*AAPS PharmSciTech* 2002; 3 (4) article 32 (http://www.aapspharmscitech.org).

# The Effect of Cosolvents on the Formulation of Nanoparticles From Low-Molecular-Weight Poly(l)lactide

*Submitted: September 16, 2002; Accepted: November 7, 2002*

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**ABSTRACT** The aim of this study was to formulate nanoparticles from poly(l)lactide by a modified nanoprecipitation method. The main focus was to study the effect of cosolvent selection on the shape, size, formation efficiency, degree of crystallinity, x-ray diffraction (XRD) reflection pattern, and zeta potential value of the particles. Low-molecular-weight (2000 g/mol) poly(l)lactide was used as a polymer, and sodium cromoglycate was used as a drug. Acetone, ethanol, and methanol were selected as cosolvents. Optimal nanoparticles were achieved with ethanol as a cosolvent, and the formation efficiency of the particles was also higher with ethanol as compared with acetone or methanol. The particles formulated by ethanol and acetone appeared round and smooth, while with methanol they were slightly angular. When the volume of the inner phase was decreased during the nanoprecipitation process, the mean particle size was also decreased with all the solvents, but the particles were more prone to aggregate. The XRD reflection pattern and the degree of crystallinity were more dependent on the amount of the solvents in the inner phase than on the properties of the individual cosolvents. The zeta potential values of all the particle batches were slightly negative, which partially explains the increased tendency toward particle aggregation.

**KEYWORDS:** nanoparticles, nanoprecipitation, poly(l)-lactide, sodium cromoglycate, XRD, zeta potential.

## INTRODUCTION

Controlled release formulations of pharmacologically active substances in which biodegradable polymers are used as carriers provide interesting options for stable and convenient drug formulations [1-6]. When colloidal particles of nanometric scale are formulated from a biodegradable polymer and a drug, a modified drug delivery/drug targeting or an improved pharmacokinetic profile of the compound is feasible. Also, improvements in selectivity, protection of the drug against fast metabolism, and more effective diffusion through biological barriers may become attainable [7].

Nanoprecipitation has been used to formulate nanoparticles by many researchers [8-10]. Complex hydrodynamic processes at the interfacial area lead to the creation of the nanoparticles [8,11]. Polylactide(s) have been widely used in earlier nanoparticle studies, although the low-molecular-weight fractions of polylactic acid (PLA) have been used in few studies (L. Peltonen, P. Koistinen, J. Hirvonen, unpublished data, 2002). The interest in using low-molecular-weight PLA is based on a shorter degradation time as compared with longer chain analogues: PLA of $M_w$ 2000 g/mol, with a controlled drug release lasting for a few hours, has been shown to be suitable, for example, in pulmonary sustained-release formulations [12,13].

In this article, PLA nanoparticles were formulated by a modified nanoprecipitation method. The main focus was to study the effect of selected cosolvents on the size and shape and several physicochemical properties (aggregation tendency, x-ray diffraction [XRD] pattern, zeta potential) of the particles. Sodium cromoglycate, a compound used in the treatment of bronchial asthma, was used as a model drug. Chloroform was used as a solvent for the polymer, PLA ($M_w$ = 2000 g/mol). The cosolvent (acetone, ethanol, or methanol) was used as a "poor" solvent (a driving solvent) for the polymer. The function of the cosolvent was also to aid in the formation of a homogeneous dispersion from the aqueous drug solution and the polymeric chloroform solution. At the interface, the cosolvents that do not possess any affinity to the polymer are the first to diffuse out from the polymeric quasi-emulsion droplets. Chloroform with a high affinity to the polymer diffuses out only later from the diminished droplets and, at the same time, the polymer starts to precipitate at the interface. These 2 diffusion steps, which are greatly affected by the properties of the solvents and the interactions between the solvents and the

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polymer, play a crucial role in the successful formulation of nanoparticles (L. Peltonen, P. Koistinen, J. Hirvonen, unpublished data, 2002).

