



## The Aggregation of Cyclodextrins as Studied by Photon Correlation Spectroscopy

G. GONZÁLEZ-GAITANO<sup>1\*</sup>, P. RODRÍGUEZ<sup>1</sup>, J.R. ISASI<sup>1</sup>, M. FUENTES<sup>1</sup>, G. TARDAJOS<sup>2</sup> and M. SÁNCHEZ<sup>1</sup>

<sup>1</sup>Departamento de Química y Edafología, Facultad de Ciencias, Universidad de Navarra, 31080, Pamplona, Spain;

<sup>2</sup>Departamento de Química-Física I, Facultad de Ciencias Químicas, Universidad Complutense, 28040, Madrid, Spain

(Received: 7 May 2002; in final form: 1 October 2002)

**Key words:** cyclodextrins, dynamic light scattering, photon correlation spectroscopy, self-aggregation

### Abstract

Photon correlation spectroscopy has been used to study the aggregation processes of natural and some modified cyclodextrins (CDs) in diluted aqueous solutions.  $\alpha$ -,  $\beta$ -, and  $\gamma$ -CD form large, polydisperse aggregates in water, although the aggregation capability is different depending on the macrocycle considered.  $\gamma$ -CD solutions filtered through 0.2  $\mu\text{m}$  give a single-modal distribution of aggregates of 224 nm in size. The monomeric  $\gamma$ -CD can be isolated by filtering through 0.1  $\mu\text{m}$ .  $\alpha$ -CD displays a bimodal distribution (monomer + aggregates) with both pore sizes. At the concentrations studied (0.012 M) the contribution in mass of the aggregates with both CDs is negligible.  $\beta$ -CD is much more persistent in its aggregation, even after sieving its solutions through 0.02  $\mu\text{m}$  filters, and time dependent. The aggregation displayed by CDs with partial substitution of the OH groups (Methyl- $\beta$ -CD and Hydroxypropyl- $\beta$ -CD) is much weaker, indicating the implication of the hydrophilic rims of the CDs in the process. High temperatures, addition of urea or electrolytes and ionisation of the OH groups by raising the pH, prevent the aggregation.

### Introduction

An aspect of CDs not completely understood is their ability to self-aggregate in water. The number of papers dealing with this subject in the literature is scarce and nowadays certain controversy still exists regarding the conditions in which these assemblies form, their structure or the driving force of the process [1–5]. However, the comprehension of these aspects is important for a number of reasons. For example,  $\gamma$ -CD solutions at concentrations above 1% display an increasing opalescence with time, which leads to precipitation, what precludes their use in ophthalmic preparations. This turbidity has been ascribed to the self-aggregation of  $\gamma$ -CD monomers [6]. Another example can be found in a recent paper of Polarz *et al.* who report the use of concentrated CD solutions as nanomolds in order to synthesise porous silica materials [7]; unexpectedly, instead of finding a material with imprinted pores of the size of a CD molecule, they obtained patterns of worm-like pores, which are assigned to some type of assemblies formed by axial stacking of CDs in solution. The presence of CD aggregates can also be a drawback in macromolecular characterisations when dealing with CDs, polymers of CDs or polyrotaxanes, specially if light scattering detection is used, leading to wrong values of the molecular weights calculated. In the same way, concentrated CD solutions could mislead UV or fluorescence spectroscopic data due to intensity loss caused by scattering.

