Maltodextrins as drying auxiliary agent for the preparation of easily resuspendable nanoparticles

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Abstract:

The drying of biodegradable polymeric nanoparticles (NP) is mandatory to improve their physical and chemical stability over time. Spray- or freeze-drying can induce irreversible aggregation of NP and therefore the use of drying auxiliary agents is required. The ability of four grades of maltodextrins differing in dextrose equivalent (DE) (*i.e.* DE2, DE6, DE12 and DE38) to protect PLGA NP from stresses was studied. High M_w maltodextrins (DE2) was not functional for obtaining an easily resuspendable dried product, since it needs a prolonged time to fully hydrate. Maltodextrins at intermediate DE showed a poor ability to protect NP from irreversible aggregation probably because too sensitive to environmental variation. DE38, which did not alter ζ-potential of NP, allowed to obtain an easily resuspendable nanosuspension independently of the drying process. The effectiveness of such material was attributed to the easiness of spray-dry a low viscous solution and to the ability of substitute the water molecules' hydrogen bonds with NP during freeze-drying.

Keywords:

freeze-drying; maltodextrins; nanoparticles; resuspendability; spray-drying.

1. Introduction

One of the main obstacles limiting the availability of products based on hydrophobic colloidal suspensions is their physical and chemical instability upon long-term storage. Thus, it is mandatory to convert these systems into solids which can be easily and rapidly reconstituted preserving the initial physicochemical characteristics of the product. Among the possible drying processes, spray-drying and freeze-drying are the methods of choice [1-5], even if both processes present serious criticisms when applied to polymeric nanoparticles [6, 7] made of materials with a low melting point and/or glass transition temperature. As an example, in the case of poly(caprolactone) nanoparticles, the exposure to a high temperature during spray-drying can be prohibitive since it can jeopardize their structural integrity, leading to degradations or coalescences, despite the short residence time in the drying chamber [7]. Regarding the lyophilization, freezing is considered the most aggressive and critical step since it can induce the nanoparticles instability. The freeze-concentration and the formation of ice-ice and ice-container interfaces caused spatial confinement of nanoparticles and the mechanical compression can damage the susceptible ones [8]. The freezing can also modify the physical state of the material constituting the nanoparticle affecting the integrity of the system. As an example, the freezing of a poly(D,L lactide acid-co-ethylene oxide) colloidal system induced the crystallization of the polymer with the formation of intra- and inter-particle bridges causing irreversible aggregation [9]. Moreover, *in vivo* pharmacokinetic study indicated that the particle size increment from 100 to 160 nm due to freeze-drying of poly(lactide-*co*-glycolide) (PLGA) and poly(caprolactone) nanoparticles induced a larger residence time and higher drug hepatic levels of CyA loaded as a model drug [10].

Difficulties in drying nanoparticles made of amorphous polymers are also dependent on the chemical affinity with the water molecules which can be responsible of polymer plasticization. The consequent decrease of the glass transition temperature (T_g) of the system causes processing problems, namely particle agglomeration, caking and stickiness. As an example, the moisture content can reduce the T_g of PLGA from about 45 °C to about 15 °C [11, 12]. Hence, the optimization of the formulation and the experimental conditions of both processes is crucial to obtain a dried product with the desired qualities and preserve the original physicochemical properties of nanoparticles. Indeed, the nature of drying auxiliary agents as well as the concentration play an important role to mitigate thermal stresses and assure a fast resuspension [8, 13], avoiding the use of a high-energy process to properly disperse nanoparticles, which precludes the practical utility in clinical settings.

Maltodextrins (MDX) appear especially useful to this purpose due to their good aqueous solubility, low viscosity and high T_g which provide a stable glassy matrix at room temperatures [14].

Based on these considerations, this work aims to evaluate the ability of four types of MDX differing in molecular weight on protecting poly(D,L lactide-*co*-glycolide) nanoparticles from thermal and mechanical

stresses induced by spray-drying or freeze-drying. In this regard, an uncapped poly(D,L lactide-*co*-glycolide) with a low T_g was selected as critical material and nanoparticles were prepared by the solvent displacement method avoiding the use of any stabilizer, which can interfere with the effect of MDX.

2. Materials and method

2.1. Materials

Uncapped poly(lactide-*co*-glycolide) (PLGA) at lactide/glycolide ratio of 50:50 and having a T_g at about 36.5 °C was purchased by Evonik Industries (G). MDX with a dextrose equivalent (DE) of 2, 6, 12 and glucose syrup with a DE38 (Glucidex®) were kindly gifted by Roquette (F). All solvents were of analytical grade, unless specified.