## MATERIALS AND METHODS

### *Materials*

PLA (2000 g/mol) was from ICN Pharmaceuticals (Aurora, Ohio). Organic solvents were chloroform, methanol, acetone (analytical grade, Riedel-deHaën, Seelze, Germany), and ethanol (Ph Eur, Primalco, Rajamäki, Finland). Sodium cromoglycate (ICN Biomedicals Inc, Aurora, Ohio) was the model drug, and propylene glycol (Ph Eur, University Pharmacy, Helsinki, Finland) was used as a stabilizing agent. The water used was filtered by Milli-RO 12 Plus (Millipore, Molsheim, France).

### *Preparation of the Nanoparticles*

The preparation method for the nanoparticles was modified from the nanoprecipitation method by Fessi et al [8], and it has been described in detail elsewhere (L. Peltonen, P. Koistinen, J. Hirvonen, unpublished data, 2002). Briefly, the method was performed as follows: 2.5 mg of the drug was dissolved in water; then acetone, ethanol, or methanol was added as a cosolvent. Twenty-five milligrams of PLA and 150 mg of propylene glycol were dissolved in chloroform, and this solution was added to the drug solution to form a dispersion. The dispersion was added to 5 mL of aqueous ethanol solution (70%). After 5 minutes of mixing, the organic solvents were removed by evaporation at 35°C under normal pressure, and the suspension was filtered (filter 20-25 µm, Whatman International Ltd, England). The amount of the cosolvents in the inner phase varied from 0.2 mL to 1.0 mL. The solvent compositions of the more successful nanoparticle batches (I-V), which were selected for the characterization and physicochemical studies, are presented in **Table 1**.

### *Characterization of the Morphology of the Nanoparticles*

The surface morphology (roundness, smoothness, and formation of aggregates) and the size of nanoparticle formulations were studied by scanning electron microscopy (SEM). The particle samples were sputtered for 20 seconds with platinum (Agar Sputter Coater, Agar Scientific Ltd, Essex, UK) and analyzed with a scanning electron microscope (DSM 962, Zeiss, Jena, Germany).

### *XRD Experiments*

XRD patterns were measured using an XRD theta-theta diffractometer (Bruker axs D8, Karlsruhe, Germany). The XRD experiments were performed in symmetrical reflection mode with $CuK_\alpha$ radiation (1.54 Å) using Göbel Mirror bent gradient multilayer optics. The scattered intensities were measured with a scintillation counter. The angular range was from 3° to 45° with steps of 0.05°, and the measuring time was 5 seconds/step.

Crystallinities of the nanoparticle samples were estimated by fitting the intensity of the crystalline component and the intensity of the amorphous component to the experimental intensity curve. The crystallinity values of the samples were obtained as the ratio of intensity integrals of the crystalline component and of the studied sample. The intensity curve from which the Bragg peaks had been subtracted was used as the amorphous model intensity curve, and the intensity curve from which the amorphous model intensity had been subtracted was used as the crystalline model intensity curve.

### *Zeta Potential*

The zeta potential values of the samples were measured by a Coulter DELSA 440-analyzer (Doppler Electrophoretic Light Scattering Analyzer, Langley Ford Instruments, Amherst, Massachusetts), which measures the distribution of electrophoretic mobility by using electrophoresis and laser Doppler velocimetry. Particles moving in an applied electric field are illuminated by a laser beam, and the velocities of the particles are obtained from the Doppler frequency shifts of the scattered laser light onto 4 photodiodes, which are fixed at 4 different angles (8.6°, 17.1°, 25.6°, and 34.2°). Since zeta potential is directly related to the electrophoretic mobility of the particles, the analyzer calculates the individual potentials from the measured velocities. Measurement time in all of the analyses was 60 seconds, frequency range was 500 Hz, and measurements were repeated at least 2 times.

## RESULTS AND DISCUSSION

### *Particle Formation and Morphology*

#### *Acetone*

In the case of acetone as a cosolvent, the only successful batch was the one that contained 0.3 mL of both acetone and water, and 1.2 mL of chloroform (batch I in **Table 1**, **Figure 1A**). However, even with this batch the amount of aggregated polymer remained high. One explanation for this phenomenon is the tendency of the polymer to aggregate when the volume of the inner phase is decreased as compared with the outer phase [14].

