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ORIGINAL CONTRIBUTION

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Formation and stabilization of a biodegradable polymeric colloidal suspension of nanoparticles

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Abstract The formation of a colloidal suspension of nanoparticles was obtained, in a very simple manner, by transferring a solution of poly-ε-caprolactone in a good solvent (L1) into a non-solvent (L2). Photon Correlation Spectroscopy (PCS) measurements confirmed by microscopic observations were used to determine the morphological aspects of the preparations. The influence of several factors on nanoprecipitation was examined: polymer concentration, L1/L2 ratio, dielectric constant of the final mixture. An experimental model of the phenomenon, which takes into account the flocculation concentration and the L1/L2 ratio, is proposed. It allows the optimal conditions for nanoparticles formation to be determined.

Key words Nanoparticles – poly-ε-caprolactone – ternary diagram – flocculation – dielectric constant

Introduction

A submicronic colloidal suspension of poly ε Caprolactone (PCL) was prepared by precipitation of the macromolecular material in an insolubilizing medium. This process [1–2] consists of the introduction of the polymer dissolved in a liquid L_1 into the precipitating medium, a liquid L_2 non solvent of the polymer. According to the operating conditions polymer precipitation will lead either to the formation of more or less large aggregates or to the formation of submicrometer sized particles. In this latter case, it is said that polymer nanoprecipitation has occurred.

PCL is one of the polyesters used in the medical field [3–4] due to the lack of toxicity of the polymer itself or of its degradation products. Its complete biodegradability, the possibility of its concomitant degradation with included active drug [5], and its ability to be sterilized are also factors in its favor.

This work describes some parameters determining the nanoprecipitation process and particularly the polymer concentration in the organic solvent, the solvent/non solvent ratio, the percentage of non-solvent in the overall formulation and the dielectric constant of the final medium.

Phase diagrams were established in order to determine the zones of nanoparticle formation. These latter were characterized by size measurements by Photon Correlation Spectroscopy (PCS).

Materials and methods

Materials

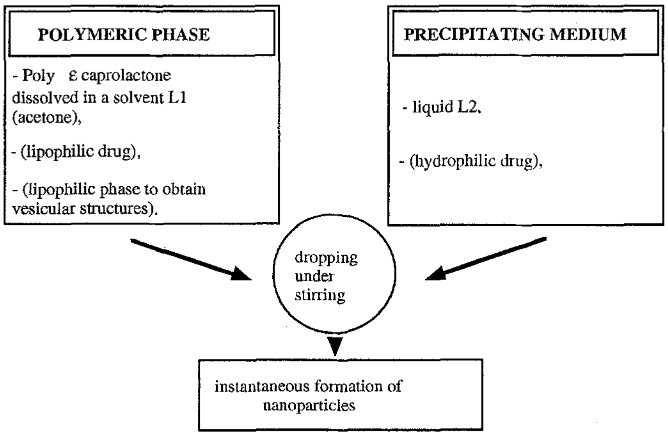
– PCL	(Aldrich Chemie) purity 99 p 100 was used without further purification. The mean molar mass was 140 000 daltons. It was soluble in the chosen solvent L1: warmed acetone under stirring.
– Solvents	Normapur Acetone Prolabo (France)
– Apparatus	The apparatus used for Transmission Electronic Microscope (TEM) observations is a microscope Philips EM 301, 80 Kv The N4 Nanosizer used to obtain size measurements is manufactured by the Coulter Company (G.B). Ultracentrifugation was performed in a L755 Beckmann using a T165 rotor. UV Spectrometric *b* measurements were performed with a Perkin Elmer Lambda 5.

Batches manufacturing

In order to simplify the interpretation of the results, nanoprecipitation parameters were simplified and standardized for the all batches (Fig. 1). The PCL polymer phase is dissolved in a solvent L1, in this case acetone. It is dropped into a precipitation medium L_2 under stirring. This precipitating medium can be an ethanol water mixture so as to adjust the final medium dielectric constant. Nanoparticles are instantaneously formed and their size can be measured immediately after mixing the two solutions.