2.2. MDX characterization

Size exclusion chromatography (SEC) was performed using an HP1100 Chemstation (Agilent, US) equipped with refractive index signal as a detector. A combination of two columns in series, Superchrome Biobasic SEC300 (300×7.8 mm, 5µm, 300 Å) and 120 (300×7.8 mm, 5µm, 120 Å), was operated at the flow rate of 0.4 mL/min and temperature of 35 °C. Samples at the concentration of 5 mg/mL were eluted using a mobile phase constituted of a 0.05 M phosphate buffer at pH=6.8 and a 0.25 M KCl solution and the injection volume was 50 µL. The weight- average molecular weight (M_w) and the number-weight molecular weight (M_n) was calculated using dextrans as standards, in the range from 1-410 KDa. Dispersity index (DI) is reported as the ratio between M_w and M_n.

Modulated differential scanning calorimetry experiments were performed using a DSC1 Star^e System (Mettler Toledo, CH) equipped with a refrigerated cooling system (RCS) to determine the T_g of MDX. Samples of about 10-15 mg exactly weighted were transferred to pin-holed aluminium pans, sealed and subjected to heating cycles from 10 to 100 °C, 150 °C or 200 at °C at the 5 K min^{-1} (period = 90 s; amplitude = 0.5 °C). The temperature range was fixed based on preliminary DSC analysis.

The DSC cell and RCS were purged with dry nitrogen at 80 and 120 mL/min, respectively. The system was calibrated using an indium standard. All data were treated with Star^e System software (Mettler Toledo, CH) and T_g is calculated as the inflection point in the reversible curve.

Dynamic light scattering analyses (section 2.7) were performed on MDX solutions at 4 and 8% w/v, following the sample preparation as per the compatibility study (section 2.4). Size distribution plots were further analysed using the high-resolution mode to resolve multimodal or broad peaks.

2.3. Nanoparticles preparation

PLGA nanoparticles (NP) were prepared using a solvent displacement method [15]. Briefly, PLGA was dissolved in a mixture of acetone/absolute ethanol (7:3 v/v) at the concentration of 1% w/v and 1 mL was

added dropwise to 10 mL of MilliQ® water, using an electronic pipette (PIPETMAN M® - Gilson, US). The system was maintained at 4 °C under magnetic stirring at 500 rpm for 15 min before increasing the temperature to 25 °C over a 3h period.

2.4. Compatibility

An aliquot of 1 mL of MDX solution in MilliQ® water or 0.9% NaCl solution was added to 1 mL of nanosuspension to get the final MDX concentration of 2, 4 or 8 % w/v. After stirring for 3 h at room temperature, the samples were visually observed, and the particle size was determined as reported in section 2.7. The zeta potential (ζ) values were measured only for samples at 2% MDX concentration to limit interferences on electrophoretic mobility.

2.5. Spray-drying

Nanosuspension in presence of MDX was sprayed through a two-fluid nozzle, operating in a co-current manner, of a Format 4 M8 (ProCepT, Belgium). The process parameters optimized according to the results of the Design of Experiments [16] were set as follows: inlet temperature= 130 °C; feed flow rate = 6.5 mL/min; nozzle pressure = 1.7 atm; nozzle diameter = 0.4 mm; ΔP = 70 mbar. The dried powders were separated from the drying air in the cyclone (outlet temperature = 37–39 °C) and deposited in the collector.

2.6. Freeze-drying

Freeze-drying was performed using an Epsilon 2-6 LSC plus freeze dryer (Martin Christ, G).
To tailor the experimental set-up [17], the glass transition temperature of maximally cryo-concentrated solution (T_g') of a 20% w/v MDX solution was determined by DSC. Briefly, an aliquot was cooled until -40 °C at 5 K min^{-1}, kept at -40 °C for 20 min and then heated to 25 °C at 5 K min^{-1}. T_g' was calculated as the inflection point of the specific heat increment at the glass–rubber transition on the heat scan.
The influence of NP on ice melting enthalpy (ΔH) of MDX solutions was evaluated by modulated differential scanning calorimetry, heating the frozen samples from -40 °C to +25 °C (period = 90 s; amplitude = 0.5 °C).
Two different freeze-drying cycles were designed based on T_g'. In particular, samples containing DE2, DE6 and DE12 were frozen at -30 °C for 2 h; then, the main drying was carried out at 10 °C and 0.22 mbar for 6 h and the secondary drying at 35 °C and 0.22 mbar for 4 h. In case of DE38, the experimental conditions were: freezing at -40 °C for 2 h; main drying at 0 °C and 0.10 mbar for 6 h; secondary drying at 35 °C and 0.10 mbar for 6 h. Afterwards, the vacuum was broken by air injection and samples were stored at room temperature until reconstitution.