The small volume of the inner phase (due to the increased polymer concentration and, therefore, viscosity of the inner phase) increases the particle size because of the higher number of collisions of the particles and

**Table 1.** Composition and Physical Properties of the 5 Batches

| | I | II | III | IV | V |
|---|---|---|---|---|---|
| Water, mL | 0.3 | 0.3 | 0.15 | 0.3 | 0.15 |
| Acetone, mL | 0.3 | | | | |
| Methanol, mL | | 0.3 | 0.7 | | |
| Ethanol, mL | | | | 0.3 | 0.7 |
| Chloroform, mL | 1.2 | 1.2 | 2.0 | 1.2 | 2.0 |
| Approximate mean particle size, nm | 260 | 200 | 1100 | 270 | 500 |
| Aggregated amount, % | 12 | 9 | 6 | 12 | 4 |
| Zeta potential, mV | -4.1 | -4.2 | -3.4 | -7.4 | -7.8 |
| Crystallinity, % | 41 | 41 | 59 | 30 | 44 |

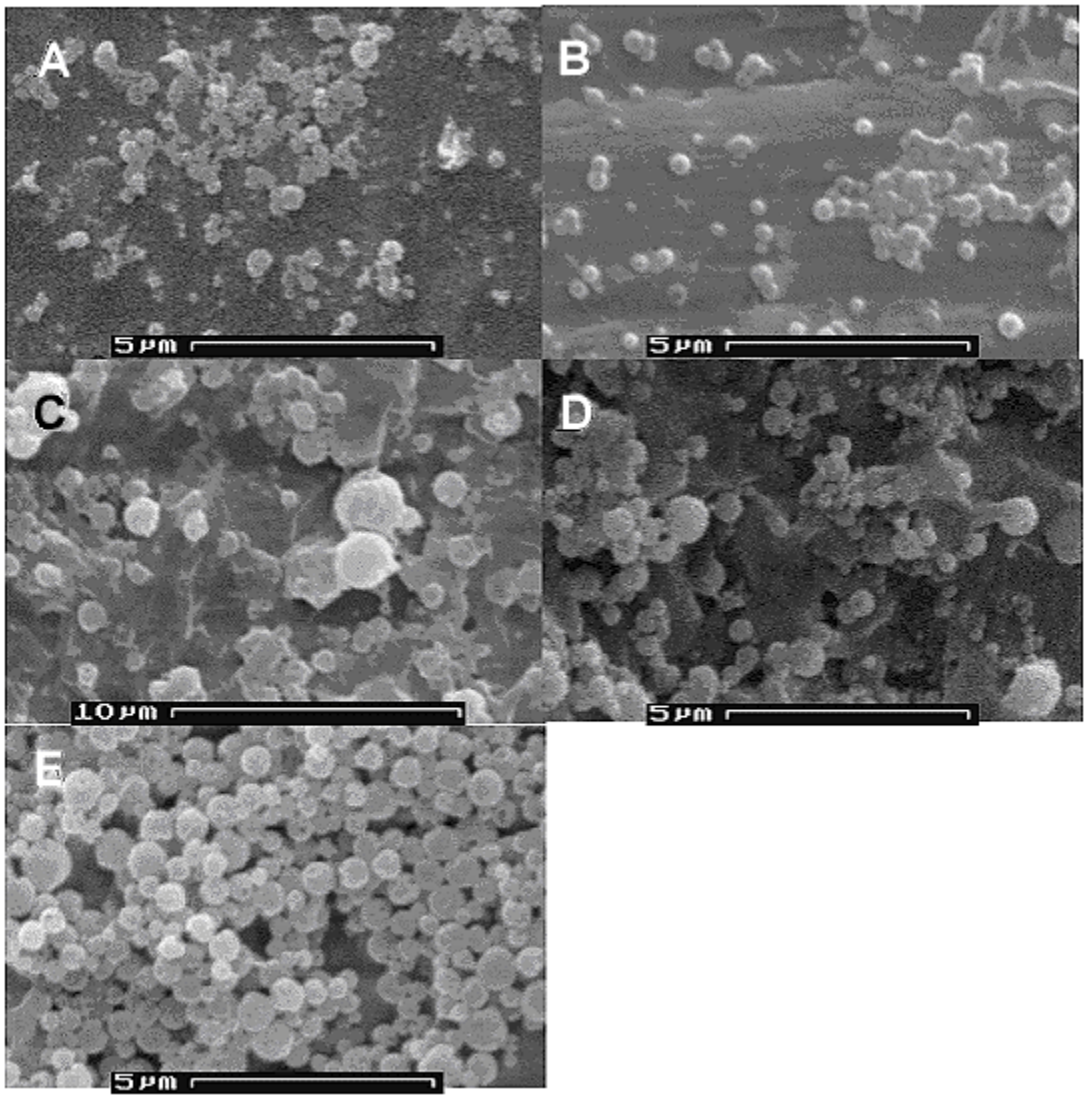


**Figure 1.** SEM photographs of nanoparticles: (A) batch I, (B) batch II, (C) batch III, (D) batch IV, and (E) batch V.

because of the fusion of the semifinished particle walls at the interface [15-17].

The optimal formulation with ethanol as cosolvent (water 0.15 mL, cosolvent 0.7 mL, chloroform 2.0 mL, batch V) was not successful with acetone because the amount of water as compared with the amount of acetone was too low to maintain the drug in a soluble form. When the amount of water was increased to 0.4 mL, the sodium cromoglycate did remain in a dissolved state, but the formation of nanoparticles was still not at a satisfactory level.

In the case of acetone, it was obvious that a high aggregation tendency was found with all the compositions tested. The smallest and near-spherical particles were formed with a low volume of the inner phase. It has been shown earlier that the mechanism of particle formation with acetone and dichloromethane as an inner phase depends on the relative volume of the solvents and independent solvent behavior [16].

With acetone alone, the rapid precipitation of PLA was the key factor, while when dichloromethane was also present, the rapid migration of acetone to the outer phase was critical for particle formation. The situation may, in part, be similar for the acetone and chloroform as cosolvents.

The viscosities of the acetone-chloroform solutions at the inner phase were very low (**Table 2**). The low viscosity may be another reason for the polymer aggregation at the interface. Probably the quasi-emulsion droplets were not stable enough, and the diffusion of acetone was too fast. Also, the polymer-acetone interactions might be problematic. Murakami et al [18] noticed that the driving solvent should be a "poor" solvent for the polymer in order to avoid aggregation. Although the solubility of poly(l)lactide in acetone is low, there might have been some polymer-acetone interactions that did not promote the precipitation of the polymer optimally.

