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Research paper

Atomic force microscopy imaging of novel type of polymeric colloidal nanostructures

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Abstract

Polymeric nanoparticles were prepared by the interfacial poly-condensation of the lipophilic monomer, phtaloyldichloride and the hydrophilic monomer, diethylenetriamine, in the presence and absence of the surfactant Pluronic® F68. The colloidal systems were analysed by dynamic light scattering and atomic force microscopy, the structures formed have two populations (150 and 350 nm) in the presence of the surfactant and one population (450 nm) in the absence of the surfactant. The results can be interpreted in terms of the formation of hollow nanocapsules that collapse on deposition and drying.

Keywords: Nanoparticles; Interfacial poly-condensation; Morphological analysis; Atomic force microscopy

1. Introduction

Several processes exist today for manufacturing colloidal polymeric systems [1,2]. Of these systems, nanoparticles have applications in various industrial fields such as cosmetics, pharmacy and agrochemical [3].

The formation of nanoparticles allows the protection of an active molecule by a polymeric envelope or matrix and the release of this product in an aimed target flowing profile [4,5].

The methods of preparation of nanoparticles may be classed in two main categories. The first and major category includes most of the methods, and is based on polymerisation reactions; the second involves the use of preformed polymers [6,7].

Recently we have developed a novel and simple concept for the preparation of nanoparticles, the methodology involves an interfacial poly-condensation reaction between hydrophilic and lipophilic monomers. The monomers used in this communication were phtaloyldichloride (PDC) and diethylenetriamine (DETA). Nanoparticles of the polyamide were prepared according to the flowing procedure methodology [7,8].

2. Methods and material

2.1. Nanoparticle preparation

All chemicals were purchased from Fluka, France and used without further purification.

Nanoparticles of the polyamide were prepared according to the flowing procedure methodology [6,8]. PDC (100 mg) and Span® 40 (40 mg) were first dissolved in acetone (20 ml), the resulting organic solution is poured into water (40 ml) containing DETA (500 mg) and Pluronic® F68 (60 mg). After magnetic stirring during 12 h, the mixture was concentrated to the desired final volume (10 ml), by removal of water and acetone under reduced pressure.

2.2. Atomic force microscopy (AFM) sample preparation

A total of 20 μl of a suspension of the nanoparticles was deposited on to a freshly cleaved mica surface, and then dried overnight.

2.3. AFM

Imaging was carried out using ultra-low amplitude non-contact mode on a Topometrix Explorer AFM, (Thermomicroscopes Inc, USA) with a 2 μm tube scanner. Scanning

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Table 1
Comparative data for nanoparticles diameter and height from AFM and DLS analysis

	Population	With Pluronic® F68		Without Pluronic® F68	
		Diameter (d) (nm)	Height (h) (nm)	Diameter (d) (nm)	Height (h) (nm)
AFM	1	150 ± 20	12 ± 2	420 ± 50	10 ± 1
	2	350 ± 30	26 ± 4	440 ± 50	18 ± 2
DLS	1	280 ± 30	–	450 ± 50	–

speeds were 0.5 Hz, a low resonance frequency pyramidal silicon cantilever resonating at 147 KHz was used (force constant = 42 n/M). The amplitude of the resonance was set manually to the minimum possible for stable imaging within the contamination layer present at the surface. Height and cross-sectional size measurements were carried out using the Line Analysis sub-program of the Topometrix SPML 4.0 imaging software package. Values given are an average of at least 30 measurements.

2.4. Particle size measurement

Dynamic light scattering (DLS) was carried out on a Malvern Zetasizer® (Malvern, UK) at a 90° analysis angle.

3. Results and discussion

Nanoparticles of the polyamide were prepared according to the flowing procedure methodology [7,8] as aqueous suspensions. Morphological examination of the colloidal suspension was performed using a comparison between AFM and DLS (Table 1).

