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Research Paper

Bead layering as a process to stabilize nanosuspensions: influence of drug hydrophobicity on nanocrystal reagglomeration following in-vitro release from sugar beads

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Abstract

Objectives In this article the feasibility of fluidized bed bead coating of nanosuspensions of drugs with significantly different physicochemical properties was investigated as a process to transform nanosuspensions into a solid dosage form. The second aim was to see how those physicochemical properties affect the coating process and the subsequent in-vitro dissolution process.

Methods Naproxen and cinnarizine were used as model drugs. A fluidized bed pellet coater with Würster insert was used to coat the nanosuspensions prepared by media milling on sugar beads.

Key findings Bead layering of cinnarizine nanosuspensions resulted in a complete dissolution in 15 min, compared to only 11% in 1 h for the unmilled powder. Naproxen also dissolved three times faster when formulated on a bead. A difference could be observed between naproxen and cinnarizine. Cinnarizine nanocrystals reagglomerate when released from the coating, resulting in a slower release when compared to the original nanosuspension. No agglomeration and no delay could be observed for naproxen. These differences are most likely caused by the difference of surface hydrophobicity between naproxen and cinnarizine.

Conclusion This study confirms that bead layering is a valuable drying technique that could complement spray drying and freeze drying, but more important is that we prove that drug physicochemical properties have a significant influence on in-vitro dissolution performance after bead layering and this is not readily predictable from the information obtained from the original nanosuspension itself.

Keywords bead layering; cinnarizine; coating; nanosuspension; naproxen

Introduction

In recent decades the screening for new active pharmaceutical ingredients (APIs) has lead to the discovery of new drugs with very high affinity and specificity for their targets. New APIs tend to have a higher molecular mass and a high degree of hydrophobicity, which makes them less water soluble.[1] Up to 40% of newly discovered APIs have a solubility problem.[2] Several formulation strategies have been developed to address low solubility/dissolution rate. Some well-known examples for oral drug delivery are the formulation of solid dispersions, self-microemulsifying drug delivery systems and micronization/nanonization.[3–5] The use of nanocrystals as a way to formulate poorly soluble drugs has matured rapidly in recent years with new formulations coming onto the market.[6]

The oral bioavailability of poorly soluble drugs is improved due to the higher solubility and dissolution rate of the nanocrystals compared to the coarse drug particles.[7] The increase in dissolution rate is the result of the high surface area of the nanocrystals. The higher solubility also has an additional positive effect on the dissolution rate. Other positive effects of the reduction in size are a reduced influence of the fasted/fed state after intake[8] and less gastrointestinal irritation, as reported for NSAIDs like naproxen.[9]

Nanosuspensions can be produced using two distinct strategies: a top-down strategy and a bottom-up strategy.[7] Examples of the top-down method are media milling or the use of high-pressure homogenization. The other strategy is to grow small crystals from solution to

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avoid a subsequent size reduction step. The fact that nanocrystals are always produced in suspension has some drawbacks, for example the API could be prone to chemical degradation.[10] On the other hand, particles can grow over time due to Ostwald ripening, which is the growth of the big(ger) crystals while the smaller ones dissolve. This phenomenon is driven by the fact that small crystals are more soluble than big ones, as is shown by the Ostwald–Freundlich equation:[7]

$$\ln(S/S_0) = (2v\gamma)/(rRT) = (2M\gamma)/(\rho rRT)$$

This equation assumes spherical particles: r is the particle radius, v is the molar volume, ρ is the density, γ is the surface tension, S is the solubility at the absolute temperature T, S_0 is the solubility of large crystals (with infinite radius), M is the molecular weight of the solid and R is the gas constant.

Nanosuspensions are also thermodynamically unstable due to the large increase in surface area. The increase in surface area results in an increase in free energy, which the system will try to minimize again by agglomeration.[7,11] In an attempt to address these drawbacks nanosuspensions are very often dried to obtain a powder that can be further processed into an oral formulation.

