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Improved Entrapment Efficiency of Hydrophilic Drug Substance During Nanoprecipitation of Poly(l)lactide Nanoparticles

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ABSTRACT

The purpose of this research was to improve the entrapment efficiency of a model hydrophilic drug substance, sodium cromoglycate, loaded inside polylactic acid nanoparticles by a modified nanoprecipitation method. The effect of formulation parameters was studied to improve the entrapment efficiency of the drug substance inside the nanoparticles. Several parameters (changes in the amount of model drug, solvent selection, electrolyte addition, pH alteration) were tested in order to increase the loading of the hydrophilic drug in the hydrophobic nanoparticles. Lowering of the pH was the most efficient way to increase the drug loading; up to approximately 70% of the sodium cromoglycate used in the particle formation process could be loaded inside the particles. The loading efficiency without the pH change was around 10% to 15% at maximum. The crystallinity values and crystal habits of the sodium cromoglycate nanoparticles were studied (x-ray diffraction) before and after the lowering of the pH. The change in pH conditions during the nanoprecipitation process did not affect markedly the crystallinity properties of the drug substance. According to this study, it is possible to improve the entrapment efficiency of hydrophilic sodium cromoglycate inside of the nanoparticles by small changes in the process parameters without alterations in the physical properties of the original drug substance.

KEYWORDS: drug loading, nanoparticles, nanoprecipitation, pH, PLA (polylactic acid)

INTRODUCTION

Biodegradable polylactide (PLA) and polylactide-co-glycolide (PLGA) polymers have been intensively evaluated for the controlled release of pharmacologically active substances.[1-6] Micro- and nanoparticle formulations of these polymers have been formulated with various methodologies.[7-10] Fessi and coworkers[11] managed to efficiently capsulate drug substances inside nanocapsules by a nanoprecipitation method. Later it was observed that the entrapment of hydrophilic drug substances inside the polymer capsules is a very difficult task using the nanoprecipitation method.[8,12,13] The reason for this difficulty is that the hydrophilic drug substances have very low affinity to the polymer. In addition, the interactions between the polymer and drug are weak, and the drug substance has a tendency to move from the organic phase to the outer aqueous phase and not in the precipitating nanoparticles.

The purpose of this study was to increase the entrapment efficiency of hydrophilic sodium cromoglycate into poly(l)lactide nanoparticles. The model drug, sodium cromoglycate, acts as a preventive reducer of bronchoconstriction in the lungs. The entrapment efficiency was modified by changes in the solvent selection, the amount of the model drug substance (sodium cromoglycate), solvent selection, the pH values of the outer and inner phases, and, finally, by the addition of salt to the aqueous phases. The stability of the drug substance after the most successful drug loadings was studied by x-ray diffraction methods.

MATERIALS AND METHODS

Materials

The polymer used was PLA (MW ~100 000 g/mol, Fluka, Buchs, Switzerland). Organic solvents were chloroform, dichloromethane (DCM), acetone, methanol (analytical grade, Riedel-deHaën, Seelze, Germany), and ethanol (Ph Eur, Rajamäki, Primalco, Finland). Sodium cromoglycate (MW 512 g/mol, ICN Biomedicals Inc, Aurora, Ohio) was used as a model drug, and propylene glycol (Ph Eur, University Pharmacy, Helsinki, Finland) was used as a stabilizing agent. The water used was purified Millipore water (Millipore, Billerica, MA).

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Preparation of the Nanoparticles

The preparation method was a variation of the nanoprecipitation method[11,14,15]: the drug substance, 5 mg, was dissolved in water, 0.3 mL with acetone or 0.6 mL with methanol; then cosolvent (acetone or methanol, 1 mL) was added into this solution. A cosolvent was needed in order to make the inner phase more homogeneous. PLA, 50 mg, and propylene glycol, 50 mg, were dissolved in 4 mL of chloroform or DCM, and this solution was added into the drug solution to form a dispersion. The dispersion was added into 10 mL of aqueous ethanol solution, and the organic solvents were removed by evaporation under reduced pressure.

Characterization of the Nanoparticle Morphology

The surface morphology (roundness, smoothness, and formation of aggregates) and the size distribution of nanoparticles 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 an SEM (DSM 962, Zeiss, Jena, Germany).