The AFM measurements were carried out on samples of the nanoparticles deposited on to a freshly cleaved mica surface, and then dried overnight. In order to obtain accurate height information for the systems, imaging was carried out using ultra-low amplitude non-contact mode on a Topometrix Explorer AFM.

In the use of 'true' non-contact mode AFM imaging the amplitude of oscillation of the cantilever is maintained at a constant value, this in contrast to the 'tapping' mode in which the is a periodic high amplitude beat. In the first case if the tip is positioned within the contamination layer, at about 2-nm above the surface capillary interactions between the tip and the surface are minimised, in the second case the tip is positioned above the contamination layer and during the high amplitude beat penetrates into the contamination layer to image the surface, here capillary force effects remain.

The images obtained from AFM of samples prepared either with or without Pluronic® F 68 are shown in Figs. 1a,b, respectively. Image cross sections for typical nanoparticles are given in Figs. 2a,b, along with height and size measurements. Some point-sample interactions are observed in the slight horizontal 'banding' observed in both cases.

From AFM imaging two groups of nanoparticle population are observed in both cases. In the presence of Pluronic® F 68 both the observed diameters (350 nm) and heights (26 ± 4) of the larger population are roughly double those of the smaller population (150 and 12 nm). This may arise from aggregation of the nanoparticles on deposition and drying, however careful analysis of the images shows no boundaries or smaller sub-particles. Interestingly the DLS values of 280 nm is an approximate arithmetic average of the two values, given the use of a mono-modal analysis in

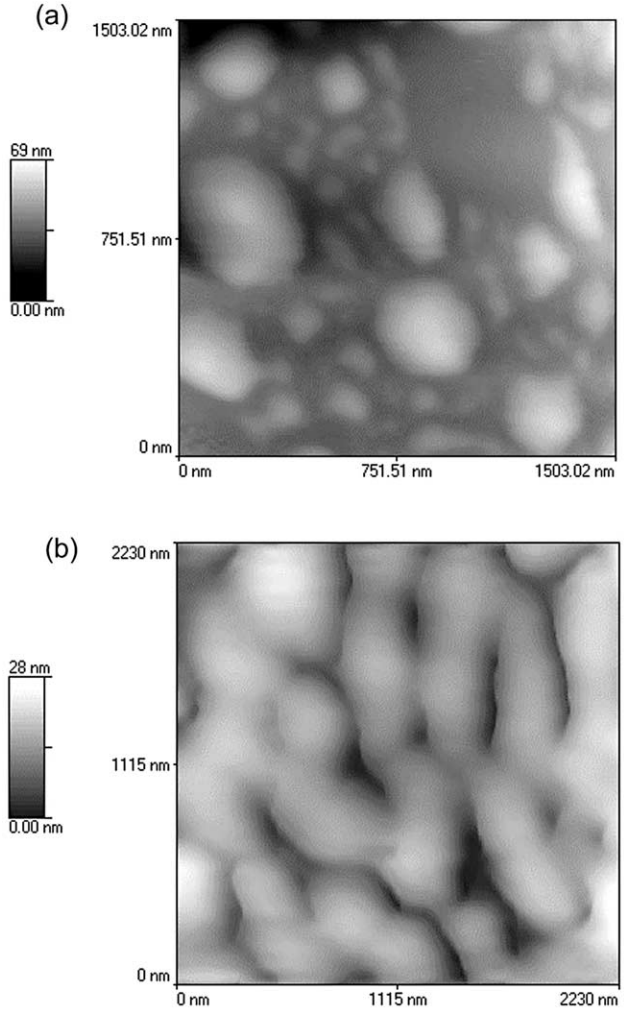

Fig. 1. Non-contact mode AFM images of nanosystems: (a) in the presence of Pluronic® F68; and (b) in the absence of Pluronic® F68.