In this work we have investigated the formation of CD aggregates in water, in diluted conditions, by photon correlation spectroscopy (PCS, DLS, or QELS). This technique has proven to be advantageous in the study of the sizes of macromolecular systems, or the associations of small molecules to form supramolecular assemblies [8]. The basis of this technique lies in the measurement of the autocorrelation function of the scattered intensity, that is, the convolution of the intensity signal as a function of time with itself. The longer the correlation with time, the slower the movement of the particles through the solution, a property that can be quantified by means of the diffusion coefficient. Both variables, size and diffusion, are related by the Stokes–Einstein equation:

$$R_h = \frac{kT}{6\pi\eta_0 D_0}, \quad (1)$$

where  $T$  is the absolute temperature,  $k$  the Boltzmann constant,  $\eta_0$  the viscosity of the solvent, and  $D_0$  the diffusion coefficient at infinite dilution. This is obtained by means of a mathematical procedure known as regularised inverse Laplace transformation, which yields the size distribution of the particles responsible for the autocorrelation function measured. The strong point of this technique is its sensitivity both to the concentration and to the size of the particles, specially to the latter, allowing the detection of aggregation in an early stage.

\* Author for correspondence. E-mail: gaitano@unav.es

## Experimental

$\beta$ -CD was kindly supplied by Roquette (lot E0568),  $\alpha$ -CD and  $\gamma$ -CD by Wacker (lots 60P068 and 1016, respectively). Water contents were 11.10, 8.29, and 7.91% respectively, as determined by thermal analysis. Methyl- $\beta$ -CD was from Cyclolab (CY-2004.1, batch 337, DS 12-13, water content less than 0.5%) and HP- $\beta$ -CD from RBI (H-107, lot UCD-697B, water content 7.4%). Freshly deionized water was used in the preparation of the CD solutions, with a maximum concentration of 0.012 M (about 1.5%). They were filtered prior to the measurements with 0.2  $\mu\text{m}$  filters (Albet, cellulose acetate), 0.1  $\mu\text{m}$  or 0.02  $\mu\text{m}$  (Whatman, inorganic membrane).

DLS measurements were performed at a scattering angle of 90° using a DynaPro-MS/X photon correlation spectrometer, equipped with a 248 channel multi-tau correlator and a Peltier effect temperature unit. The wavelength of the laser was 825.2 nm. The size distribution was obtained from the intensity autocorrelation function by regularization analysis, implemented in the Dynamics<sup>TM</sup> software package, and the hydrodynamic radii were calculated from the diffusion coefficients by means of the Stokes–Einstein equation. Temperature was  $25.0 \pm 0.1$  °C unless otherwise stated.

## Results and discussion

### Natural and substituted CDs

Figure 1 shows the correlogram and the corresponding regularization analysis for a 0.012 M  $\gamma$ -CD solution filtered through 0.2 and 0.1  $\mu\text{m}$  and immediately measured. With the 0.2  $\mu\text{m}$  filter, a polydisperse single modal distribution is observed, yielding a mean hydrodynamic radius of  $112 \pm 37$  nm. By filtrating through 0.1  $\mu\text{m}$ , a fast mode due to the monomeric  $\gamma$ -CD, at 0.84 nm in radius, is now detected, together with a slow mode due to the tail of the distribution that can pass through the pores.  $\alpha$ -CD, however, renders a bimodal distribution even with the 0.2  $\mu\text{m}$  filter, the faster mode corresponding to the monomeric  $\alpha$ -CD (0.74 nm) and the slow one ( $68 \pm 20$  nm) attributable to aggregates (Table 1). The trend in the hydrodynamic radii for the monomers is reasonable in the light of the actual dimensions of CDs [9]. The diameters of the aggregates are in accordance with those of Coleman *et al.* [1], although they do not report the detection of monomeric CD. In the mentioned work, few details are offered regarding the conditions in which the experiment has been carried out, specially about filtering and data analysis. However, care must be taken in this subject, since a single modal analysis of the scattering data would impute all the measured intensity to the aggregates, leading to the conclusion that CDs in water are extensively aggregated. In view of Figure 2 for  $\alpha$ -CD, 0.2  $\mu\text{m}$  filtered, although the intensity contribution for the slow mode is *ca.* 97%, its mass contribution must be very low. The mass distribution can be calculated approximately, by considering that the scattering intensity of a particle  $i$  is