### *Methanol*

As compared with acetone, methanol is a more polar solvent with higher viscosity (**Table 2**). Methanol also has a lower affinity to PLA than acetone. With a low inner phase volume (batch II, **Table 1**), round particles 200 nm were formed (**Figure 1B**, **Table 1**). Also, the amount of aggregated material was lower with methanol as compared with acetone.

Unlike in formulations including acetone, batch III—with a minimum amount of water and an increased amount of the driving solvent (methanol)—was somewhat successful (**Figure 1C**). Although the drug was dissolved in a very small amount of water, addition of methanol did not precipitate it. The polarity of alcohol and the capability of methanol to form hydrogen bonds increased the solubility of sodium cromoglycate, both in the water-alcohol and in the organic phases [19].

Therefore, the formed polymer-drug dispersion was clear and homogeneous. However, the formed particles were large (approximately 1100 nm, **Table 1**), with a large size distribution, and they were slightly angular as compared with the particles formed from batch II. The amount of aggregated polymer was decreased markedly, but the formed particles still seemed to be partially adhered to each other. As found in earlier studies, the relative amount of the inner phase solvent as compared to the outer phase solvent clearly affected the particle formation [20-22].

In this study, when the volume of the inner phase was increased as compared to the volume of the outer phase, the particle size was increased. The high aggregation tendency of the particles was obvious when the volume of the inner phase liquids was low. This result is clearly similar to the case of acetone: increased viscosity of the inner phase and fusion of the semifinished particles.

It seemed that the polarity of the driving solvent had no marked effect on the formation of nanoparticles. The decreased aggregation was mainly caused by the decreased affinity of the driving solvent to the polymer and, accordingly, the improved organization of the polymer chains. Also, the higher viscosity of methanol may impede aggregation by stabilizing the nanoparticles more efficiently.

