. We add that, as far as the inclusion complex is concerned, the amorphization induced by coprecipitation will also contribute to the observed dynamical transition.

In the whole explored T-range, the mobility of the inclusion complex is definitely lower than that of the physical mixture. This result strongly supports a picture where the "host−guest" interactions are responsible for a constrained average dynamics of the inclusion complex.

A hindering of the vibrational dynamics for the Gen/β-CyD inclusion complex was already observed by FTIR-ATR spectroscopy.[26] The spectral changes (bumping and diminishing in intensity) revealed, upon complexation, for the O−H, C═O, C═C, C−O−C, C−O stretching and C−H bending vibrations, were there explained by hypothesizing that, during the complexation process, Gen enters into the β-CyD torus-shaped cavity, disturbing some of the previous existing bonds, while the interstitial and intracavity water molecules change their hydrogen bonding environment. The present findings show that the "host−guest" interactions affect as well the relaxational motions on the picosecond time scale.

We finally focused on the important aim at characterizing the average rigidity of the samples in the high-temperature region, where large structural fluctuations take place and the systems become less and less stable. It may be argued that, as the systems approach the instability region, the double-well method will lose its validity as the atoms will tend to explore most of the conformational space accessible to them. In other words, in the high-temperature range, the energy landscape accessible to the average hydrogen atom will resemble a confining harmonic potential well:[45]

$$V(r) = br^2/2 \tag{5}$$

It should be remarked that this potential is only apparently harmonic, as actually describes the overall shape of the multiple minima of the conformational substates theory.[52] This approximation holds provided that the experimental time-window is quite large (high energy resolution), in such a way that the atoms have the time to explore the conformational landscape. It is as well reasonable to suppose that eq 5 may be applied for temperatures such that $p_2/p_1 \geq 0.5$, i.e. above 280 K where the conformational landscape is rather homogeneously populated.

Under these conditions, the configurational mean square displacements are then given by

$$\langle u^2\rangle_c = k_B T/3b \tag{6}$$

The b parameter is a measure of the system rigidity, since it quantifies the linear response of the system fast fluctuations as a function of temperature, sampled within the present experimental time resolution. A fit of the $\langle u^2\rangle_c$ trend in the high-T range, by using eq 5, allows for a direct estimation of b. We remark that a similar approach has been already used also to describe the proteins dynamical features in terms of an effective force constant.[53]

Table 1 shows the estimated T_d and b values.

The stiffness, as quantified by the b parameter, increases on passing from physical mixture to inclusion complex. It is now possible to make some hypotheses with the aim at providing new insight on the connection between the stiffness parameter b and thermal stability, which may be quantified by the melting temperature T_m of a sample. As a matter of fact, it is widely reported in literature[54] that the occurrence of complexation with β-CyD (that, as well-known,[55] melts and decomposes at around 563 K) is accompanied by the disappearance of the endothermic peak that is characteristic of the encapsulated molecule (in the case of Gen, a sharp melting peak has been revealed[56] by differential scanning calorimetry at around 582 K). This disappearance, which can be can be ascribed to both the new amorphous state resulting from complexation and the strong "host−guest" interaction, indicates a greater thermal stability of the inclusion complex with respect to the single components. On the contrary, in the case of physical mixture each component generally behaves independently and the thermogram shows the presence of peaks of both pure compounds.

Then, on the basis of the aforementioned studies, and taking into account previous EINS results[57] where higher T_d and b values are accompanied by higher T_ms, we can hypothesize that higher T_d and b values for Gen/β-CyD inclusion complex with respect to physical mixture will correspond to a greater thermal stability of the latter with respect to the former. With this respect, a differential scanning calorimetry analysis on our samples will be helpful to clarify this behavior.

The present results support an emerging scenario where the mechanism through which complexation process improves the

TABLE 1: Dynamical Transition Temperature (T_d) and Rigidity (b) for All the Investigated Samples

sample	T_d (K)	b (N/m)
Gen		2.40 ± 0.1
β-CyD	220	0.70 ± 0.03
Gen + β-CyD physical mixture	220	0.82 ± 0.04
Gen/β-CyD inclusion complex	230	0.92 ± 0.05

drug thermal stability, i.e. the activation of "host−guest" interactions and the formation of an amorphous phase, is closely related to the hampering of the molecular thermal fluctuations in the picosecond time scale. On these grounds, the stiffness parameter seems to be of relevance in pharmaceutical field, since starting from a physical characterization of the "host−guest" specific interactions in the solid phase, it will be in principle possible to attempt an enhancement of the technological performance in use (toughness or stability) of solid drug/cyclodextrin formulations, such as conventional tablets or extended-release matrix, or pellets prepared by agglomeration techniques.

Conclusions

The T-dependence of molecular mobility of Gen/β-CyD inclusion complex in the picosecond time scale ($\tau \leq 150$ ps) has been investigated through elastic incoherent neutron scattering and compared with those of the single components and the physical mixture. The aim was to understand the effect on the thermal fluctuations of the "host−guest" interactions activated during the complexation process and to establish a possible connection between the thermal stability of the complex and the dynamical response to the temperature variation.

Within the experimental time window, the pure drug appears to behave mainly like a harmonic solid at all the analyzed temperatures, whereas for β-CyD, physical mixture and inclusion complex the activation of additional degrees of freedom is the signature of the so-called dynamical transition, revealed at a temperature $T_d \cong 220-230$ K. This phenomenon, that can be described by a two-site jump model, appears to be driven by the hydration due to the presence of crystallization water molecules. In particular, it has been correlated with the relaxation of the hydrogen bonding network between biomolecules and crystallization water molecules, which, in turn, can be associated with the onset of translational modes. In the case of inclusion complex, its amorphous state will also play a role in the revealed transition.

The specific "host−guest" interactions, involved in the formation of the Gen/β-CyD inclusion complex, not only modify the structural properties of the complex, but as well its average dynamics in the picosecond time scale. In fact, the thermal fluctuations of the complex are sensibly less large than those of the physical mixture. This smaller amplitude, together with the reduced dynamical response vs T of the complex, are probably connected to the well-known improvement of the thermal stability of drugs upon complexation. The molecular rigidity related to the hampering of the picosecond time scale dynamics can be of great concern in order to optimize the drug design of cyclodextrin-based pharmaceutics.

Additional quasi elastic and inelastic neutron scattering studies on these systems to better describe the nature of the motions are in progress.

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