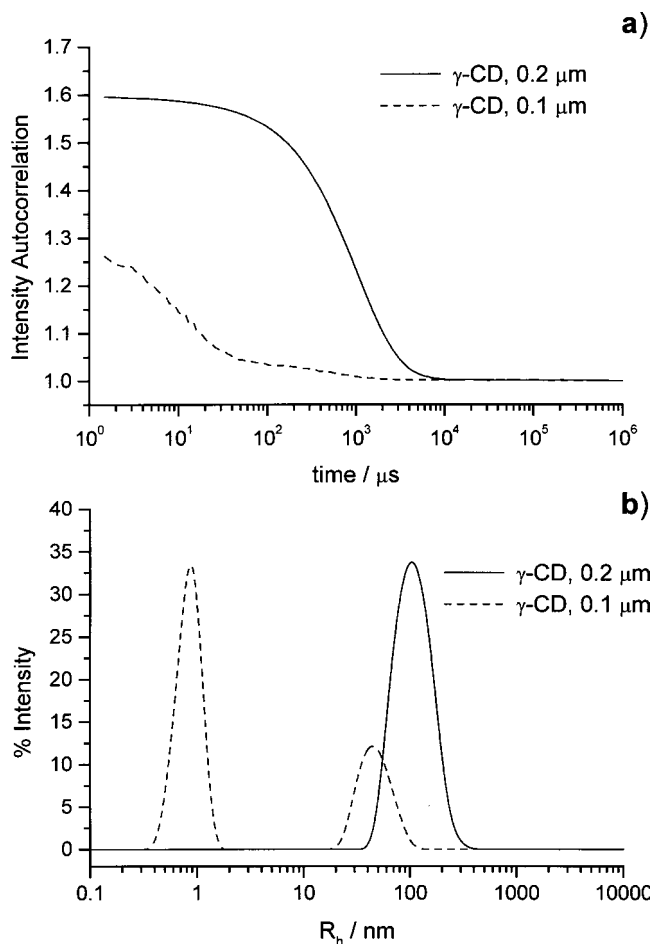


Figure 1. (a) DLS correlograms for  $\gamma$ -CD 0.012 M at 25 °C filtered through 0.1  $\mu\text{m}$  and 0.2  $\mu\text{m}$ ; (b) size distributions for the curves of (a).

Table 1. Mean hydrodynamic radii and polydispersities (in nm) of 0.012 M CDs solutions at 25 °C

	0.1 $\mu\text{m}$ filter*	0.2 $\mu\text{m}$ filter
$\alpha$ -CD	$0.69 \pm 0.11$ $126 \pm 36$	$0.74^{**}$ $68 \pm 20$
$\beta$ -CD	$0.78^{**}$ $124 \pm 32$	$174 \pm 38$
$\gamma$ -CD	$0.84 \pm 0.13$ $48 \pm 13$	$112 \pm 37$
RAMEB	–	$0.85 \pm 0.16$ $60 \pm 17$
HP- $\beta$ -CD	–	$0.86 \pm 0.14$ $62 \pm 12$

\* $\beta$ -CD was filtered with 0.02  $\mu\text{m}$ ;

\*\*polydispersity very small.

proportional to both its molecular weight,  $M_i$  and its weight concentration,  $c_i$ .

$$I_i = K M_i c_i. \quad (2)$$

The molecular weight of the  $i$  particle can be estimated from the following equation, where  $v$  is its partial specific volume,  $a$  is a shape parameter equal to 2 for coils and 3 for spherical particles, and  $N_a$  is the Avogadro number:

