ELSEVIER

International Journal of Pharmaceutics 120 (1995) 1–11

international journal of pharmaceutics

Research Papers

Direct imaging of modified β-cyclodextrin nanospheres by photon scanning tunnelling and scanning force microscopy

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Received 22 July 1994; revised 13 September 1994; accepted 27 October 1994

Abstract

The morphological and topographical properties of nanospheres are of major importance for characterizing these systems. The quality, and consequently the applications, of such dispersions are determined by the mean particle size, their distribution by size and the topography of the nanospheres. This work is devoted to the application of contemporary analytical techniques: photon scanning tunnelling microscopy (PSTM), scanning force microscopy (SFM) and no-contact scanning force microscopy (NC-SFM) to image nanospheres prepared from chemically modified β-cyclodextrins (β$CD-C_6$) without any sample preparation which could introduce artefacts. We have shown that the spherical geometry and high degree of monodispersity of nanospheres from β$CD-C_6$ were confirmed by PSTM, but that NC-SFM was an excellent technique for the imaging of nanospheres prepared from β$CD-C_6$.

Keywords: Cyclodextrin; Nanosphere; Photon scanning tunnelling microscopy; Scanning force microscopy ; Surface topography

1. Introduction

The cyclodextrins are a family of water-soluble hydrophobic cavity-forming oligosaccharides ('hosts') which can accommodate in their cavities a wide range of water-insoluble and water-soluble drugs ('guests') to form water-soluble inclusion complexes in the absence of covalent bonding, to enhance the solubility, dissolution rate, stability, protection from oxidation, and, consequently, the bioavailability of a wide variety of organic molecules (Duchêne and Wouessidjewe, 1990).

Recently, we developed a new concept in drug delivery (Skiba et al., 1993a,b) which takes advantage of certain properties of cyclodextrins (Uekama and Irie, 1987; Szejtli, 1992) and nanoparticles (Couvreur, 1985; Speiser, 1991; Puisieux et al., 1994) in order to circumvent problems associated with the use of either system alone, and to improve their performance in drug delivery.

The morphological evaluation of nanoparticulate drug carriers is technically difficult because of the very small size (nanometer range) of the particles in the final colloidal suspension, and the necessity of staining or making a replica, which could introduce artefacts.

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SSDI 0378-5173(94)00349-1

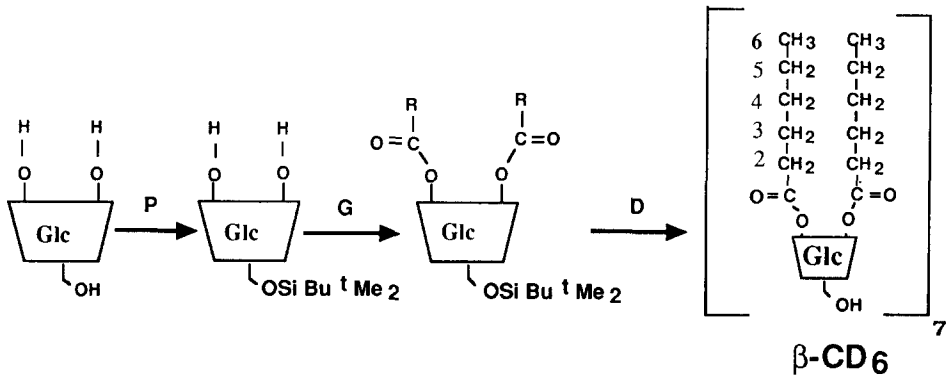


Fig. 1. Synthesis of βCD-C_6.

Photon scanning tunnelling microscopy (PSTM) and scanning force microscopy (SFM) have opened up new horizons for exploring the surfaces of biomaterials, since they extend the high resolution of scanning tunnelling microscopy (STM) to non-conducting materials. However, the nanospheres could be dislodged from the support when SFM is used in the standard contact mode, resulting in a deformed image of the morphology and surface topography of the nanospheres (Sommer et al., 1993).