2.7. Dynamic light scattering

The Z-average diameter (D_H) and the size distribution (PDI) of samples were evaluated by photon correlation spectroscopy using a dynamic light scatter (DLS, Zetasizer Nano ZS, Malvern Instrument, UK),

equipped with a backscattered light detector, operating at 173° and 25 °C. The results calculated using the Dispersion Technology Software (Malvern Instruments, UK) are reported as intensity distribution.

2.8. Zeta potential

Zeta potential (ζ) of nanosuspension was assessed by M3-PALS (Phase Analysis Light Scattering) technique, using Zetasizer Nano ZS (Malvern Instrument, UK) at 25 °C.

2.9. Resuspendability

An aliquot of 1 mL of MilliQ® water or 0.9% w/v NaCl was used to reconstitute 5 mg of the dried powders. Samples were gently shaken at 100 rpm and 25 °C in a benchtop incubation shaker (Sartorius Certomat IS, G) and the size distribution was evaluated by DLS after 5, 30 and 60 min.

MDX were considered suitable drying agent if the quality of DLS measurement resulted good and D_H (expressed as peak size) of the reconstituted NP was not significantly different with regards to the values from the compatibility study (α=0.05, ANOVA One-way). For this purpose, the size of the main peak was considered, accounting also for its percentage on the whole size distribution.

The ζ of NP reconstituted in water without signs of aggregation was also evaluated after 60 min of shaking.

3. Results and discussion

3.1. Characterization of MDX and evaluation of their physical compatibility with PLGA NP

Molecular weight distribution and thermal properties are two of the main parameters that can potentially impact the ability of MDX to act as a drying agent. The physicochemical features of the selected MDX are summarized in **Table 1**. According to the literature data, M_n and M_w of MDX decreased increasing the DE value [18], following an exponential relationship (R^2>0.99). A similar dependence was found in the case of T_g: in a homologous polysaccharide series with different M_n, the decreased length of polymeric chains, namely the increased concentration of reducing sugars in MDX, determines the transition from glassy to rubbery state at lower temperatures, compared to high M_w products [19]. Moreover, the change in heat capacity associated with the T_g (ΔC_p, **Table 1**) was related to DE [20]: the higher the M_n, the smaller the ΔC_p. No statistical differences between ΔC_p of DE6 and DE12 were found, in agreement with the similarity in terms of T_g and M_n (**Table 1**).

PLGA NP were prepared avoiding the use of surfactants or steric stabilizers which can generally remain adsorbed onto NP surface [21] and cooperate to preserve NP size during drying [22]. The selected process conditions allowed to obtain monodispersed PLGA NP (PDI = 0.059±0.012, n=3 batches) with a mean

hydrodynamic diameter lower than 170 nm (D_H = 157±7 nm, n=3 batches) and a ζ-potential of about -31 mV.

PLGA NP were compatible with all aqueous MDX solutions as no aggregates or phase separations were evident after 3h of stirring. However, for MDX concentration higher than 2%, DLS analyses evidenced a slight increment of NP D_H as a function of the MDX grade and concentration. This was concomitant to a shift of ζ-potentials towards higher values and was due to the adsorption of MDX on NP surface (**Table 2**). The change of D_H became more evident increasing the ionic strength of the dispersant medium. PLGA NP as such were physically stable in 0.9% NaCl solution, but adding MDX, only DE2 and DE38 at all the concentrations tested, were able to maintain an acceptable monodisperse distribution of NP, even if D_H increased (**Fig. 1**). At intermediate DE values, the formation of aggregates was dependent on MDX concentration. In other words, DE6 was compatible with NP only at 2%, despite the significant increase of D_H, and the same behaviour was evident in case of 4% DE12 solution. Furthermore, increasing the DE12 concentration to 8%, D_H of PLGA NP shifted to the submicron range (**Fig.1**).

