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# Interactions of amphiphilic calix[4]arene-based Solid Lipid Nanoparticles with bovine serum albumin

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**Abstract**

The interaction of Solid Lipid Nanoparticles (SLN) based on amphiphilic calix[4]arene with one of the major circulatory protein, serum albumin, has been investigated by Photon Correlation Spectroscopy (PCS) and Atomic Force Microscopy (AFM). The carrier systems have shown the ability to interact with bovine serum albumin (BSA), which forms a capping layer up to 17 nm in depth. AFM imaging revealed that the SLNs are protected by this layer against flattening on surfaces.


*Keywords:* Solid Lipid Nanoparticles; Calixarene; Bovine serum albumin (BSA); Photon Correlation Spectroscopy (PCS); Atomic Force Microscopy (AFM)

## 1. Introduction

Solid Lipid Nanoparticles (SLN) have generated increasing interest (Demirel and Yazan, 2000; Mehnert and Mader, 2001; Müller et al., 2000) as alternative carrier systems, for bioactive molecules, to such classical colloidal transporters such as liposomes (Lian and Ho, 2001), polymeric (Mitra and Maitra, 2000) and protein-derived nanoparticles (Weber et al., 2000).

They possess a combination of the advantages of high monodispersity, long temporal stability, relatively good stability with regard to temperature, possible dehydration and reconstitution, high loading factors for incorporated molecules and low toxicity, either intrinsic or from a lack of toxic biodegradation side products (Mehnert and Mader, 2001).

We have for some time been working on the preparation of SLNs based on amphiphilic supramolecular macrocyclic skeletons, focusing initially on cyclodextrin-based amphiphiles (Coleman and Kasselouri, 1993; Skiba et al., 1993; Sommer et al., 1993), but more recently on calix-arene-based amphiphiles (Houel et al., 2002; Shahgaldian et al., 2002). The calix[*n*]arenes (Gutsche, 1998), synthetic oligo-macrocycles of methylene-bridged phenolic rings, possess a number of advantages over the semi-natural cyclodextrins, these being ease of chemical modification using low toxicity chemical reactions, and without the need of vacuum drying (Shahgaldian et al., 2001), low production cost and without the known toxicity of the cyclodextrins (Bost et al., 1997).

The above characteristics probably make the calix-arene-based SLNs better targets for application as medicinal transport systems compared to the cyclodextrin-based SLNs.

For their application and especially for their use as intravenous transport systems, it is important to understand the interactions of the SLNs with ions (Houel et al., 2002), small molecules (Da Silva et al.,

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submitted), metabolites and circulatory proteins found at high concentrations in physiological fluids.

The main circulatory proteins are the serum albumins, present at high concentrations up to 46 g/l and the globulins concentrations up to 27 g/l (Diem and Lenter, 1972). The serum albumins are of particular interest for their ability to anchor both hydrophilic and hydrophobic surfaces and participate in a cascade of protein adhesion at surfaces presented to sera (Peters, 1996).

In this article, we wish to present a study by Photon Correlation Spectroscopy (PCS) and Atomic Force Microscopy (AFM) of the interaction of SLNs derived from three amphiphilic calix[4]arenes: *p*-dodecanoylcalix[4]arene (**1**), *p*-dodecanoyl-25-(ethoxy carbonyl methyloxy)-calix[4]arene (**2**) and *p*-dodecanoyl-25-(2-carboxy-methyloxy)-calix[4]arene (**3**).

It will be shown that bovine serum albumin (BSA) adheres, by Langmuir type surface adsorption, to the surface of the SLNs to generate a layer up to 17 nm in depth around the SLNs. However, even at BSA concentrations up to 40 g/l no protein or SLN aggregation occurs. AFM non-contact mode imaging shows that the SLNs are present as discrete non-aggregated systems in dried gels of BSA and that the SLNs cause no restructuring of such gels.

## 2. Experimental

### 2.1. Sample preparation

**1**, **2** and **3** have been synthesised as previously described (Da Silva et al., submitted; Shahgaldian et al., 2001). All chemicals were purchased from Acros Organics (France), BSA from Sigma (France) in an essentially fatty acid free form (≥96%).