In the present study, we report SFM imaging of nanospheres from chemically modified β-cyclodextrins (βCD-C_6), produced using (tapping

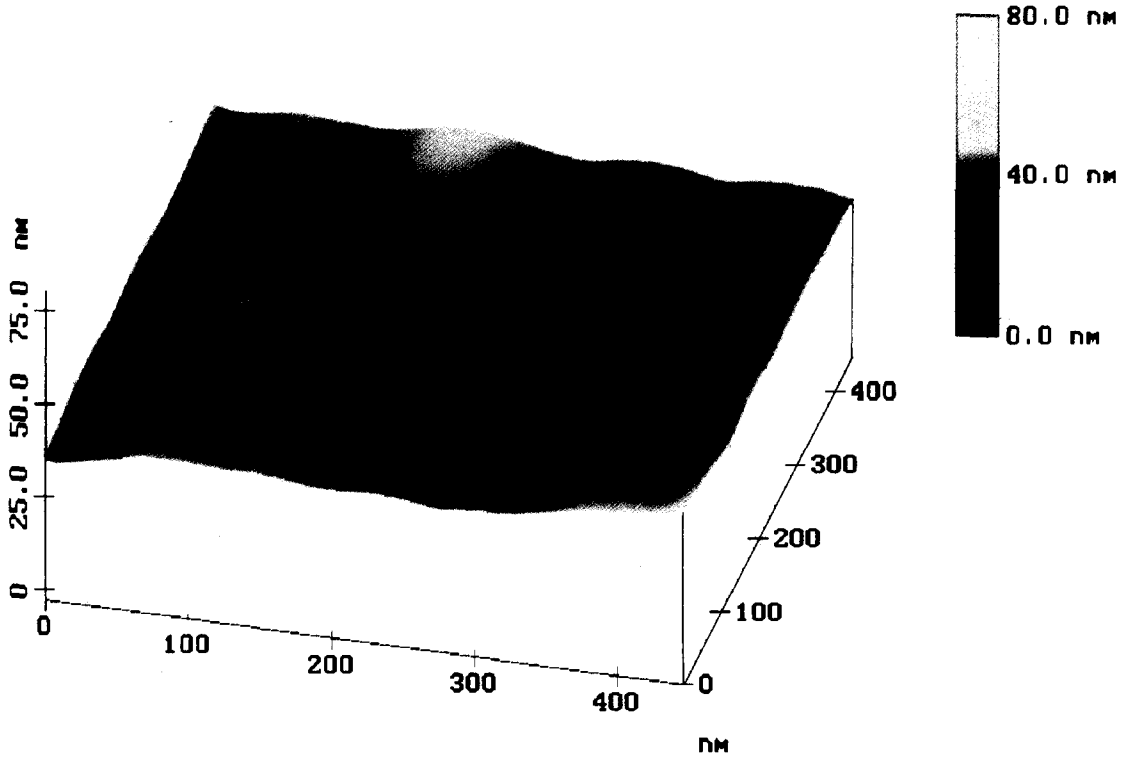


Fig. 2. Contact-mode SFM three-dimensional image of nanospheres made from βCD-C_6.

mode) no-contact scanning force microscopy (NC-SFM) and PSTM imaging and compare these images with those produced by contact mode SFM.

2. Experimental

2.1. Materials

Chemically modified β-cyclodextrin (βCD-C_6) was synthesized in the Laboratoire de Chimie Organique (CNRS ER45). Poloxamer 188 (Pluronic PE F68®) as surfactant was obtained from BASF (Wyandotte, USA). Acetone was chosen as the organic water-miscible solvent on the basis of previous work and pharmaceutical acceptability with regard to toxicity (Allémann et al., 1992).

2.2. Synthesis of βCD-C_6

The chemically modified β-cyclodextrin (βCD-C_6) was synthesized from natural β-cyclodextrins ($(\alpha 1,4\text{-Glc})_7$) according to the method of Zhang et al. (1991) which can be recapitulated simply by protection of primary hydroxyl groups (P), grafting of fatty acid chains of six carbons to secondary hydroxyl groups (G), and a deprotection (D) strategy as shown below (Fig. 1).

2.3. Preparation of nanospheres

The concept of the preparation method was based on the spherical crystallization technique (Kawashima et al., 1982) developed by Skiba et al. (1993a). The βCD-C_6 was dissolved in acetone at 25°C and the resultant solution was poured slowly, through a silicon tube fitted with a fine tip, into 50 ml of distilled water containing a Pluronic PE F68® and subjected to magnetic stirring. The nanospheres were formed immediately and the colloidal suspension obtained was then concentrated by evaporation under vacuum to about one-fifth of the initial volume.