Drug Entrapment Efficiency

For the drug entrapment efficiency tests, the nanoparticle suspension was divided to 4 different samples. The successfulness of the particle formation in the analyzed batches was ensured by SEM measurements. Before starting the chemical (spectrophotometric) analyses for the drug entrapment efficiency, the repeatability of measurements between different batches was ensured by repeated analyses. The total amount of the drug in the suspension was analyzed by drying the samples and dissolving the sample in 5 mL of chloroform. After the polymer was dissolved, 20 mL of water was added and the mixture was mixed carefully in a separation funnel. Thereafter, the amount of drug in the water phase was detected by a UV-spectrophotometric method at 238 nm (Ultrospec III, Pharmacia LKB Biotechnology, Uppsala, Sweden). The test was repeated with another nanoparticulate sample.

The amount of the drug in the water phase of the suspension was analyzed by filtering the nanoparticle sample through a 0.22-µm filter (Millipore) and by measuring the concentration of the drug in the filtered sample by the UV-spectrophotometric method. The test was again repeated with another sample. The amount of drug inside the particles was calculated by subtracting the amount of drug in the aqueous phase of the suspension from the total amount of the drug in the nanoparticle suspension. The entrapment efficiency (%) of drug was calculated by the following equation:

$$\text{Drug Entrapment}\,(\%) = \frac{\text{Mass of Drug in Nanoparticles}}{\text{Mass of Drug Used in Formulation}} \times 100 \qquad (1)$$

Variable-Temperature X-Ray Powder Diffraction Experiments

X-ray diffraction patterns were measured using a variable-temperature x-ray powder diffraction (VT-XRPD) (Bruker AXS D8, Karlsruhe, Germany). The VT-XRPD experiments were performed in symmetrical reflection mode with CuK_α radiation (1.54 Å) using Göbel Mirror bent gradient multilayer optics (Bruker AXS). The scattered intensities were measured with a scintillation counter. The angular range was from 10° to 25° with the steps of 0.1°, and the measuring time was 5 s/step and 10 s/step at room temperature. The measurements were performed at different temperatures ranging from room temperature to 169°C (the melting point of the polymer is ~150°C[15]) and cooling back to room temperature with the heating and cooling rates of 0.2°C/s.

Data Analysis

Crystallinity of the samples was estimated by fitting the intensity of the crystalline component and the intensity of the amorphous component to the experimental intensity curve. The crystallinity of the samples was obtained as the ratio of the integrals of the intensities of the crystalline component and the studied sample. The intensity curves of the melted samples were used as the amorphous model intensity curve, and the intensity curve, where the amorphous model intensity was subtracted, was used as the crystalline model intensity curve.

RESULTS AND DISCUSSION

Solvent Selection

The starting point from our previous studies[14,15] was that approximately 10% of the hydrophilic drug substance that was originally used in the particle formulation process could be blocked inside of the formed nanoparticles.

In previous studies (Peltonen L, Koistinen P, Karjalainen M, Häkkinen A, Hirvonen J, unpublished data, June, 2003), the authors successfully formulated PLA nanoparticles with either chloroform or DCM as the solvent for PLA (the solubility properties of the L-form of PLA restrict the selection of possible organic solvents). In this study, both chloroform and DCM were evaluated as the organic solvent for the PLA polymer. Acetone and methanol were tested as cosolvents. The authors started with a composition, where the organic

Table 1. Optimized Modifications To Increase the Entrapped Amount of Sodium Cromoglycate Inside the Poly(l)lactic acid Nanoparticles*

Modified Property		Drug Entrapped/ %	Mean Particle Size/nm
Solvents	Chloroform/acetone	11.5	470
	Chloroform/methanol	12.2	770
	DCM/methanol	14.1	780
Amount of drug as related to the amount of polymer (%)	5	16.3	700
	10	14.2	770
	15	8.4	820
	20	8.4	760
Outer phase	Outer phase 96% ethanol saturated by NaCl	13.5	1300
	1 drop of crude HCl added to the outer phase	69.6	1200

solvent was chloroform (4 mL) and the cosolvent acetone (1 mL). With this composition, the particles were round and smooth, and they were very small in size (Figure 1A, Table 1). These were desirable quality parameters for nanoparticles, but the smaller the particles the lower the entrapped amount of drug substance.[16,17] In the earlier studies, it was observed that a high amount of water also decreased the amount of drug entrapped.[16-18] For this reason, the entrapment efficiency of sodium cromoglycate was slightly lower with acetone than with methanol (Table 1), although the difference was small.