SLNs have been prepared as previously described (Shahgaldian et al., 2003).

To 1 ml of SLN suspensions, was added an equal volume aqueous solutions of BSA in order to obtain final protein concentrations of 2, 4, 10, 20, 30 and 40 g/l. The blanks were prepared by adding 1 ml of pure water to the SLN suspensions.

### 2.2. PCS

PCS experiments was carried out using a Malvern 4700 spectrometer and 7132 256-channel correlator and a 40 MW He–Ne laser (633 nm). All values were measured at an angle of 90° in 10 mm diameter cells. The system was thermostated at 25 °C. Measurements were carried out on diluted samples (1/10), to make the viscosity negligible. All measurements were repeated five times and the variance of the measurements was less than 5%.

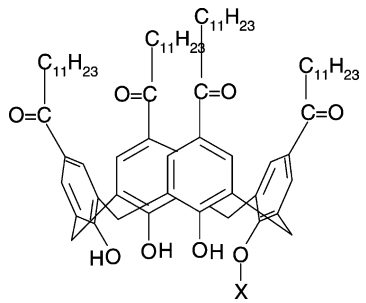
### 2.3. AFM imaging

Imaging was carried out using a Thermomicroscope Explorer AFM equipped with a 100-μm tripod scanner, in non-contact mode, using high resonant frequency ($F_0$ = 320 kHz) pyramidal cantilevers with silicon probes at a scan frequency of 1 Hz. Images are processed with the SPM Lab 5.01 software package and are presented unfiltered.

Samples were prepared by deposition of 10 μl of the samples on freshly cleaved mica plates and were dried at 20 °C overnight.

## 3. Result and discussion

Scheme 1 presents the general formulae for **1**, **2** and **3**. SLNs of these molecules have been prepared by the solvent displacement method and mixed with varying concentrations of BSA, the variations of the hydrodynamic diameter of the SLNs, measured by PCS are presented in Fig. 1, for clarity and to permit comparison, the values are given as ratios of the value of the average hydrodynamic diameter in pure water. In the absence of BSA, SLNs average hydrodynamic diameters are 150, 183 and 193 for **1**, **2** and **3**, respectively.




Scheme 1. Formulae of **1** (X = H), **2** (X = –$CH_2$COOEt) and **3** (X = –$CH_2$COOH).

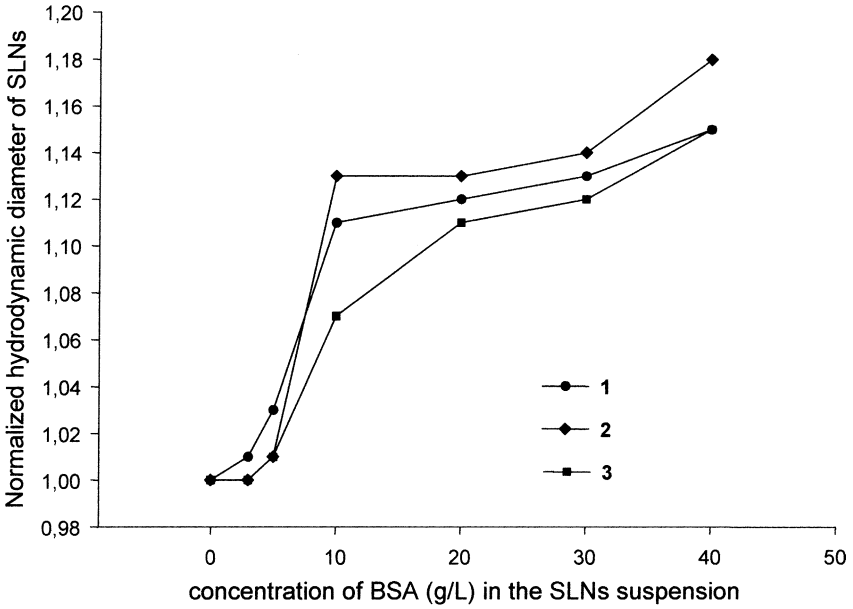



Fig. 1. Average hydrodynamic diameters of **1**, **2** and **3** as a function of the concentration of BSA in the suspension, for clarity and to permit comparison of the results, the values are given as ratios of the value of the average hydrodynamic diameter in pure water for each compound.