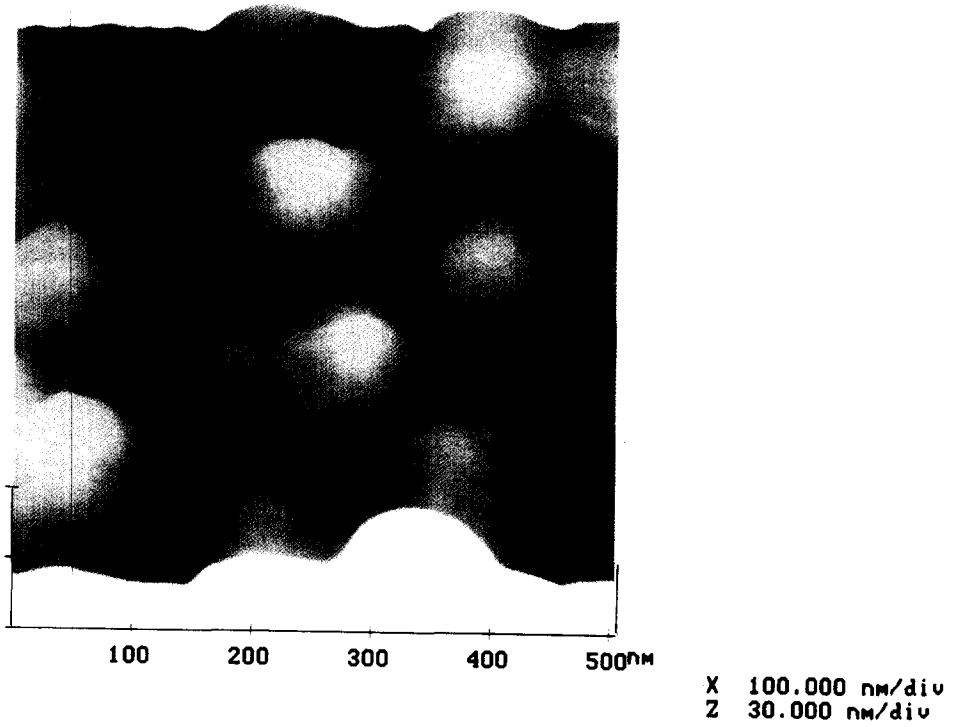


Fig. 3. No-contact-mode SFM three-dimensional image of nanospheres made from βCD-C_6.

2.4. Particle size determination

Particle size was estimated by quasi-elastic light scattering using monochromatic laser ray diffusion (Nanosizer N4MD Coultronics, S.A.) France). Experimental conditions were: temperature, 20°C; reference angle, 90°; viscosity, 0.899×10^{-3} Pa s; refractive index, 1.330.

2.5. Scanning force microscopy (SFM)

Scanning force microscopy (SFM) (Nanoscope III model from Digital Instruments USA), also known as atomic force microscopy (AFM), measures the surface topography by scanning a tip across a surface and measuring the attractive or repulsive force between the tip and the surface. Similar to STM, and for the same reasons, it is possible to operate the microscope at constant height or at constant measurement. The scanning tip is fixed on a cantilever with a known spring constant. The cantilever displacement provides an estimate of the overall interaction force between the tip and the surface (Golovchenk, 1986; Hansma et al., 1988; Marti et al., 1988; Smith et al., 1989).

The cantilever displacement was initially measured by an STM tip placed on the top of the SFM tip in a sort of double assembly. Nowadays this measurement is generally performed by mea-