A survey of the literature shows that two major techniques are currently used to dry nanosuspensions: spray drying and freeze drying.[10,12] Although satisfactory results have been obtained with both techniques, powders are produced that need further processing into capsules or tablets. This downstream processing is not always straightforward because of problems with the flowability, bulk density and hygroscopicity of these powders. Beads that are the result of a layering process using a fluidized bed with Würster insert can easily be filled into capsules, making the downstream processing very straightforward. Although sometimes referred to as an option to dry nanosuspensions,[13] very few articles can be found in the literature investigating this process. Möschwitzer and Müller[14] made use of this technique but not for an immediate release dosage form as the pellets were additionally given an enteric coating. In a paper by Olver and co-workers a bead formulation was applied in a clinical trial. The article mainly focuses on the clinical outcome of the study and no information about the process or the influence of drug physicochemical properties was included.[15] The complete absence of information (also in other publications[16]) on the layering process and the influence of stabilizer type and drug properties is a significant gap that needs to be addressed.

The aim of the present paper is therefore twofold. First, the effect of stabilizer type and concentration on the bead coating process was studied. Second, the influence of drug surface hydrophobicity on the in-vitro release and dissolution of drug nanocrystals from successfully layered beads was investigated. For the investigation of the release properties, two model drugs (naproxen (Nap) and cinnarizine (Cin)) were selected based on their significant difference in surface hydrophobicity,[17] with cinnarizine having the higher hydrophobicity. Hydroxpropylmethylcellulose (HPMC) and alfa-D-tocopherol polyethylene glycol 1000 succinate (TPGS) were used as non-ionic polymeric stabilizer and non-ionic surface-active stabilizer, respectively.

Materials and Methods

Materials

Both naproxen (D(v,50) 42.9 μm) and cinnarizine (D(v,50) 97.6 μm) were obtained from Fagron NV (Waregem, Belgium). HPMC 5mPa.s was obtained from Colorcon Inc. (West Point, PA, USA) and TPGS from Isochem (Gennevilliers, France). Polysorbate 20 (Tween 20) was obtained from Applichem GmbH (Darmstadt, Germany). The sugar beads (pharm-a-spheres 710–850 μm) were kindly donated by Hanns G. Werner GmbH (Tornesch, Germany). Demineralized water (>18 MΩ) was produced with an Elga maxima ultra pure water system (Elga Ltd, Bucks, England). All other solvents and reagents were of HPLC or analytical grade.

Preparation of crude suspensions

Samples were prepared by weighing 80 g of API in a polypropylene bottle of 1000 ml (Nalgene, Rochester, NY, USA). Subsequently the stabilizers were added in concentrations ranging between 5 and 50 wt% in relation to the API. Demineralized water was added under continuous stirring on a magnetic stirrer plate to a final weight of 800 g, resulting in suspensions with 10% (w/w) of drug.

Media milling of nanosuspensions

The crude suspensions were homogenized using a mixer before starting the milling experiment and during milling itself. Nanosuspensions were subsequently prepared from these crude suspensions by media milling using a Dyno-Mill Multilab (WAB, Bachofen, Switzerland) in combination with an LP-A2 inox peristaltic pump (Siemens, Munich, Germany). The composition or identity of nanosuspensions is indicated by the drug and stabilizer used, for example CinTPGS10 for a nanosuspension containing cinnarizine as drug and TPGS as stabilizer in a 10% concentration (w/w towards the drug). The milling chamber (300 ml; flow-through set-up) was composed of silicon carbide; the accelerator (64 mm diameter) was composed of zirconia. Milling was performed for 2–4 h using yttrium-stabilized zirconia beads (0.3 mm; Tosoh Corp., Tokyo, Japan) at 2390 rpm, with a bead load of 190 ml. The pump speed for the flow trough was set at 1.00. The temperature was maintained below 40°C using circulating cooling water around the milling chamber.

Particle sizing of crude API and nanosuspensions

The particle size distribution of the starting APIs and of the nanosuspensions was determined with laser diffraction using a Malvern Mastersizer Micro Plus (Malvern Instruments Ltd, Worcestershire, UK). The measurements were performed on ultrasonicated suspensions (ultrasonication until stable obscuration) of the API in about 500 ml of a very dilute polysorbate 20 solution (ca. 0.1%) in case of the unmilled APIs. The pump speed was set at 1600. For the nanosuspensions the measurements were performed in about 500 ml of demineralized water with and without ultrasonication (1 min). The reported values are the 50% volume percentile (D(v,50)) and the 90% volume percentile (D(v,90)) calculated from volume distributions obtained using the Mie model. A dispersant refractive

index of 1.33, a real particle refractive index of 1.15 and an imaginary particle refractive index of 0.1 were used.