Apart from the two constituent liquids of the phases L1 and L2 and the PCL, no other component (surfactant, buffer) enters into the preparation. Therefore the stability of a nanoparticle suspension cannot be attributed to any substance adsorbed on their surface.

Fig. 1 Batches manufacturing



Samples were stored at ambiant temperature and protected from light.

Particle size measurement

– microscopic observations

TEM analysis was made on nanoparticles treated by negative staining with phosphotungstic acid. This technique was used once to confirm the PCS measurements of the nanoparticle size.

– Photon Correlation Spectroscopy (PCS)

This technique is concerned with the time dependence of the fluctuations in the intensity of the light scattered by a suspension of particles. These fluctuations are conveniently described by time-dependent correlation functions [6]. The instrument used (N4 Nanosizer) gives the number of counts per seconds (C/S) for 90° angle selection and accumulates the value of the scattered-light correlation function in 80 digital counters.

For a system of rigid, monodisperse, compact particles the correlation function is

$$C(t) = A \cdot \exp(-2\Gamma t) + B ,$$

where A and B are constant, $C(t)$ is related to the fluctuation relaxation time τ by

$$\Gamma = Dq^2 = 1/\tau ,$$

q is the scattering fluctuation wave vector amplitude. D is the diffusion coefficient which allows to determine the particle size through the Stokes–Einstein equation:

$$D = k \cdot T/3\pi \cdot \eta \cdot d ,$$

where k is the Boltzmann constant, T is the absolute temperature, and η is the viscosity of the medium in which the particles of diameter d are suspended.

Contrary to the manufacturer's directions recommending performing size measurements in a pure solvent, particle sizes were measured in a water/acetone mixture, sometimes added with ethanol. Since the nature of the final mixture has some consequences on the particle size, on the refractive index and on the viscosity of the medium, and since these two last parameters are essential in the correlation treatment data obtained by the Nanosizer, we calibrated the apparatus with two known suspensions:

- a PCL suspension (mean diameter 215 ± 9 nm)
- a polystyrene latex (Rhone Poulenc) mean diameter 160 ± 10 nm.

These two suspensions were filtered on millipore membranes (pore size 0, 45 μm) before use. From the relationship between the number of counts per second (C/S) and the percentage of acetone in the sample, it was possible to determine correction factors to apply to size measurements of nanoparticles related to the solvents. We found that these differences were negligible with regard to the following correction related to the morphology of the particles.

C/S is in itself a useful parameter for evaluating our preparations, since a decrease can indicate the beginning of flocculation. As the light-scattering intensity of a particle increases in proportion to its surface area [7], we divided C/S by the square of the particle diameter for concentrations lower than those leading to flocculation. This corrected value of C/S is referred to as C/C.

Results and discussion

Morphological aspect of the preparations

We tried to determine the threshold percentage of solvents allowing nanoparticle formation, according to the ternary phase diagram (water, acetone, PCL) presented in Fig. 2.

Two zones were evident.

- Zone a, in which the PCL concentration is higher than the polymer solubility in acetone alone.
- Zone b, the zone of nanoparticle formation which is divided into three different regions: bI, bII, bIII, each of them subdivided in A, B or C (Table 1).