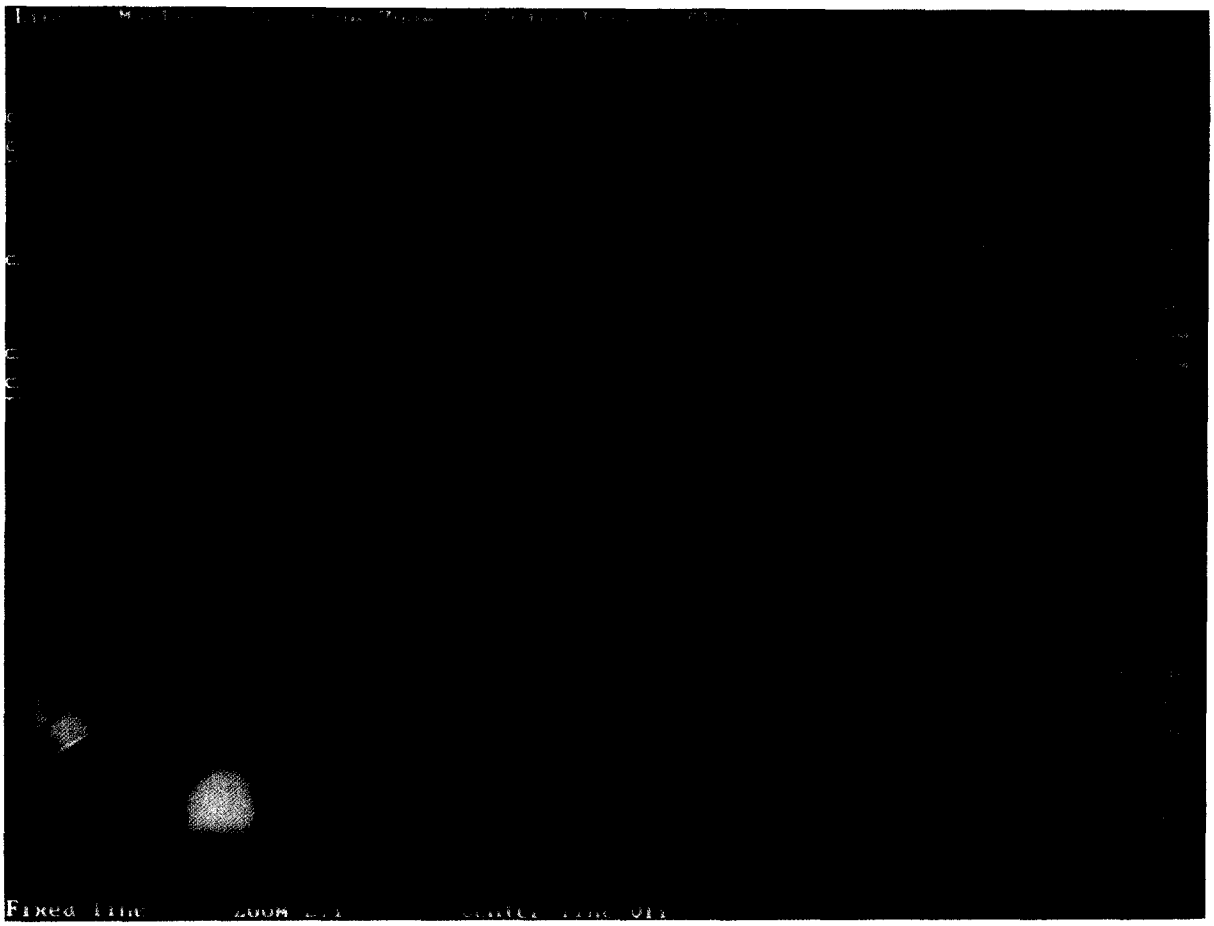


Fig. 4. Transect plot of the image shown in Fig. 3. Regular curvature of the nanospheres from βCD-C_6 with a diameter of approx. 137 nm is shown. Independent size measurements by laser light scattering of the nanosphere population from which this image was taken yielded an average size of 165 ± 33 nm (Fig. 2).

suring the change in the reflection of a laser beam reflected by the top of the cantilever. A four-quadrant photodiode detector allows the cantilever position to be recorded, as well as its torsion, which indicates the frictional force during the scan.

SFM has been found to be easier to manipulate than STM but gives a lower resolution, typically 1 Å compared with 0.1 Å for STM. The main interest stems from the ability of SFM to scan non-conducting samples.

No-contact scanning force microscopy (NC-SFM) is a complex imaging technique used only when an image surface is too soft to image with contact SFM (Sommer et al., 1993).

2.6. Photon scanning tunnelling microscopy (PSTM)

Photon Scanning Tunnel Microscopy (PSTM) (Société Spiral, Dijon, France) is the optical equivalent of scanning tunnelling microscopy. A fine optical fibre is placed in the evanescent field produced by the sample which is placed on a prism illuminated from below at the critical incident angle. This technique allows the samples to be observed with a lateral resolution at least 20-times better than that of conventional optical microscopes. As the optical fibre scans the surface of the object, topological features are translated into variations in light intensity. When these are recorded, they allow surface features to be detected which are smaller than the wavelength of light. Although its resolution is less than that of STM or SFM, this technique gives a true topographical map of the sample.

The images obtained by PSTM contain a mixture of information about the topography and local variations in the refractive index of the sample.