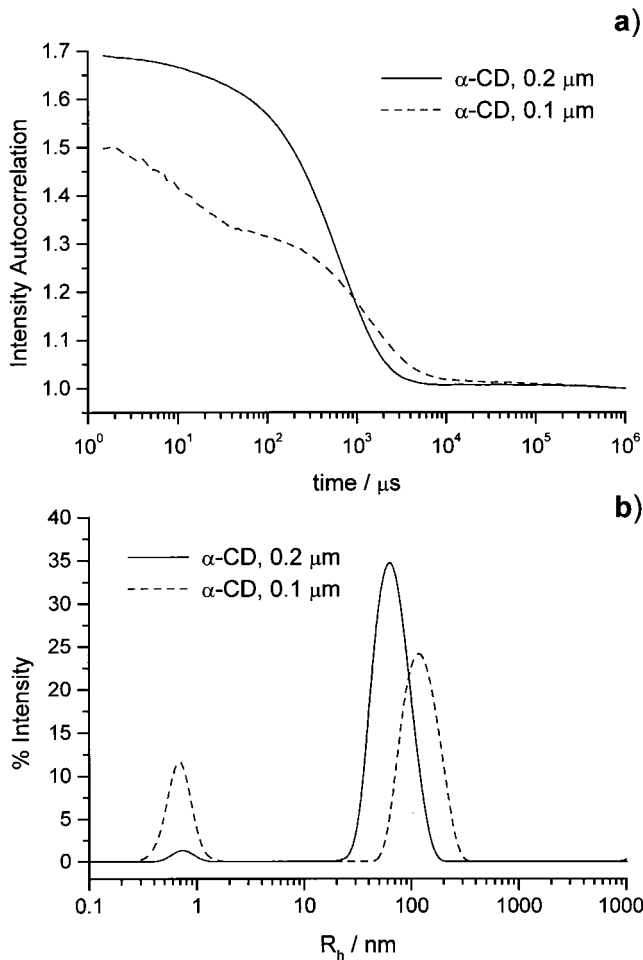


Figure 2. (a) DLS correlograms for  $\alpha$ -CD 0.012 M at 25 °C filtered through 0.1  $\mu\text{m}$  and 0.2  $\mu\text{m}$ ; (b) size distributions for the curves of (a).

$$M_i = R^a N_a (4\pi/3v) \quad (3)$$

and Equation (2) becomes

$$I_i = K' R_i^a c_i, \quad (4)$$

the mass distribution, expressed in terms of the concentration, can be deduced from the intensity after substituting in Equation (4):

$$\frac{c_i}{\sum c_i} = \frac{I_i/R_i^a}{\sum (I_i/R_i^a)}. \quad (5)$$

The mass distribution thus defined will be strongly dependent on the radius of the particle.<sup>1</sup> In this way, the mass contribution of the  $\alpha$ -CD aggregates would be 0.8% assuming coils or 0.001% for spheres, that is to say, practically negligible.  $\gamma$ -CD filtered through 0.2  $\mu\text{m}$  does not permit this calculation, since the scattered intensity due to the aggregates is so large that a single, slow mode, can be detected. Yet, by comparing the scattering due to the monomeric  $\gamma$ -CD after filtration through 0.1  $\mu\text{m}$ , to that of the monomeric

<sup>1</sup> A more rigorous calculation should include a shape factor,  $P(\theta)$ , which takes into account the angle dependence of the scattered light due to the shape of the particle. The assumption that  $P(\theta) = 1$  is only valid for particles smaller than the wavelength of the incident radiation.

$\alpha$ -CD, both intensities fall within the same order of magnitude, what leads us to the conclusion that the  $\gamma$ -CD must be essentially in its monomer form after filtering, as  $\alpha$ -CD is.

Another point to consider when studying aggregation is its evolution with time. In the mentioned paper of Szente *et al.* [6] the kinetics of opalescence of  $\gamma$ -CD solutions, and the effects of additives and filtration, have been investigated. The authors observe that, after filtration through 0.22  $\mu\text{m}$ , haziness still occurs. We have performed an analogous experiment with a 0.012 M solution of this same CD using DLS, after filtration through 0.1 and 0.2  $\mu\text{m}$ . The intensity changes of both solutions with time have been plotted in Figure 3. For the wider pore, the distribution is single modal, and the intensity increases with time, up to reaching a plateau, with particles of *ca.* 140 nm in radius. On the contrary, the solution filtered with 0.1  $\mu\text{m}$  do not display so significant increase of intensity, and basically the monomeric  $\gamma$ -CD is observed, together with a small proportion of aggregates. 0.2  $\mu\text{m}$  pores let the aggregates, or a considerable part of them, pass through the filter. These assemblies could act as nuclei of crystallisation, being the responsible of the turbidity reported in [6]. Thus, by using 0.1  $\mu\text{m}$  filters, a stable solution can be prepared with no presence of precipitates.