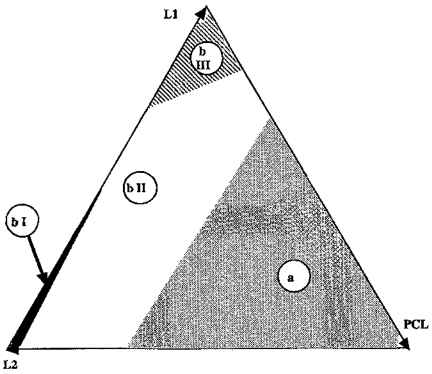


Fig. 2 Ternary diagram showing the domain of nanoparticles formation (bI)

In region bI, the phases bIA to bIC are observed with a low polymer concentration (0.5 to 1.5%) while the other phases only existed in the presence of high proportions of acetone. The behavior observed in the different regions was as follows:

Region bI nanoparticle suspension

All the polymer is transformed without any apparent flocculation. Nanosizer observations allowed us to distinguish the following aspects within this region:

- domain bIA. The final preparation is perfectly limpid. Nanosizer observations showed particle size of 150 to 200 nm. The standard deviation is about 50 nm and the polydispersity is nearly 10%.
- domain bIB. The observed Tyndall effect confirmed PCL nanoparticles formation (PCL is the only compound able to scatter light in the colloidal state). Only one peak is observed at 150 nm with a standard deviation of 67 nm. For this preparations the diameter measured by TEM analysis gave 150 ± 20 nm.
- domain bIC. The preparation has a milky appearance, due to a high nanoparticle concentration or to large particles masking the Tyndall effect. Two different populations of particles can be detected. The larger particles are the result of nanoparticle aggregation. This region bIC preceds flocculation and is visible to the naked eye.

Region bII flocculation

The growth of aggregates detected in region bIC leads to flocculation of the suspension.

- bIIA. Soon after nanoprecipitation, the preparation begins to flocculate. Sedimentation follows about 10 days later, leaving nanoparticles in suspension.
- bIIB. Both flocculation and sedimentation are fast. The supernatant is completely clear and no particle is detected by the Nanosizer.

Table 1 Macroscopic aspect of the preparations according to the formulation

bI – Suspension of nanoparticles			bII Floculation		bIII Dissolution of the PCL	
Undetectable but using Nanosizer	Nanopart. detected by Tyndall effect	– milky appearance, – large particles	Nanoparticles remaining in the supernatant	The supernatant is completely clear	– glassy aspect, – partial dissolution	– transparent – depots of polymer
bIA	bIB	bIC	bIIA	bIIB	bIIIA	bIIIB

Region bIII polymer dissolution

The proportion of acetone in the final mixture is high enough to maintain the polymer in solution. In the polymer concentration range used here (0.5 to 15%), the concentration of acetone consists of at least 75% (w/w) of the mixture.

- bIIIA. The solution appears glassy and opalescent. After a few days a translucent precipitation forms on the bottom of the container. This does not give an interpretable result in the Nanosizer.
- bIIIB. The solutions are transparent. When the concentration of PCL in the acetone is high, precipitation leads to the formation of macroscopic depots of polymer.

Characterization of the zone of nanoparticle formation (Region bI)

When observed with the naked eye the transition from phase bIA to bIB seems to be a continuous transformation. In contrast, a characteristic transition point can be detected between phase bIB and bIC, by a sudden decrease in the parameter C/S determined by the Nanosizer (Fig. 3). This indicates the start of flocculation and allows us to determine the threshold level of different solvents bordering the zone of nanoparticle formation.

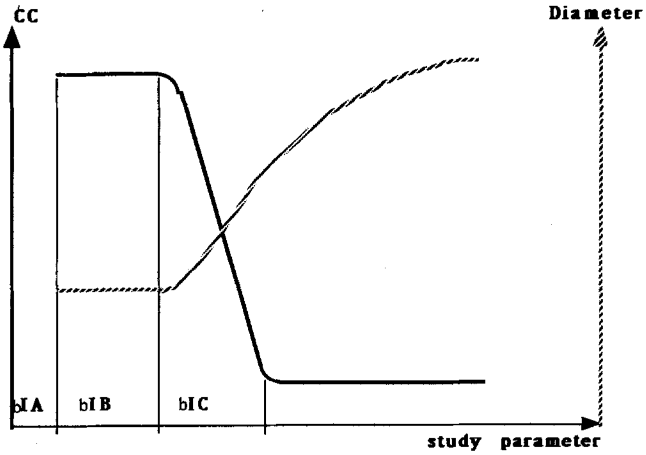


Fig. 3 The transition phase from phase bIB to phase bIC

Factors determining nanoparticle formation

Preliminary experiments allowed us to identify certain factors determining whether colloidal precipitation will occur. The most important are: the initial polymer concentration, the volume ratio (r) of the solvents L1 and L2, and the nature of the final mixture, particularly its dielectric constant.