In all the cases, the diameters increase with increasing the concentration of BSA. For **1**-based SLNs, the size increases from 152, 153, 162, 163, 164 to 166 nm for concentrations of 3, 5, 10, 20, 30 and 40 mg/ml, respectively. These slight but significant variations in the size of the SLNs can be assumed to be due to the formation of a BSA capping layer at the surface of the SLNs as observed by Moselhy et al. (2000) for poly(NIPAm/MAA) nanoparticles. Assuming the circular shape of the SLNs, the layer formed at a concentration of BSA of 40 g/l can be estimated at 8 nm in depth. This is equivalent to a double layer of BSA molecules whose size is 4 nm × 4 nm × 14 nm (Peters, 1996). The average hydrodynamic diameters for **2**-based SLNs are constant for concentrations up to 3 g/l but an increase in size is observed for concentrations of 5, 10, 20, 30 and 40 g/l in BSA, respectively, 186, 207, 206, 210 and 216 nm. This increase is more important than those observed for **1**-based SLNs, showing an increase in the affinity of the BSA for the surface of the SLNs. Here, the protein layer can be estimated at 17 nm in depth. Two adsorption mechanisms are possible: (i) the interaction places the BSA in the same geometry as for **1**-based SLNs, there is then a 4-layer adsorption or (ii) a single layer along the major axis of BSA (14 nm). In the case of **3**-based SLNs, the average diameters are 194, 195, 205, 213, 216, 222 nm for concentrations of 3, 5, 10, 20, 30, and 40 g/l of BSA. This increase of size is again more important than those observed for **1**-based SLNs and can be compared to those observed for **2**-based SLNs. The protein layer can be estimated at 15 nm, this is best explained by an adsorption along the major axis. The difference in the adsorption in the adsorption mechanism will arise from variations in the hydrophilicity at the exterior of the SLNs and also from possible specific binding. This would especially be expected with SLNs which should interact with the known fatty acid binding sites of BSA (Arg117, Lys351 and Lys475) (Peters, 1996).

In all cases, the increase in size is not important enough to be explained by a flocculation or aggregation process but by the formation of a BSA capping layer. Moreover, it can be postulated that the affinity of BSA for the surface of **2**- and **3**-based SLNs is more important than for **1**-based SLNs.

Fig. 2 presents AFM images of **1**-based SLNs with and without BSA at a concentration of 20 g/l.

The image of the **1**-based SLNs shows moderately flattened objects of 250 nm in diameter and 55 nm in