The case of  $\beta$ -CD is somewhat different. When filtering with 0.2  $\mu\text{m}$ , only the mode due to the aggregates can be detected, with a mean radius of 174 nm. However, when filtered through 0.1  $\mu\text{m}$ , a broad slow mode arises, together with the monomeric  $\beta$ -CD at 0.77 nm. The contribution of the slow mode increases quickly with time, until the vanishing of the signal due to the free  $\beta$ -CD. This is accompanied by a remarkable increase of the overall scattered intensity. Filtering with 0.02  $\mu\text{m}$  has the same effect. The intensity for a 0.02  $\mu\text{m}$  filtered solution has been traced (Figure 4), together with the calculated radius of the aggregates. The increase in the scattering is mainly due to the growth of the particles, reaching a constant mean value of 140 nm, approximately two hours after filtering.

The substitution of the OH groups has direct consequences on the aggregation. Thus, with RAMEB, even when filtering through 0.2  $\mu\text{m}$ , the dominant mode observed is that due to the monomer, with a negligible contribution of aggregates. The same occurs with the HP- $\beta$ -CD (Table 1). These evidences are a confirmation that the assembling is due to the OH groups of the rims. Häusler *et al.* [2] report a strong increase in the viscosity of HP- $\beta$ -CD solutions, at concentrations beyond 50% which are ascribed to self-aggregation. However, at the concentrations studied here (*ca.* 1.5%), we did not observe such aggregation.

#### Effect of additives and temperature

It is well known that chaotropic agents such as urea, electrolytes or an alkaline pH, enhance the solubility of natural CDs [10], a fact that must be directly connected with the self-aggregation. We have chosen three additives to study their effectiveness in the dispersion of the assemblies: NaCl, urea and NaOH 1M. The resultant CD solutions (0.012 M in CD) have been filtered through 0.2  $\mu\text{m}$ . With all the additives,