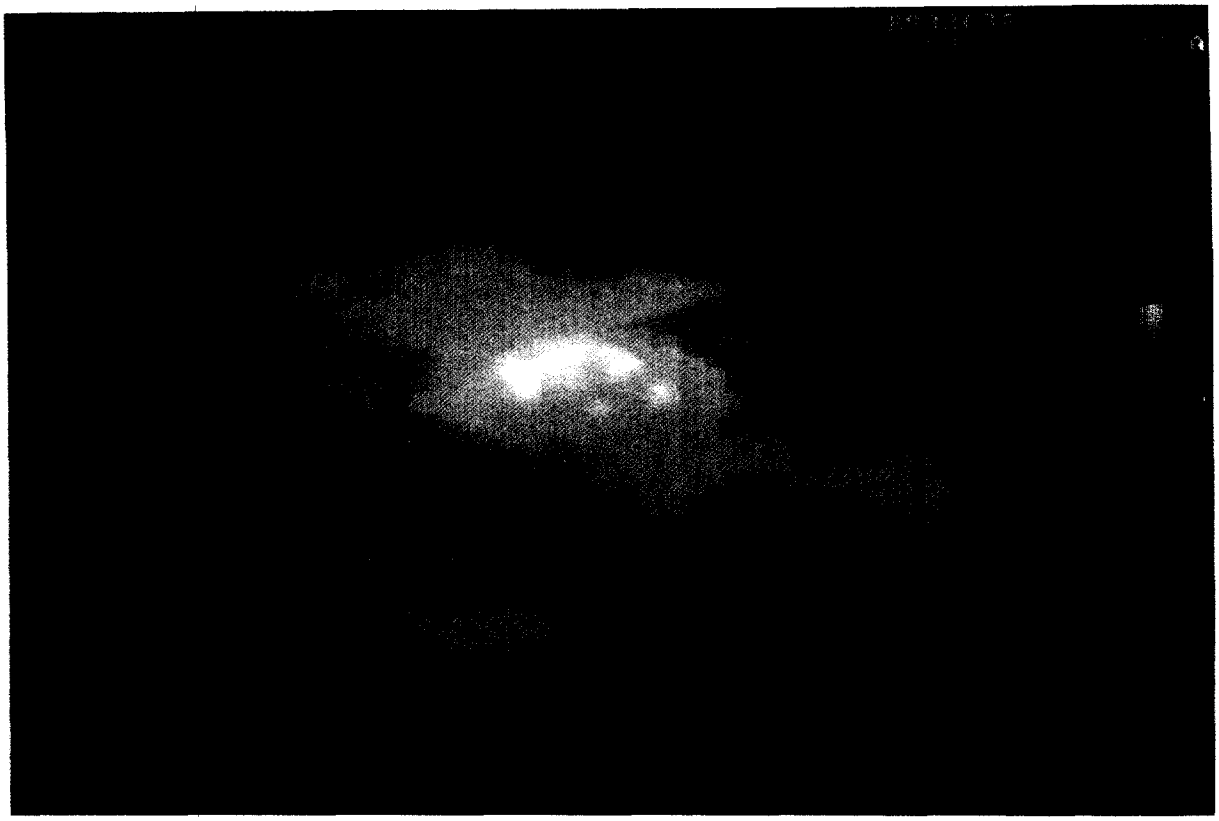


Fig. 5. PSTM three-dimensional image of a group of nanospheres made from βCD-C_6 with a diameter of 147.7 nm by vertical measurement. Topography image, 10 μm xy scan.

In the spectroscopic mode, regular networks appear flat. A comparison of NC-SFM and PSTM images for the same sample allows a better interpretation.

2.7. Preparation of samples: PSTM / SFM

The samples were prepared by placing 50 μl of the nanospheres from βCD-C_6 (Sommer et al., 1993) onto a freshly cleaved mica surface and allowing the water to evaporate (approx. 1 h air-drying at ambient temperature). The sample was mounted on (i) a Digital Nanoscope III and scanned in the two modes (SFM, NC-SFM) and (ii) Prototype PSTM (Spiral R&D).

All specimens were scanned at ambient temperature and pressure.

3. Results and discussion

3.1. Particle size determination

The estimated average diameter of nanospheres was 165 ± 33 nm, the profile corresponded to a normal distribution and the sample could be considered monodisperse as shown by the cumulative results and the polydispersity index calculated by software from the autocorrelation functions.

3.2. SFM / NC-SFM

The contact mode of SFM provides excellent lateral resolution when the short sensing tip used comes into contact with the surface of a sample. Fig. 2 shows the image obtained at *xz* scan di-

Fig. 6. PSTM three-dimensional image of a group of nanospheres made from βCD-C_6 with a diameter of 147.7 nm by vertical measurement. Topography image, 5 μm *xy* scan.