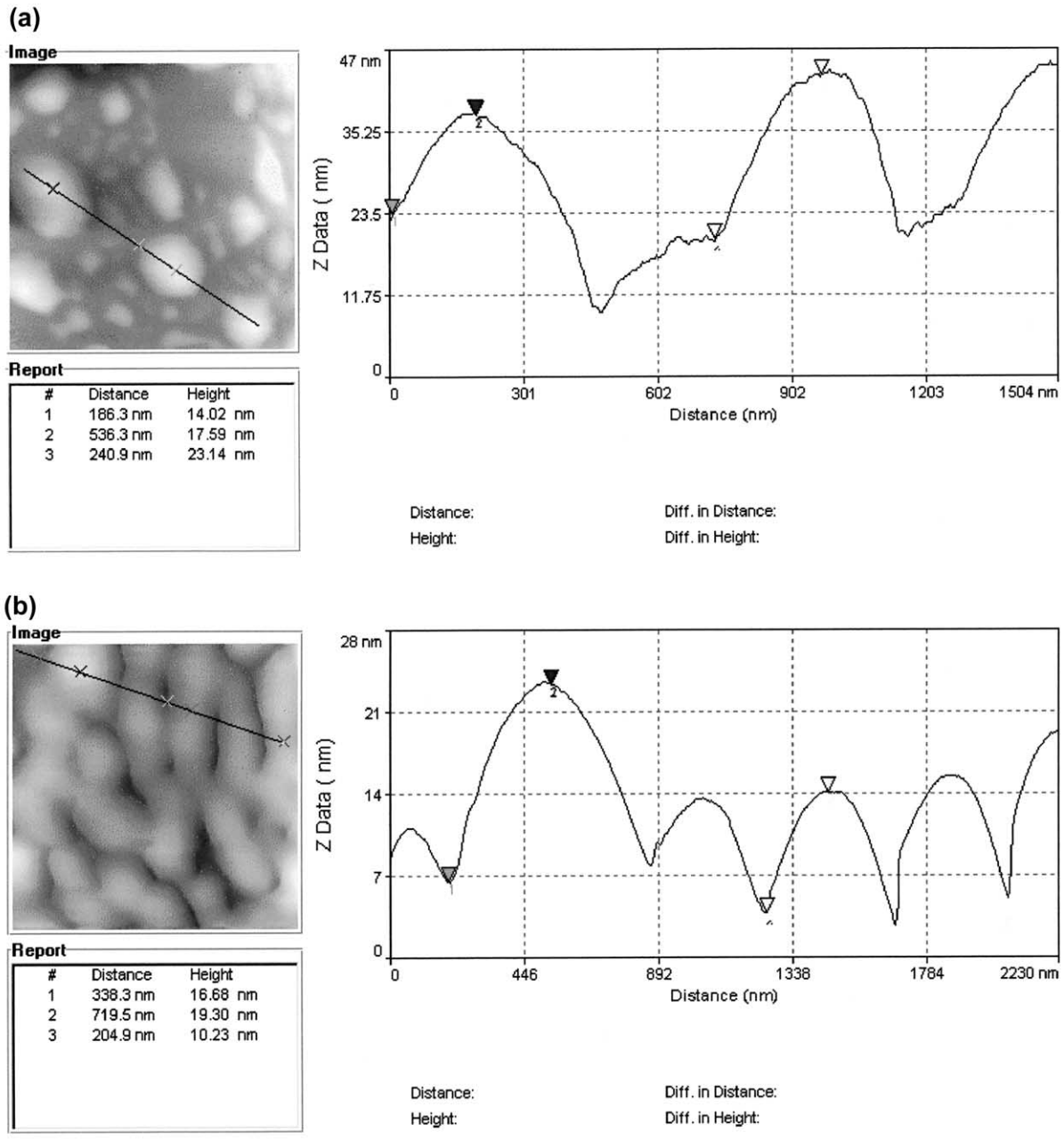

Fig. 2. Cross section for nanosystems from AFM line analysis: (a) in the presence of Pluronic® F68; and (b) in the absence of Pluronic® F68.

this case and the high degree of poly-dispersity we believe that both the small population and the larger population may coexist in suspension.