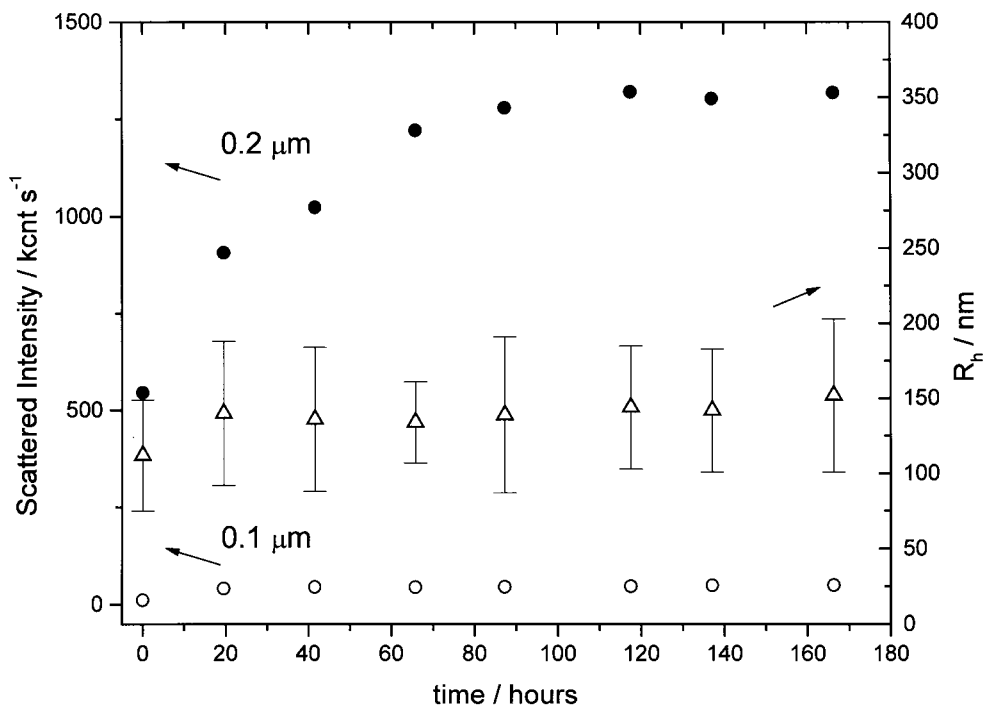


Figure 3. Scattered intensity of a solution of  $\gamma$ -CD 0.012 M, filtered through  $0.2 \mu\text{m}$  ( $\bullet$ ), and  $0.1 \mu\text{m}$  ( $\circ$ ), at  $25^\circ\text{C}$ . The mean radius and polydispersity of the aggregates are represented by ( $\Delta$ ) and error bars, respectively.

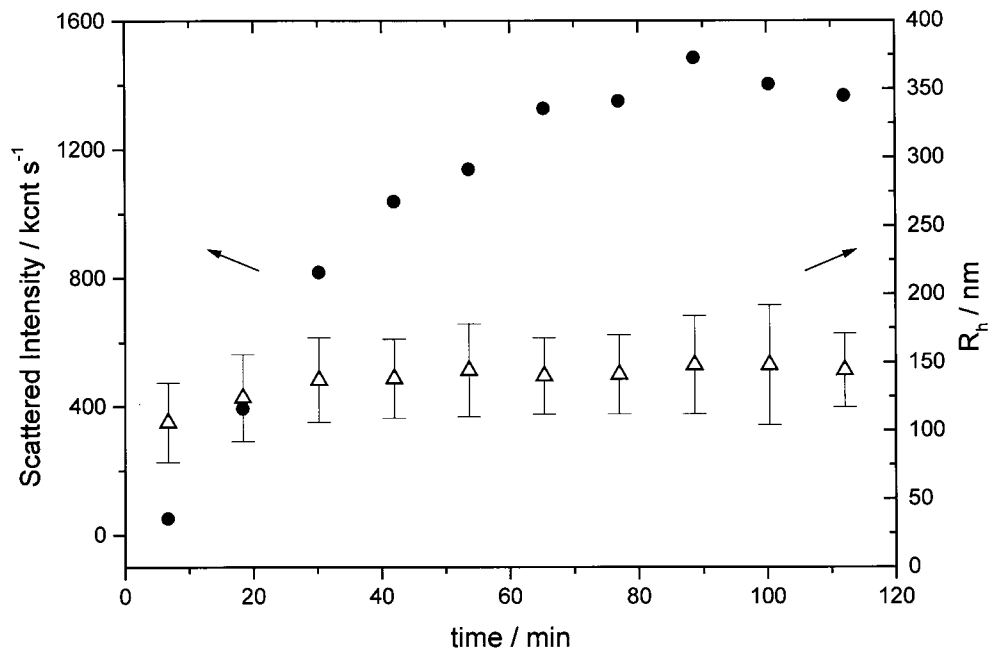


Figure 4. Scattered intensity of a solution of  $\beta$ -CD 0.012 M, filtered through  $0.02 \mu\text{m}$  ( $\bullet$ ), at  $25^\circ\text{C}$ . The mean radius and polydispersity of the aggregates are represented by ( $\Delta$ ) and error bars, respectively.