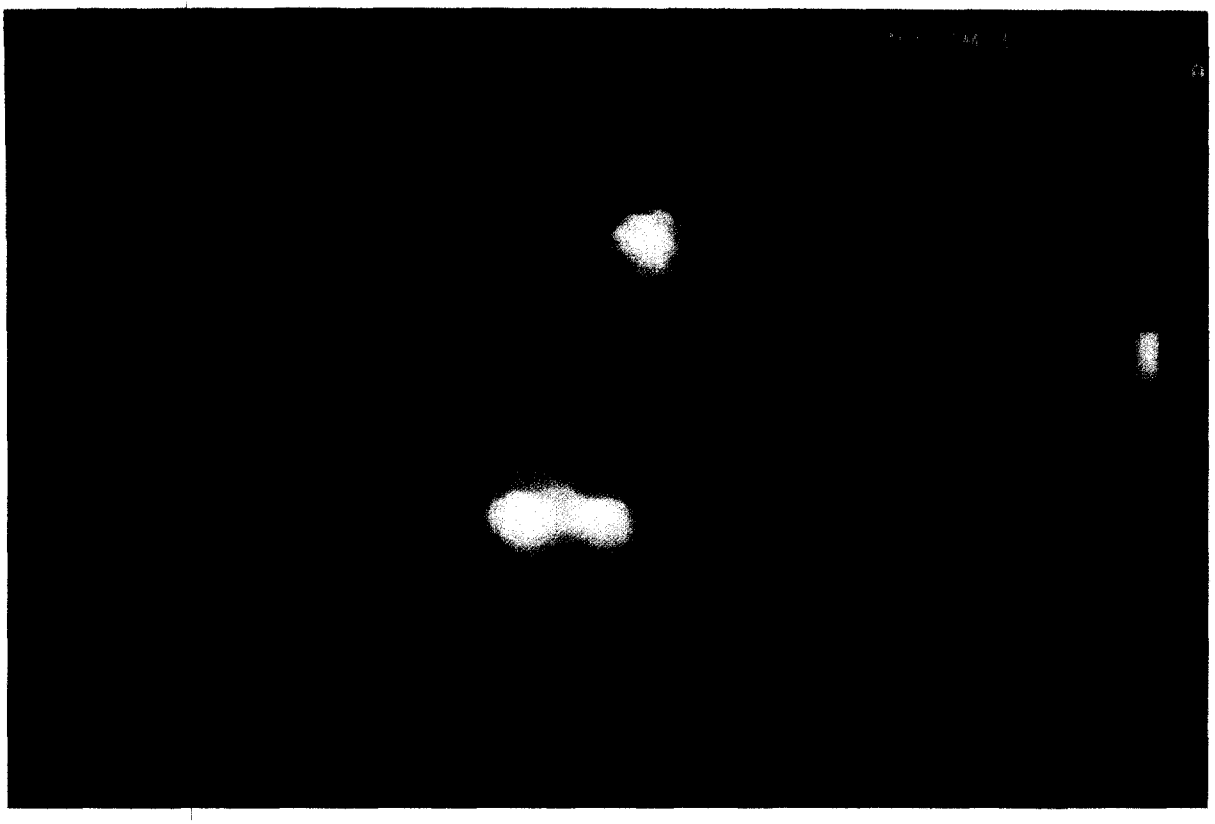


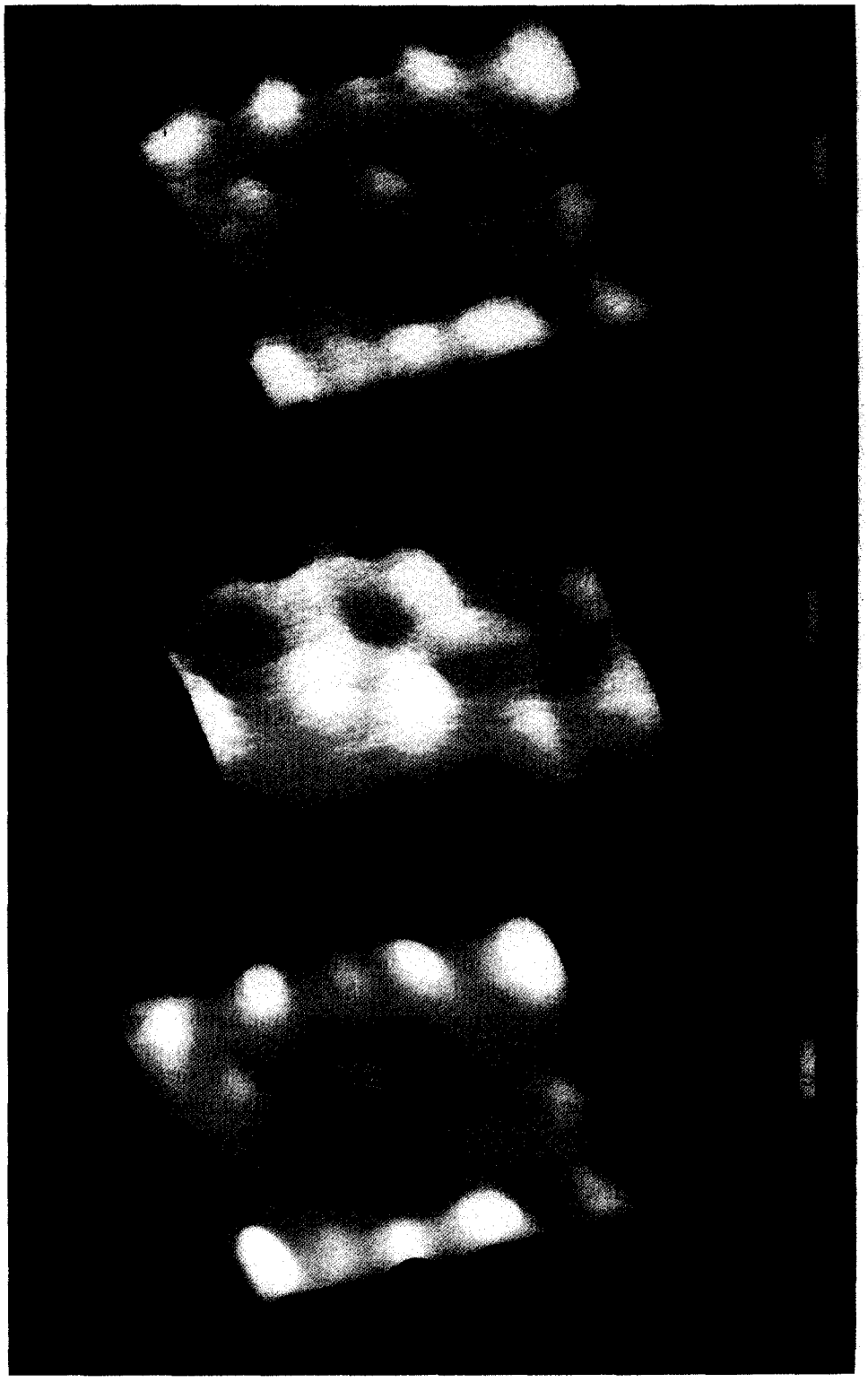
Fig. 7. PSTM three-dimensional image of a group of nanospheres made from βCD-C_6 with a diameter of 147.7 nm by vertical measurement. Topography image, 2 μm *xy* scan.