### *Ethanol*

Ethanol is a less polar solvent than methanol, but the viscosity of ethanol is much higher than the viscosity of methanol or acetone (**Table 2**). Ethanol was clearly the best cosolvent in this study: the number of nanoparticles formed was the highest, and the particles were the most perfectly round. With batch IV, the size deviation still remained relatively high (**Figure 1D**, **Table 1**). It was also noticed that a few particles were not really separate but partly attached.

With batch V (**Table 1**), equally sized, round particles were achieved (**Figure 1E**). These particles were clearly separated from each other, and the surface morphology of the particles was very smooth. With this batch, the efficiency of particle formation was high, and typically over 90% of the polymer in the batch formed round particles that were not prone to aggregation. Similar to when methanol was the cosolvent, with this batch the particles were larger (around 500 nm) and the aggregated amount was lower as compared to batch IV.

When batches with a different amount of ethanol (from 0.2 mL to 1.0 mL) were prepared, it became obvious that neither the presence of ethanol in the inner phase nor the increase in the total volume of the inner phase was

**Table 2.** Physical Properties of the Solvents

| | Density, g/mL (20°C) | Dielectric Constant (25°C) | Viscosity, cP (20°C) |
|---|---|---|---|
| Water | 0.998 | 80.1 | 1.00 |
| Acetone | 0.790 | 20.7 | 0.36 |
| Methanol | 0.791 | 32.7 | 0.59 |
| Ethanol | 0.789 | 24.6 | 1.10 |
| Chloroform | 1.489 | 4.8 | 0.57 |

the key factor. Instead, the relative amounts of the individual inner phase solvents were very important. For example, when the amount of water was minimized as compared to the amount of ethanol, the drug and polymer phases formed a very stable dispersion, and it was possible to add the dispersion into the outer phase very homogeneously. Ethanol, like methanol, acts as a cosolvent that does not precipitate the drug in the inner phase even when the solvents are present at high concentrations. The special properties (high viscosity, interactions with the PLA polymer) of ethanol as a solvent played a very important role, too. When the same batches were studied with methanol as a cosolvent, the particle formation was far less effective.

The high viscosity of ethanol as compared to the other cosolvents (**Table 2**) had a positive effect on the formation of nanoparticles. This may be explained by the mutual interactions between the solvents and the polymer (PLA). The polarity of the driving solvent could not be the most important factor (ethanol has greater polarity than acetone but lower polarity than methanol, **Table 2**). More likely, the specific polymer-solvent interactions explain the observations. Murakami et al [18,23] noticed that the aggregation of poly(lactide-coglycolide) polymer was effectively disturbed by the addition of ethanol or methanol. The positive impact of alcohol was explained by the presence of alcohol in the inner phase, which speeds up the precipitation of the polymer and increases the phase separation of the polymer during the solvent diffusion. It was also noticed that the amount of ethanol needed to achieve this point was lower than with a similar amount of methanol. Ethanol is presumably a "poorer" solvent for PLA as compared to acetone or methanol, and it promotes more actively the precipitation of the polymer. In the optimal batch (batch V), the amount of ethanol in the inner phase was approximately 25%, which is quite close to the optimal value found in the study of Murakami et al [23].

The amount of methanol might need to be increased much more in order to achieve the same effect as with ethanol.

## *XRD*

**Figure 2** presents the measured XRD patterns of the samples with batches I to V. The diffraction pattern of batch I included reflections at about 4.8°, 9.5°, 12.1°, 16.7°, and 19.1° (2θ), corresponding to Bragg distances of 15.5 Å, 9.3 Å, 7.3 Å, 5.3 Å, and 4.7 Å, respectively. These reflections are near the reflections (010, -110, 020, 200, and -220) of monoclinic sodium cromoglycate [24], where a = 11.9Å, b = 15.8Å, c = 3.68Å, and β = 110°. The distances of the reflections differed from 0.1 to 0.6 Å, indicating that the size of the unit cell is not exactly similar. Similar changes in the crystal structure of sodium cromoglycate have been reported earlier with the changes in water stoichiometry [24,25].