a remarkable fall in the intensity is observed, together with the appearance of the peak due to the monomers. The slow mode, corresponding to the aggregates, renders a mean size of *ca.* 80 nm, although it is negligible in its mass contribution. The case is more remarkable for  $\beta$ -CD, which does not show the aggregation kinetics observed in pure water. For instance, the addition of urea 4, 6 and 8 M, to a  $\beta$ -CD solution reduces the intensity from 4.1 Mcnt/s in water to 0.17, 0.10 and 0.07 Mcnt/s, respectively. With  $\beta$ -CD/NaCl 4 M solution, the intensity falls down to 11 kcnt/s, the slow mode

vanishes, and only the CD peak (0.73 nm) can be observed. All these solutes produce the same effect: the weakening of the intermolecular forces that held together the CDs in the aggregates. Urea is a hydrogen-bond-disrupting agent which competes with other CDs in the formation of hydrogen bonds, and the same thing occurs with electrolytes such as NaCl. NaOH 1 M has the same effect in all the CDs, the only difference being the slight increase in the hydrodynamic radii of the monomeric CD (0.73, 0.87, and 0.88 nm for  $\alpha$ -,  $\beta$ -, and  $\gamma$ -CD, respectively), consequent with the thicker

solvating sphere caused by the OH ionisation. Another factor that can disrupt the aggregates is thermal agitation. Raising the temperature to 55.0 °C significantly reduces both the intensity and the contribution due to the aggregates in all the CDs studied. Nevertheless, the efficiency seems poorer than that achieved with the additives.

## Conclusion

Natural CDs form large and polydisperse aggregates in solution, of *ca.* 200–300 nm in size, although their contribution in mass is small compared to that of free CD. The self-aggregation capability depends on the CD considered. For  $\alpha$ -CD and  $\gamma$ -CD, the aggregates can be easily removed by filtering through 0.1  $\mu\text{m}$  filters. The resultant solutions are stable with time and do not show further aggregation.  $\beta$ -CD, however, is very persistent in the formation of aggregates, even when filtering through 0.1  $\mu\text{m}$  or 0.02  $\mu\text{m}$ , and displays a fast aggregation kinetics. Substituted CDs, such as RAMEB or HP- $\beta$ -CD do not display significant aggregation, neither do natural CDs at high pH, what confirms that the aggregation occurs with the intervention of the hydrophilic rims. In the same way, the presence of salts, chaotropic agents as urea, or high temperatures notably reduce the presence of aggregates.

## Acknowledgements

Authors acknowledge financial support from the Ministerio de Ciencia y Tecnología (fund num. BQU2001-1426-C02-02), the Ministerio de Educación y Cultura (fund num. BJU2000-0264), the Gobierno de Navarra, and the Asociación de Amigos de la Universidad de Navarra for the doctoral grant for P.R.

## References

1. A.W. Coleman, I. Nicolis, N. Keller, and J.P. Dalbiez: *J. Incl. Phenom. Mol. Recognit. Chem.* **13**, 139 (1992).
2. O. Häusler and C.C. Müller-Goymann: *Starch* **45**, 183 (1993).
3. N. Azaroual-Bellanger and B. Perly: *Magn. Reson. Chem.* **32**, 8 (1994).
4. J.M. Valleton, S. Alexandre, A.W. Coleman, and A. Kasselouri: *Thin Solid Films* **284–285**, 765 (1996).
5. G.G. Gaitano, W. Brown, and G. Tardajos: *J. Phys. Chem.* **B 101**, 710 (1997).
6. L. Szente, J. Szejtli, and G.L. Kis: *J. Pharm. Sci.* **87**(6), 778 (1998).
7. S. Polarz, B. Smarsly, L. Bronstein, and M. Antonietti: *Angew. Chem. Int. Ed.* **40**(23), 4417 (2001).
8. R. Pecora: *Dynamic Light Scattering: Applications of Photon Correlation Spectroscopy*, Plenum Press, New York (1985).
9. J. Szejtli: *Cyclodextrin Technology*, Kluwer Academic Publishers, Dordrecht (1988).
10. D.Y. Pharr, Z.S. Fu, T.K. Smith, and W.L. Hinze: *Anal. Chem.* **61**, 275 (1989).