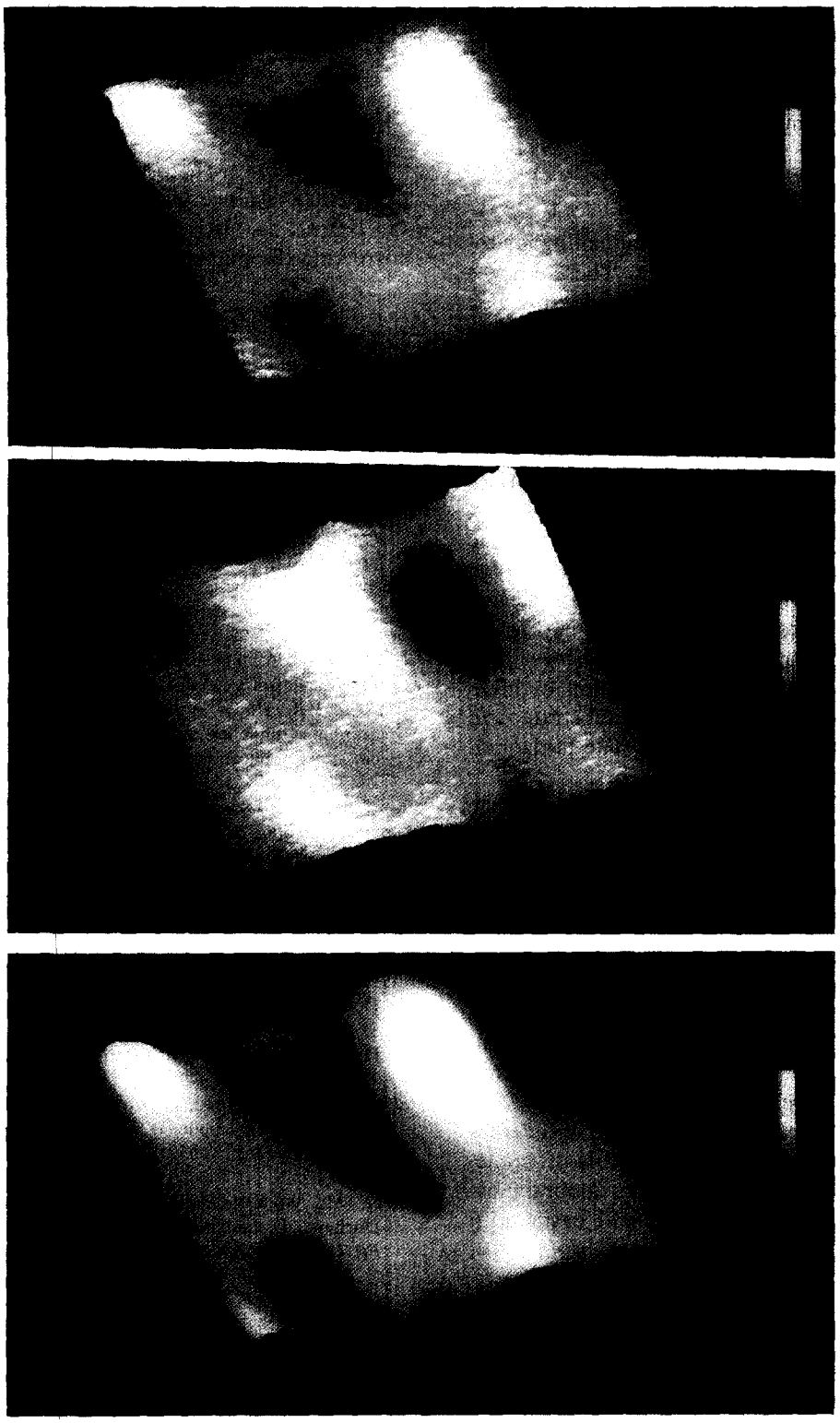
mensions of 500 nm. The analysis is carried out in constant deflection mode, in which the deflection of the cantilever is held constant and the displacement of the tip is measured. The cantilever used was made of Si_3N_4 and had a spring constant of 0.12 N m^{-1}, and a tip diameter of approx. 40 nm. Even with such a low force applied to the surface, deformations of the nanospheres were clearly evident. These were elongated and flattened by the tip during the scan (Fig. 2).