This data can be explained considering that the apparent persistence length of hydrated MDX linearly increases as a function of DE, until a maximum value is reached around DE10 and, then, it decreases by increasing the de-polymerization degree of MDX [23]. This would explain the relative lower ζ-potential values of DE6 and DE12 with respect to those measured after adding DE2 and DE38. Moreover, it is also recognized that the apparent persistence length of high-M_w polysaccharides is influenced by the increase of ionic strength, which modified stiffness and the conformation shape of MDX [24]. Moreover, sodium chloride, which it is known to compress the electrical double layer at the NP surface in a concentration dependent way and to reduce the ζ-potential [25, 26].

In other words, the PLGA NP aggregation might be related to mobility of polysaccharide chains which also exert a slight impact on ζ-potentials shielding (**Table 2**). . DLS analyses performed on MDX dispersion in water or 0.9% NaCl supported this hypothesis. Indeed, DE6 and DE12, due to their limited capacity to form ordered structures [28], generated clusters which underwent to reorganization at high ionic strength increasing their size (**Fig. 2b-c**). Conversely, DE2, because of the ability to form "*helical coils*" [24-27], segregated in clusters stable to environmental variations, such as ionic strength, (**Fig. 2a**) Finally, DE38 is too small (M_n ~ 1500 Da) to form clusters in agreement with the lack of any populations detectable by DLS (data not shown).

Considering that the irreversible aggregation of PLGA NP depended on the DE6 and DE12 concentration, their potentiality as drying auxiliary agent became less relevant for the purposes of this study. In general, PLGA NP/MDX samples, which underwent an increase of D_H higher than 300 nm in water or sodium chloride solution, were not worthy of further investigation.

3.2. Drying of nanoparticles

3.2.1. Spray-drying

The performances of 2% MDX solutions were tested using the optimized conditions [16]. In all the cases, the yield ranged from about 50 to 75 % and the outlet temperature was around 40 °C, not exceeding the T_g of raw PLGA (T_g ~36.5 °C).

The data of reconstitution in water evidenced that the higher the DE value, the faster the recovery of the original NP D_H (**Fig. 3a**): only DE38 allowed to obtain a monodispersed distribution after 5 min; DE12 and DE6 based solids required 30 min and 60 min, respectively; whilst DE2 was unable to guarantee the formation of a stable colloidal system.

This different ability can be related to the MDX viscosity which controls the NP motion towards the surface of the droplets during drying. Hence, it can be assumed that DE2 slowed water evaporation and diffusion rate of PLGA NP within the droplets which were slowly transported by means of convection flow. Thus, the probability of interactions among constrained NP increased, causing the formation of irreversible aggregates. In case of DE38 the possibility of NP rearrangement was limited by the almost instantaneous drying which assured that NP would remain separated as in the feed. This hypothesis is in line with MDX M_w and the time needed for a complete NP reconstitution: DE2 promoted NP aggregation, while DE6 and DE12 favoured the dispersion of NP over relatively prolonged time, according to their M_w, and DE38 allowed the fastest recovery of NP size.

Regarding the ζ-potential, the values in presence of DE12 and DE6 were superimposable to those measured during the compatibility study (**Table 2**); meanwhile in presence of DE38 (ζ = -28.9±3.9 mV) the value was not statistically different compared to NP as such (t-test $p > 0.05$).

Furthermore, DE38 was the only MDX that permitted to re-suspend NP also in 0.9% NaCl within few minutes (**Fig. 3b**).

These results confirmed that the formation of MDX clusters had a detrimental effect on the reconstitution of spray-dried NP. Therefore, MDX at higher concentrations were not tested.

3.2.2. Freeze-drying

A DSC investigation was preliminary carried out to determine the thermal properties of MDX solutions and, consequently, to establish the optimal set-up of the freeze-drying conditions.

For all MDX solutions at 20% w/v, two thermal events were observed scanning the sample from -40 °C to 25 °C: an inflection point on heat flow signal attributed to the T_g' of maximally cryo-concentrated solution and an endotherm related to the melting of frozen water (T_m'). As expected, the higher the MDX molecular weight, the higher the T_g' [29, 30] and the higher T_m' [31] (**Table 3**).

Typically, T_g' is used as a reference for designing both freezing and primary drying steps of freeze-drying process, since this value represents the temperature at which the system undergoes to drastic changes in

viscosity, heat capacity and molecular mobility as the glassy matrix is formed. Changes in heat capacity ($\Delta C_p'$) at T_g' also permitted to evaluate the temperature dependence of MDX molecular mobility. Among all tested materials, the lowest $\Delta C_p'$ of DE38 indicated the formation of the strongest glass due to the limited chain mobility during the transition, which also occurred at the lowest temperature (**Table 3**) [32].