Influence of the ratio L1/L2 (r)

For a fixed polymer concentration (26 g/l) the influence of r was studied in the range 0.33 to 5.000. Table 2 shows that above $r = 0.333$, the flocculation is so intense that the size of the particles remaining in suspension cannot be determined.

Influence of the polymer concentration

For a fixed ratio r, the polymer concentration has varied from 1 g/l to 40 g/l. Figure 4 shows that after a certain limit flocculation occurs. This corresponds to the beginning of phase bIC and is accompanied by a sudden decrease in C/C.

Influence of the dielectric constant ε of the final mixture

As well as the ratio L1/L2, it is possible that the importance of r in nanoparticle formation is related to the dielectric constant of the final water/acetone mixture, since the two components have very different ε values. To study the influence of this parameter, we measured ε as a function of Cf (in mg/ml), the concentration of polymer in L1 at which

Table 2 Effect of the ratio L1/L2 (= r)

r	ε	Phase	Diameter (nm)	SD (nm)	CC
0.133	73.0	bIB	115	29	17.2
0.167	71.5	bIB	130	40	15.7
0.333	65.2	bIC	150	67	13.5
1.000	50.3	bIIA		Floculation	
1.600	43.5	bIIA		Floculation	
2.500	37.6	bIIA		Floculation	
5.000	30.6	bIIB		Floculation	

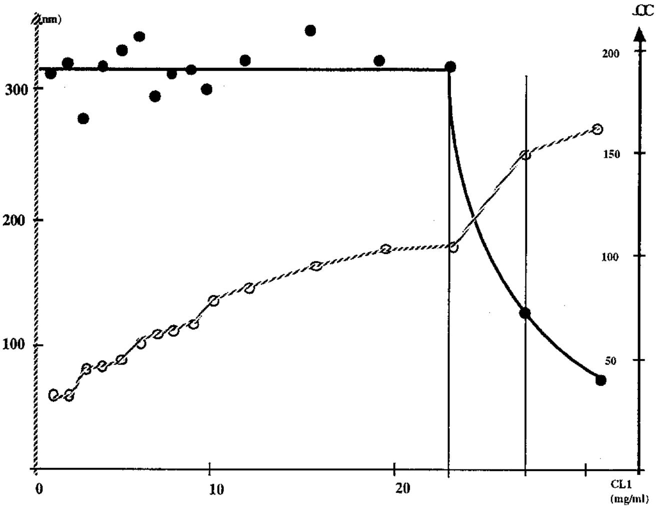


Fig. 4 Variation of the Corrected Count (C/C) and of the diameter versus the concentration of the polymer in L1 (acetone). $r = 0.1667$

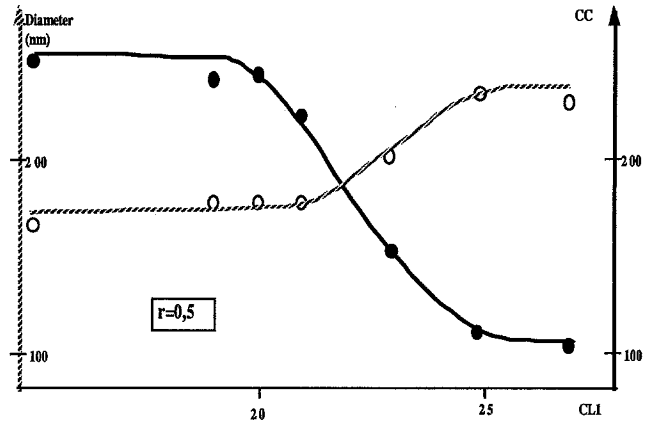


Fig. 5 Variation of the Corrected Count (C/C) and of the diameter of the particles versus the concentration of the polymer in L1 (acetone). Determination of *Cf*