NC-SFM provides topographical images of a sample by monitoring the attractive van der Waals' forces between the tip and the sample. The cantilever and tip hover a few nanometers above the surface, never touching it and never contaminating it. Whereas dragging can deform soft or elastic surfaces, NC-SFM interaction forces are in the piconewton range, making it ideal for imaging soft or elastic surfaces. The resolution of NC-SFM is as good as or superior to that of contact SFM (Fig. 3). The advantages in the NC-SFM mode are very low compressive forces and the almost complete absence of lateral forces responsible for the fluidity of images of the very soft or elastic surfaces of nanospheres in the standard contact mode.

In Fig. 4 the nanospheres are clearly imaged as distinct individual objects, allowing size analysis to be undertaken as shown in the cross-section. The two nanospheres measured had diameters of

Fig. 8. PSTM three-dimensional image of one nanosphere made from βCD-C_6 with a diameter of 143 nm by horizontal measurement. (a) Topography image, 1.4 μm *xy* scan. (b) Topography, inverse image, 1.4 μm *xy* scan. (c) Topography, image treated by Fourier transformation, 1.4 μm *xy* scan.





127 and 147 nm giving a mean value of 137 nm. Independent size measurements by laser light scattering of the nanosphere population from which this image was taken yielded an average size of 165 ± 33 nm.

3.3. PSTM

Fig. 5–7 are photon scanning tunnelling micrographs of βCD-C_6 nanospheres. These images were obtained in the topographic mode, in which the light intensity is held constant and the probe follows the contours of the surface. In the centre of Fig. 5, a cluster of eight nanospheres can be seen. Fig. 6 and 7 show an enlargement of this region. The mean diameter of the nanospheres from vertical measurements was 147.7 nm and a narrow size distribution was also noted. In Fig. 8, about 10 nanospheres can be seen. The reverse image (Fig. 8b) allows the nanospheres, which appear brilliant in Fig. 8a, to be distinguished from the dark substrate. The image in Fig. 8c after Fourier transformation shows the nanospheres perfectly after elimination of high-frequency noise arising from the substrate and the residue of the aqueous phase. The horizontal diameter was 143 nm.

Fig. 9 is a physical enlargement of the particle visible in the centre of Fig. 6a. It is surrounded by a halo 20 nm in thickness, which is probably the surfactant. The horizontal diameter is 130 nm.

4. Conclusion

This investigation reports the first successful imaging of βCD-C_6 nanospheres by PSTM and NC-SFM. The results indicate that NC-SFM is capable of imaging direct observations and makes in situ measurements possible. Details of ripple, topography, and spherical geometry unobtainable by PSTM or SFM can be observed easily with NC-SFM. The high degree of monodispersity of βCD-C_6 nanospheres has been confirmed by PSTM and NC-SFM.

Acknowledgements

The authors wish to thank J.L. Bijeon for his excellent assistance with the PSTM experiments. G. Barratt is also thanked for her help with correcting the manuscript.

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Fig. 9. PSTM three-dimensional image of nanospheres from βCD-C_6 with a diameter of 130 nm by horizontal measurement. (a) Topography image, 0.67 μm *xy* scan. (b) Topography inverse image, 0.67 μm *xy* scan. (c) Topography image treated by Fourier transformation, 0.67 μm *xy* scan.

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