With acetone, the amount of water needed to be kept at a considerably higher level as compared with methanol because of the tendency of drug substance to precipitate in the presence of acetone with lower amounts of water. With methanol, the amount of water could be as low as 0.3 mL (out of a total volume of 5.3 mL of the inner phase), which was not possible with acetone (sodium cromoglycate was precipitated). With chloroform/methanol the particles were nicely round shaped, and the size deviation was narrow, but the drug entrapment efficiency was slightly lower than with DCM/methanol (Figure 1B, Table 1). The main reason for this difference was probably the decreased diffusion of chloroform to the outer phase as compared with DCM. DCM is slightly more hydrophilic than chloroform (DCM is soluble in ~50 parts of water; chloroform is soluble in ~200 parts of water), and because of the faster diffusion to the outer phase, the precipitation of the polymer with DCM is faster.[19] The interfacial precipitation should be fast enough to block efficiently the drug substance inside the forming nanoparticles.[19-21]

Because the combination of DCM and methanol gave the highest entrapment efficiencies, the following studies, if not otherwise stated, were performed using DCM and methanol as the (co-)solvents (Figure 1B).

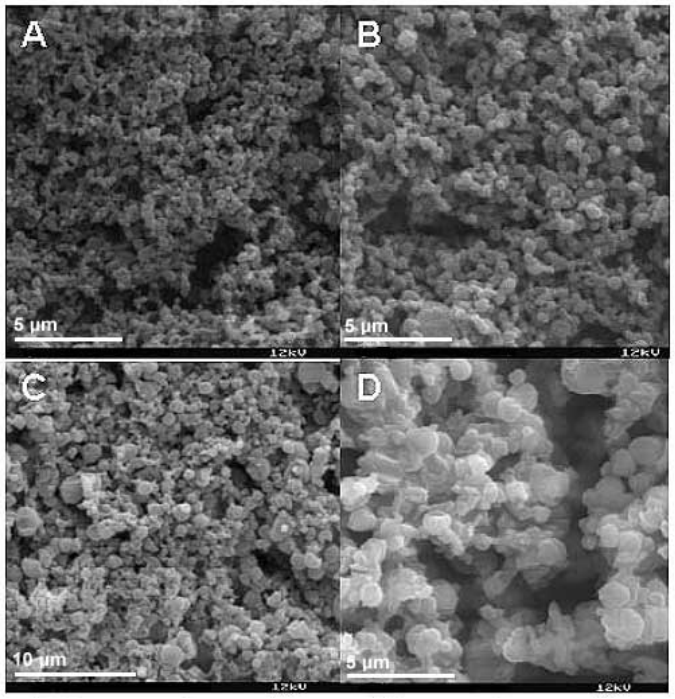

Figure 1. SEM photomicrographs of nanoparticles: (A) batch with chloroform and acetone as solvents, (B) batch with DCM and methanol as solvents, (C) batch with NaCl addition during the particle formation process, and (D) batch with HCl addition during the particle formation process.

Amount of Drug Substance

The effect of the proportional amount of drug substance on the entrapment efficiency was studied by varying the amount of sodium cromoglycate (as compared with the amount of the polymer) between 10% and 40% (wt/wt). The organic phase consisted of DCM and methanol (Table 1). The amount of the polymer was kept constant (50 mg). The drug entrapment (Equation 1) inside the particles decreased

as the relative amount of the drug substance was increased (Table 1). This observation may be explained by the fact that the increased amount of model drug formed a more porous polymer-matrix structure: inside the polymer core there were large channels and hollows filled by the drug substance, through which the drug could easily escape to the outer phase.[22] Also, because of the increased drug concentration inside the nanoparticles, the osmotic pressure difference between the outer and inner phases changed; this might cause some damage to the formed quasi-emulsion droplets and, again, make it easier for the drug substance to escape from the inner phase.[23]