flocculation begins, as we can see in the decrease in the number of particles and an increase in their size. To determine *Cf* precisely, we have used both the variations in the size of the objects and the parameter C/C, as a function of polymer concentration, at given *r* values. A typical curve is shown in Fig. 5.

After performing a number of experiments at different *r* values, relationship between *Cf* and *r* was obtained (Fig. 6). In this figure, for each value of *r*, the state of the preparation at different concentrations, expressed as CL1 in a decimal logarithm scale, is represented by a symbol. The triangles (▽) represent points before the fall in C/C, while the squares (■) show a transition state corresponding to Cf, and the (▲) triangles show the decrease in C/C.

Discussion

In Fig. 6, the transitions between phase bIB and bIC was only observed for *r* values lower than 1.2. When *r* was higher than this value, a fall in C/C can still be detected, but it corresponds to the passing from the beginning of flocculation to complete flocculation (bIC to bIIA or bIIA to bIIB).

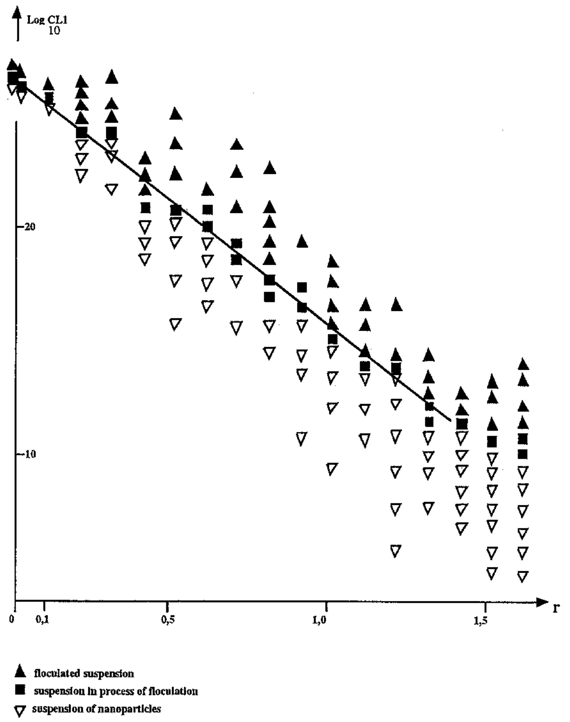


Fig. 6 Variation of the flocculation concentration Cf (■) versus *r*, the L1/L2 ratio

If one joins together the points which correspond to flocculating solutions, a linear relationship between log *Cf* and *r* was observed. Furthermore, this figure allows us to define a zone of nanoparticle formation. The maximum concentration is about 30 g/l ($r = 0$) and maximum value of *r* is 1.2. When *r* is higher than 1.2, the relation is no longer linear, the drop in C/C is not so evident, the presence of a high proportion of acetone renders dispersion of the suspension impossible, probably because of polymer dissolution by acetone and of the presence of a more or less gelified mass described above.

Modelling

The linear part of the relationship can be expressed by the following equation

$$\log_{10} Cf = -0.3r + 1.48 \ . \qquad (1)$$

Colloid Polymer Science, Vol. 273, No. 5 (1995)

The linear regression coefficient is 0.997. This equation is in good agreement with that of Souchay [8] which quantifies colloid flocculation by non-solvents by relating the tendency to precipitate γ to the particle concentration C.

$$\log_{10} C = K\gamma + A \,, \tag{2}$$

where A and K are constant.