The influence of PLGA NP on the thermal properties of MDX solutions was relevant, despite they accounted for the minority of the formulation. Indeed, the dispersed solid caused the broadening of ice melting peaks, due to the possible structural heterogeneity of nucleation, with a concomitant variation of ice melting enthalpy values [$\Delta(\Delta H_m)$]. This feature can be attributed to the formation of hydrogen bonds among PLGA and water molecules which could increase the amount of unfreezable water [12]. Interestingly, $\Delta(\Delta H_m)$ decreased concomitantly to MDX M_w, suggesting that the colloidal system caused a great deal on instability in fragile glasses which are probably more sensitive to the variation of viscous flow generated by the increase of unfrozen water. This observation agreed with the appearance of cakes obtained after freeze-drying 2% MDX solution with or without NP added. As exemplified in **Fig. 4**, DE2 based cakes collapsed only in presence of NP, while DE38 did not show visible defects.

However, it is noteworthy that the loss of structure of the DE2, DE6 and DE12 dried products was not just a cosmetic issue, as NP massively aggregated after reconstitution in water and NaCl solution. Conversely, at the 2% concentration, DE38 permitted the formation of an elegant cake, but the reconstituted samples in water and 0.9% NaCl presented a too high polydispersity (**Table 4**).

Regarding DE2 based cakes, only the increase of concentration to 8% permitted to obtain an elegant solid, but after 5 min of reconstitution the NP size increased up to about 350 nm and 500 nm in water or 0.9% NaCl, respectively (**Table 4**). DE12 matrix obtained from a 4% solution showed a good aesthetic but, again, the reconstituted sample was too dispersed, independently of the media and the time points considered (**Table 4**).

Eventually, DE38 at 4% concentration was able to avoid size variation (at all the time points considered, $p>0.05$) with the respect of reference sample (**Table 4**). Moreover, the decrease of ζ (ζ=-47.4±1.1 mV) reflected the good stability of this reconstituted sample also in 0.9% NaCl ($D_H \sim 200$ nm at 5 min, $p>0.05$ at all the time points considered).

Generally speaking, the stabilization of NP during lyophilization can be driven by two main mechanisms occurring concomitantly [33]. First, the “vitrification hypothesis” suggests that glassy matrices formed by amorphous protectants (*i.e.* saccharides) upon freezing allow the immobilization of NP preserving them from detrimental effects of ice crystals. Secondly, the “water replacement theory” proposes that the hydrogen bonds between water and NP are replaced by similar interactions occurring onto NP surface with the adsorbed excipient, thus avoiding particle aggregation or fusion.

Among the selected MDX, DE38 is a glass former material which was demonstrated to remain adsorbed to PLGA NP despite the environmental condition as both the size and the ζ-potential values of NP slightly

increased. Hence, its ability to protect NP during lyophilization can be ascribed to the concomitant occurrence of both mechanisms.

3. Conclusions

The overall data suggest that DE38 can be proposed to maintain PLGA NP stable during spray- and freeze-drying and to assure the aqueous stability after reconstitution. It should be underlined that such material was effective at low concentrations for both drying processes. This advantage is of relevance in spray-drying since it was possible to limit the amount of DE38 required to obtain an easily resuspendable nanosuspension, using a technique which permits to reduce the time and the operation costs with respect to lyophilisation [34].

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Conflict of interest

The Authors declare no conflict of interest.

Figure captions

Fig. 1 – Compatibility of PLGA NP, expressed as hydrodynamic diameter (D_H, nm) and PDI, with solutions of MDX at different concentrations (%, w/v). Compatibility results of NP with 4 and 8% of DE6 are not reported since macroscopic aggregates were evident, and the samples were not suitable to the DLS measurement.

Fig. 2 – High resolution size distribution plots of (a) 4% DE2 and (b) DE6, and (c) 8% DE12 in water (dark grey lines) and 0.9% NaCl (light grey lines).

Fig. 3 – Hydrodynamic diameter (D_H) of PLGA NP in presence of 2% MDX before spray-drying (t = 0) and after 5, 30 and 60 min of gentle reconstitution in(a) water and (b) 0.9% NaCl.

Fig. 4 – 2% MDX DE2-based freeze-dried product (a) without and (b) with PLGA NP added.

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