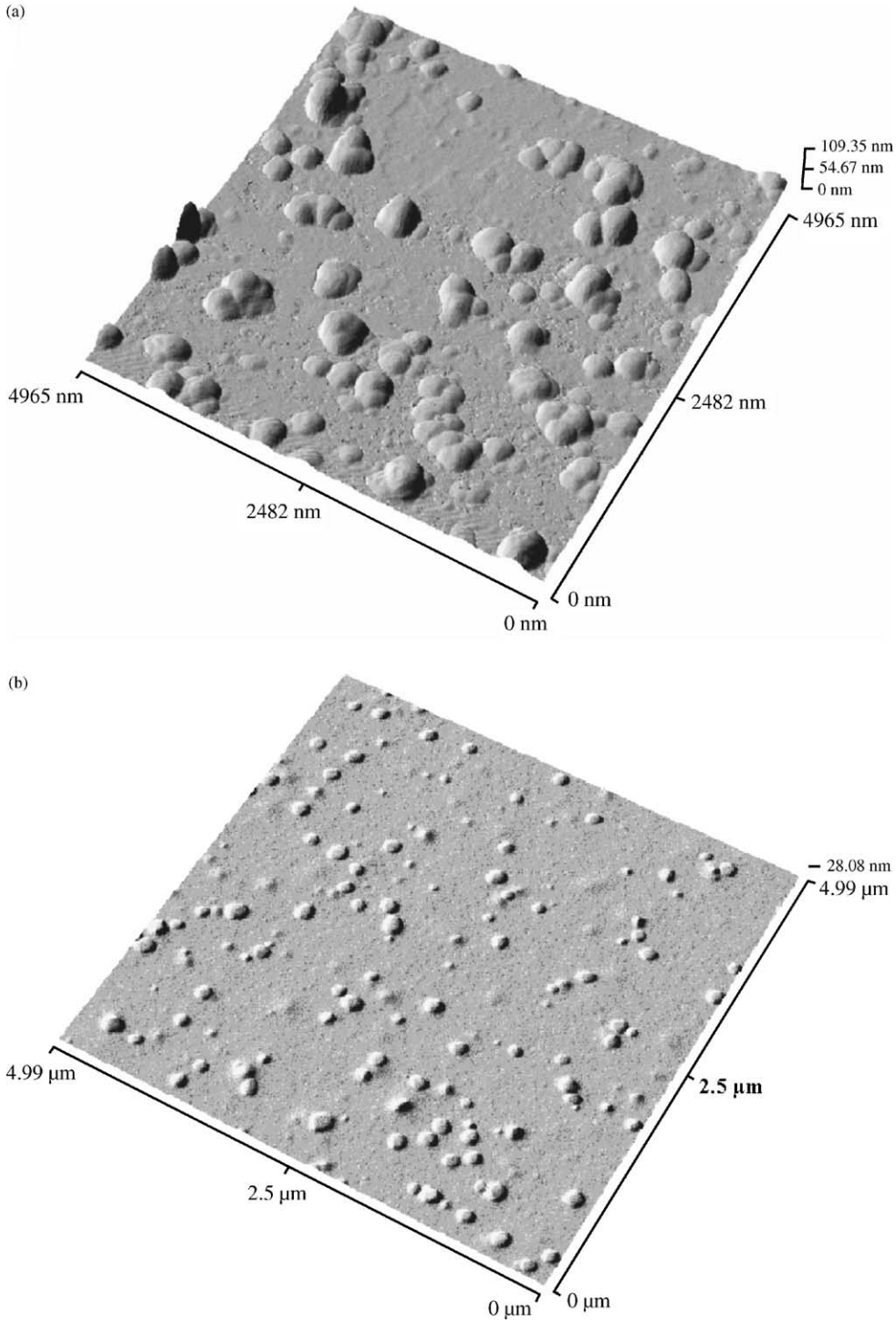



Fig. 2. Non-contact mode AFM images of **1**-based SLNs without (a) and with BSA (20 g/l) on a mica surface (b).

height; these values can be compared to those obtained by PCS. We have recently reported a study of the behaviour of BSA with various ions on surfaces, in this study it was shown that BSA on a surface and in the absence of additional salts forms flat gels, with a very low rugosity (Lazar et al., 2001). The image of **1**-based SLNs with BSA shows particles with 15 nm in height and 155 nm in diameter, homogeneously distributed on surface. The divergence between the volume calculated from the PCS and the AFM experiment can be explained by the fact that the SLNs are embedded in the gel and thus the topographic imaging used here measures only that part protruding from the film. Their more circular shape shows the protecting action of the BSA capping layer against flattening. Similar results have been obtained previously with **1**-based SLNs in gel matrices, where the interaction of polysaccharides with the SLNs has been shown to protect the particles against flattening where the gels were dried on a surface (Shahgaldian et al., 2003).

## 4. Conclusion

The study of the effect of BSA on three different *p*-dodecanoycalix[4]arene has been investigated by PCS and AFM. The results show the formation of an albumin protecting capping layer on the surface of the SLNs. The lack of SLN aggregation, even at high concentrations of BSA, opens the perspective to develop these transporters in intra-venous administration.