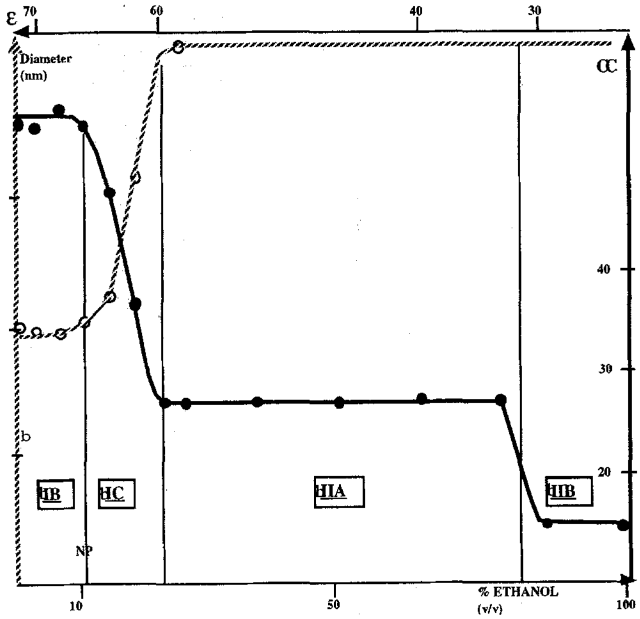
Effect of modification of the dielectric constant by adding ethanol to the aqueous precipitating phase L_2

The above relationship can also be expressed as a function of the dielectric constant of the final medium. Because the dielectric constant is a decreasing linear function of the acetone proportion, the linear decrease of r is equivalent to a linear increase of ε. The graph relating $\log_{10} Cf$ to ε is still a straight line, but the slope is not the same.

ε can also be modified by adding a second solvent to the precipitating medium L_2. We did this by adding ethanol to the aqueous phase. The dielectric constant of ethanol is 24.3 at 25 °C, so the ε constant of the mixture can vary over a wide range.

Figure 7 shows the relationship between the different regions of the phases diagram and the dielectric constant of the final medium. It is noteworthy that the transition from phase bI to phase bII takes place at relatively high values of the dielectric constant of the final medium (about 60).

Fig. 7 Variation of the Corrected Count (C/C) and of the diameter of the particles versus the percentage of ethanol



We observed that for ε values equal to or higher than 66 nanoprecipitation gave rise to individual particles (bIB) mean size of about 100 nm. The phase bIC appearing between $62 < \varepsilon < 66$ is a progressive transitory phenomenon between the flocculated stage and the non-flocculated one (phase bIIA and bIB). For ε levels down to 33, flocculation is neither complete nor immediate, while it is instantaneous and complete for lower ε values ($\varepsilon < 33$).

This study demonstrates the importance of the dielectric constant of the final medium, thus the importance of the polarity of the solvents in the formation and stability of nanoparticles.

Conclusion

Nanoprecipitation is a simple process, easy to perform, whose originality has led to the registration of international patents. The only difficulty lies in the fact that the preparation is only possible under restricted conditions: the area concerned in the ternary diagram is very narrow. Indeed, in order to observe the nanoprecipitation phenomenon alone it was decided to use no additive and not to remove the organic solvent after precipitation. As a result, the zone of formation of nanoparticles is so restricted as to compromise the possibility of development to an industrial scale (latex production) or semi-industrial scale (production of drug-delivery systems). However, nanoprecipitation applications are increasing at present because of the possibilities of removing organic solvents or water in order to concentrate the suspensions and because of the use of cryoprotectors allowing the stabilization of the submicronic colloidal suspensions.

The understanding of the nanoprecipitation mechanisms was the first aim of this study. However, the development of an experimental model of the main parameters confers on it a predictive value. So, with the help of morphological data (diameter variations according to the concentration in phase 1) or the physicochemical data (flocculation threshold according to the ratio r or the dielectric constant ε), it is possible to predict the optimal conditions for nanoparticles preparation and to anticipate the size of the obtained nanoparticles.

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