Several molecules may also disturb the interfacial precipitation of the polymer; hence the polymer core does not prevent the drug from escaping the inner phase.[13] With higher amounts of the drug substance, particle aggregation was observed from the SEM figures (data not shown). This result was probably caused by the mechanical defects (the roundness of the particles was lost due to the more porous polymer matrix or due to the channel formation) in the particles.[22,24]

In the following analyses (alterations of the properties in the inner and outer phases) the relative amount of the drug substance as correlated to the amount of the polymer was 1:10.

Properties of Inner and Outer Phases

Salt Addition

Addition of an electrolyte affects the osmotic gradient between the inner and outer phases, and this may have an impact on the drug entrapment.[17,25] In this study, sodium chloride was first added to the inner phase to make the sodium cromoglycate less soluble. Also, the properties of the outer phase were affected by adding sodium chloride to the outer phase. By the salt addition, the osmotic gradient was also altered. However, the addition of sodium chloride to the outer phase was problematic, because sodium chloride is poorly soluble in the 96% ethanol. For this reason, the ethanol solution was saturated by the sodium chloride. The salt addition slightly improved the entrapment efficiency of the drug substance; the best effect was achieved when the salt was added both in the inner and outer phases, although the salt concentration in the inner phase needed to be kept considerably low (<0.1 M) in order to avoid the precipitation of the sodium cromoglycate (Table 1).

As the sodium chloride was added to the formulation, a higher amount of large particles was formed (Figure 1C). Earlier particle formulations with a water/oil/water-double emulsion technique have shown that the particle size was decreased by the addition of salt because of the decreased osmotic pressure gradient.[18] According to our results, in the nanoprecipitation method, the pressure difference between the outer and inner phases was not particularly important for the drug entrapment efficiency. Instead, the addition of the salt altered the flow mechanics at the interfacial area and, because of this, the stability of the quasi-emulsion droplets was decreased, the particle size increased, and the solvent diffusion altered.

From the SEM figures (Figures 1B and C), an increased aggregation of the particles was noticed after the addition of sodium chloride. The addition of salt has, thus, a most marked impact on the particle surfaces, but the entrapment efficiency of hydrophilic sodium cromoglycate in PLA nanoparticles could not be markedly improved during the nanoprecipitation process.

Inner and Outer Phase pH

The pH of the water phase affects the ionization of the drug substance and, hence, the solubility. An ionic drug substance is prone to stay in the water phase, while the molecular form is more likely to be attached to the hydrophobic polymer phase, and, in this case, the drug substance is more efficiently nanocapsulated. Based on this finding, by simply adjusting and controlling the pH-value, the entrapment efficiency of drugs inside nanocapsules can be increased.[13,26]

Sodium cromoglycate was in an ionic form at the pH of purified water (~pH 6). In the ionic form, sodium cromoglycate is more soluble as compared with the molecular form and easily escapes to the aqueous phase. Ionic sodium cromoglycate is negatively charged, and this negative charge may cause repulsion forces between the drug molecule and the negatively charged carboxylic acid ends of the PLA molecules. Hence, a relatively low entrapment efficiency of the sodium cromoglycate was expected.

The pH changes in the outer phase seemed to have a more pronounced effect on the drug entrapment than those in the inner phase. The pH of the outer phase was changed by adding a few drops of crude HCl (37%) to the ethanol phase. When 1 drop of crude HCl was added to the outer phase, the pH of the aqueous ethanol was lowered from 7.7 to 1.2. When another drop was added to the outer phase, the pH was not lowered dramatically anymore; the measured pH was 0.9. The best results, up to 70% (69.6% ± 0.3%) drug entrapment inside the PLA nanoparticles, were achieved by adding a single drop of HCl (Table 1). The addition of a larger amount of HCl did not increase the entrapped amount of drug; instead the nanoparticles tended to aggregate even more.