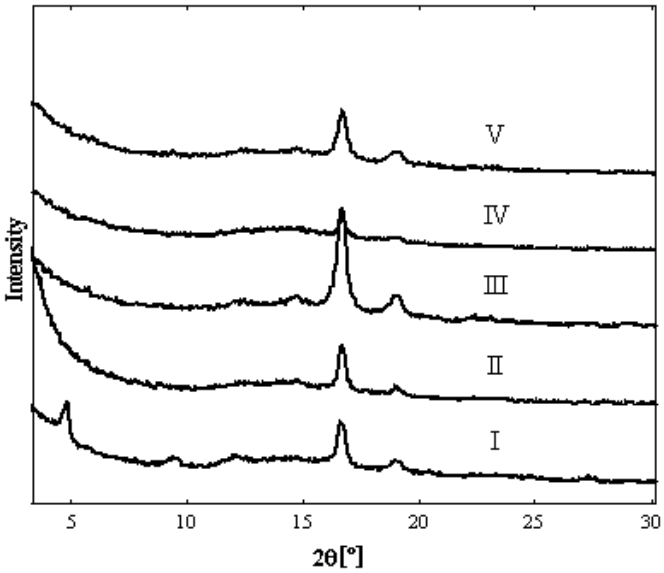


**Figure 2.** XRD patterns of the 5 batches.

The diffraction patterns of batches II and IV had reflections at about 16.7° and 19.1°. The diffraction patterns of batches III and V included these 2 reflections and weak reflections at 12.1° and 14.9°. Batch V had a weak re-

flection at 9.5°, too. The same reflections in the diffraction patterns indicate the same crystal structure. The differences between the diffraction patterns were caused by the preferred orientation of the crystals. It is common for certain crystals to grow in particular directions: obviously, this preference affects the properties of a compound. The most isotropic sample was batch I, because the diffraction pattern included most of the reflections of sodium cromoglycate. A very interesting observation is that batches II and IV as well as III and V are identical to each other with regard to the total amount of solvents. The only difference is that batches II and III contained methanol and batches IV and V contained ethanol. This observation indicates that the reflection patterns are more dependent on the amount of the solvents in the inner phase than on the properties of the individual alcohol (methanol or ethanol).

The crystallinity values of batches I through V are presented in **Table 1**. The crystallinity of batch III was the highest (59%). The crystallinity of batch V was the second highest (44%). Batches III and V contained the same amount of water and chloroform, but batch V contained ethanol and batch III contained methanol. The lowest crystallinity (30%) was in batch IV. The degree of crystallinity of the nanoparticles was the highest when the amount of water in the formulation was the lowest and when the volume of the inner phase was the highest. The most isotropic sample, batch I, was the only batch that contained acetone.

### *Zeta Potential*

The zeta potential values of the 5 batches did not differ markedly from each other (**Table 1**). The weakly negative values indicate that the PLA nanoparticles were not stabilized by electrostatic repulsion forces [26], and the relatively low values of the zeta potentials (from -3.4 to -7.8 mV) might be one reason for the high aggregation tendency of the particles. In general, the biocomponents, including plasma proteins, have a slightly negative charge [27].

Positively charged nanoparticles could, therefore, significantly interact with these proteins, which might be avoided by the negatively charged nanoparticles. On the other hand, nanoparticles with a large negative zeta potential induce unfavorable immunological reactions [27].

In this light, the weakly negative zeta potential values of the nanoparticle formulations under study were desirable.

## CONCLUSION

By the selection of the cosolvent in the inner phase, one may dramatically alter the properties of PLA nanoparticles prepared by a modified nanoprecipitation method. When acetone, ethanol, or methanol was selected as a cosolvent, the optimal particles were achieved with ethanol. When the volume of the inner phase was decreased, the particle size was also decreased, but the particles were more prone to aggregation. The solvents also have a clear effect on the crystallinity of the formed nanoparticles. The zeta potential values of all the particle batches were slightly negative, which may also partially explain the observed aggregation tendency of the nanoparticles.

## ACKNOWLEDGEMENTS

Financial support from the Finnish Technology Agency (TEKES), Focus Inhalation Inc (Finland), and Orion Pharma (Finland) is acknowledged. We thank Electron Microscopy Unit of the Institute of Biotechnology-University of Helsinki for providing laboratory facilities.

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