In the absence of Pluronic® F 68, from the AFM imaging two populations are again observed, with in the case similar diameters (420 and 450 nm) but with different heights (10 and 18 nm), in this case there is a tendency for the nanoparticles to coalesce at the surface and the height differences observed may simply result from an overlay of a second layer of the nanoparticles. In this case the observed sizes of the nanoparticles correspond more closely with those observed from DLS. This implies that a single population is present both in solution and after deposition onto mica.

Comparison of the surface areas for the data from DLS and from AFM, gives the following values; in the presence of Pluronic®, DLS = F 68, 2.5×10^5 nm^2; AFM average of the two populations = 2.37×10^5 nm^2; and in the absence of Pluronic® F 68, DLS = 6.35×10^5 nm^2 and AFM = 6.1×10^5 nm^2. Whilst these values are only approximate, they correlate reasonably well between the two physical methods.

Analysis of the data indicates that the diameter of nanoparticles is much larger than the height, approximately diameter/height = 12 for both sub-populations in the presence of Pluronic® F 68 and d/h = 45 and 25 for the two sub-populations in the absence of Pluronic® F 68. The

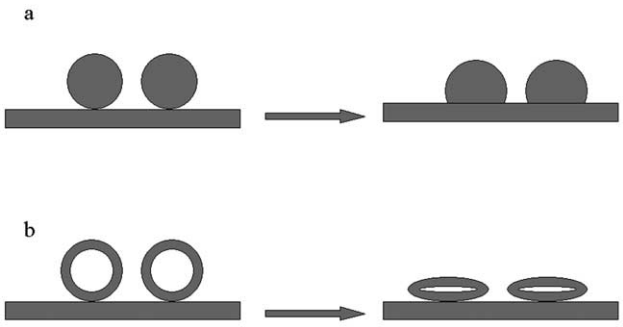

Fig. 3. Schematic representation of the evolution of: (a) nano-matrix (nanosphere); and (b) nano-vesicular (nanocapsule) systems on deposition and drying on a surface.

approximate doubling of d/h for the nanoparticles prepared in the absence of Pluronic® F 68 is again in agreement with an overlay of a second layer.

The structures produced by this interfacial poly-condensation may be either solid (nanospheres) or hollow (nanocapsules). It is has been previously shown that accurate height information can be obtained in the non-contact mode on such soft objects as liposomes [9] or solid-lipid nanoparticles [10,11].

From the above results it is possible to postulate on the structural nature of the nano-systems (Fig. 3). If the diameter was roughly equal to the height or even up to d/h = 4, the objects would be solid nano-matrix structure (nanospheres) (Fig. 3a), this has been observed for the cyclodextrin based nanospheres [12]. However, if the system is not based on a continuous matrix, collapse of the liquid pocket encapsulated by the polymer membrane will occur and the object will flatten to a height roughly twice the thickness of the polymer walls (Fig. 3b). This is the case observed for non-contact mode analysis of protein containing liposomes [9]. So, not only is it possible to define the structures produced as nanovesicular (nanocapsules), but it is also to propose that polymer wall will be approximately 6 nm thick in the presence of Pluronic® F68 and roughly 4–5 nm thick in the absence.

It is also important to indicate that the Pluronic® F68 is not necessary for formation of nano-vesicular structure by interfacial poly-condensation reaction. It is noteworthy that the presence of Pluronic® F68 was needed for wall coating formation and for suspension stabilisation. However, the presence of Span® 40 is necessary in formulation of these nano-vesicular polymeric structure, this affirmation indicates that the poly-condensation reaction between the lipophilic monomer and the hydrophilic monomer takes place at the interfacial region of a mesoscopic system.

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