Based on the SEM figures (Figure 1D), at pH 1.2 the particles were more aggregated, and the particle size was clearly increased (1200 ± 200 nm) as compared with the original

composition (Table 1). The increased tendency for aggregation is typically caused by a lowered degree of ionization of the free carboxylic acid ends of the polymer as the pH is lowered.[13] In neutral pH, these groups are negatively charged, but when the pH is lowered, the number of charged groups is decreased and the electric repulsion forces are also decreased.

VT-XRPD Results

The most successful method (pH reduction by the addition of HCl) to increase the drug entrapment efficiency was studied further. The influence of HCl addition (pH changes) to the crystallinity of the sodium cromoglycate and the PLA nanoparticles was analyzed by VT-XRPD measurements. Figure 2 presents the measured VT-XRPD diffraction patterns of nanoparticles as a function of temperature. At room temperature, the diffraction pattern included clear reflections at approximately 16.7° and 19.1° (2θ), corresponding to Bragg distances of 5.3 Å and 4.7 Å, as was also the case in our earlier study with the sodium cromoglycate nanoparticles without any alterations in pH.[14]

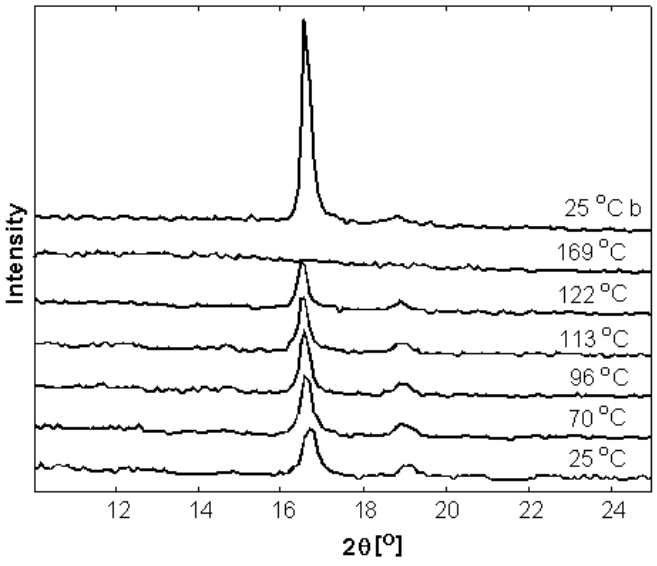

Figure 2. VT-XRPD graphs of nanoparticle batch with HCl addition at different temperatures during the heating and after the cooling (the uppermost curve, marked with letter "b").

The diffraction pattern of nanoparticles at different temperatures is presented in Figure 2. The diffraction patterns included the same reflections of 200 and -220 up to the temperature of 122°C, indicating the same crystal structure. The reflections of these diffraction patterns moved to the left as a function of temperature. This finding indicates that the distances of the Bragg planes were increased due to the heating. However, the maximum change in the distances was only 1 nm. The reflections disappeared and the samples were changed into a totally amorphous form at the temperature of 169°C, where the PLA polymer had been melted. The diffraction pattern showed the reflection at approximately 16.7° after the sample was cooled back to room temperature. Also the weak reflection at approximately 19.1° was present after cooling, indicating that the sample was recrystallized back as the same crystal structure. The reflections also moved back to starting positions, to the starting distances of the Bragg planes.

Table 2 presents the crystallinity values. Heating the sample to 122°C did not change the crystallinity values, but after the melting and cooling back to room temperature, the samples recrystallized with a higher value of crystallinity. This result probably means that the HCl or PLA and sodium cromoglycate were cocrystallized during the cooling process, so that after the melting, the total amount of crystalline material stayed at a higher level as compared with the unmelted nanoparticle sample.

Table 2. Crystallinity Values at Different Temperatures

Temperature/°C	Crystallinity/%
25	55
70	53
96	54
113	54
122	56
169	0
25	72

CONCLUSION

Nanoprecipitation method has its limits on efficiently loading hydrophilic drug substances inside the nanoparticles. However, by lowering the pH of the outer media, the drug entrapment efficiency of water-soluble sodium cromoglycate was increased from 10% to 15% to as high as a level of approximately 70%. X-ray diffraction measurements ensured the stability of the drug substance after the alterations in pH.

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