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## References

Bost, M., Laine, V., Pilard, F., Gadelle, A., Defaye, J., Perly, B., 1997. The hemolytic properties of chemically modified cyclodextrins. J. Incl. Phenom. Mol. Recognit. 29, 57–63.

Coleman, A.W., Kasselouri, A., 1993. Supramolecular assemblies based on amphiphilic cyclodextrins. Supramol. Chem. 1, 155–161.

Da Silva, E., Shahgaldian, P., Gualbert, J., Aïssa, K., Lazar, A., Pouget, E., Coleman, A.W., submitted. Interaction of amino-acids with amphiphilic calix-[4]-arenes at the mesoscopic interfaces. J. Incl. Phenom. Mol. Recognit.

Demirel, M., Yazan, Y., 2000. Solid lipid nanoparticles (SLN). J. Pharm. Sci. 25, 167–179.

Diem, K., Lenter, C., 1972. Scientific Tables. Ciba-Geigy, New York, USA.

Gutsche, C.D., 1998. Calixarene Revisited. The Royal Society of Chemistry, Cambridge, UK.

Houel, E., Lazar, A., Da Silva, E., Coleman, A.W., Solovyov, A., Cherenok, S., Kalchenko, V.I., 2002. Interfacial interactions of cations with amphiphilic dihydroxyphosphonyl-calix-[4]-arene mesosystems. Langmuir 18, 1374–1379.

Lazar, A., Shahgaldian, P., Coleman, A.W., 2001. Anion recognition effects in the structuring of bovine serum albumin films. J. Supramol. Chem. 1, 193–199.

Lian, T., Ho, R.J.Y., 2001. Trends and developments in liposome drug delivery systems. J. Pharm. Sci. 90, 667–680.

Mehnert, W., Mader, K., 2001. Solid lipid nanoparticles. Production, characterization and applications. Adv. Drug Deliv. Rev. 47, 165–196.

Mitra, S., Maitra, A.N., 2000. Colloidal nanoparticles for drug delivery and targeting. J. Surf. Sci. Technol. 16, 316–330.

Moselhy, J., Wu, X.Y., Nicholov, R., Kodaria, K., 2000. In vitro studies of the interaction of poly(NIPAm/MAA) nanoparticles with proteins and cells. J. Biomater. Sci. Polym. Ed. 11, 123–147.

Müller, R.H., Mader, K., Gohla, S., 2000. Solid lipid nanoparticles (SLN) for controlled drug delivery—a review of the state of the art. Eur. J. Pharm. Biopharm. 50, 161–177.

Peters, T., 1996. All About Albumin. Academic Press, London, UK.

Shahgaldian, P., Coleman, A.W., Kalchenko, V.I., 2001. Synthesis and properties of novel amphiphilic calix-[4]-arene derivatives. Tetrahedron Lett. 42, 577–579.

Shahgaldian, P., Cesario, M., Goreloff, P., Coleman, A.W., 2002. Para-acyl calix[4]arenes: amphiphilic self-assembly from the molecular to the mesoscopic level. J. Chem. Soc. Chem. Commun., 326–327.

Shahgaldian, P., Quattrocchi, L., Coleman, A.W., Goreloff, P., 2003. AFM imaging of calix-arene based Solid Lipid Nanoparticles (SLNs) in gel Matrices. Eur. J. Pharm. Biopharm. 55, 107–113.

Skiba, M., Wouessidjewe, D., Coleman, A., Fessi, H., Devissaguet, J.P., Duchene, D., Puisieux, F., 1993. Preparation and use of novel cyclodextrin-based dispersible colloidal systems in the form of nanospheres. PCT Int. Appl. WO 9325195.

Sommer, F., Tran Minh, D., Coleman, A.W., Skiba, M., Wouessidjewe, D., 1993. Seeing is believing: imaging amphiphilic-cyclodextrin-derived liposomes by atomic force microscopy. Supramol. Chem. 3, 19–22.

Weber, C., Coester, C., Kreuter, J., Langer, K., 2000. Desolvation process and surface characterization of protein nanoparticles. Int. J. Pharm. 194, 91–102.