Bead layering of nanocrystals using a fluidized bed pellet coater

Coating of sugar beads with nanocrystals was performed using an Aeromatic MP 1 fluid bed coater (GEA, Switzerland) equipped with a Würster insert. The instrument parameters were set to 60°C inlet temperature for HPMC stabilized nanosuspensions and 50°C for TPGS stabilized nanosuspensions. Air volume was set to between 3 and 4 (adjusted visually to fluidize the beads evenly out of the Würster insert). The atomizing air pressure was set to 1.5 bar and the feed rate of the nanosuspension was 4 g/min. These parameters were kept constant for all experiments and determined on the basis of preliminary experiments. Prior to coating of some of the nanosuspensions extra HPMC was added to the nanosuspensions to optimize the coating. Concentrations of added HPMC were 30 or 40% (w/w) with respect to the API; this will be denoted throughout the text by adding HPMC30 or HPMC40 to the nanosuspension code (for example CinTPGS20HPMC40). A quantity of 500 g of nanosuspension was coated on 500 g of sugar beads, resulting in a theoretical drug load of 10% with respect to the sugar core. In the case of added HPMC the coated amount of suspension was more than 500 g (500 g and the added HPMC) to ensure an equal load when compared to the cores.

Particle sizing of bead formulations and uncoated beads

To investigate the possibility of reagglomeration of nanocrystals after release from the beads, coated and uncoated beads were milled using a Cryomill (Retsch GmbH, Haan, Germany). Two grams of material were loaded in a stainless steal milling chamber of 25 ml together with one stainless steel ball of 15 mm. This was subsequently milled at 20 Hz for 1 min. Because of the starch present in the beads, it was necessary to also measure uncoated bead powder as a reference. The powders were introduced in about 500 ml of demineralized water and the laser diffraction measurement was started after constant obscuration was reached. For each sample a measurement with and one without ultrasonication was performed. Parameters were set at the same values as for the nanosuspensions.

Determination of the drug load

Coated beads (50 mg accurately weighed in a test-tube) were dissolved in 10 ml of pure dimethylformamide (DMF) using a rotary mixer. This results in a turbid solution because of the starch in the beads. The solutions were centrifuged using a 5804 R centrifuge from Eppendorf (Hamburg, Germany) at 4000 rpm for 5 min. Supernatant (400 µl) was diluted with 400 µl DMF in an HPLC vial prior to analysis by HPLC. All drug loads were determined six times for each sample.

Dissolution experiments

Dissolution of the bead formulations and crude API was done in test-tubes using different media for naproxen and cinnarizine. The dissolution medium consisted of demineralized water with 0.4% of sodium laurylsulphate (SLS) for naproxen and demineralized water with 2% SLS for cinnarizine (the chosen concentrations give a comparable solubility for the drugs). A sample of 10 mg of formulation (ca. 1 mg of drug) or 1 mg of crude API was accurately weighed in a test-tube and 10 ml of medium was added. Time points were 5, 10, 15, 30, 45 and 60 min. Mixing was ensured during dissolution using a rotary mixer (12 rpm) (Snijders Scientific, Tilburg, The Netherlands). Samples of 1 ml were withdrawn using a syringe and filtered through a PTFE filter of 0.1 µm mean pore diameter (Whatman Inc., Clifton, NJ, USA). The first 500 µl were discarded, and 400 µl of the filtrate was then diluted with 400 µl DMF in an HPLC vial. Dissolution media and rotation speed were selected on the basis of preliminary experiments to make discrimination between the coarse powder and the bead formulation possible. The total amount of API in the test-tubes never exceeded 85% of the solubility in the selected dissolution medium (the solubility of naproxen was 0.14 mg/ml and of cinnarizine was 0.12 mg/ml). All time points were determined in triplicate.

The dissolution of the pure nanosuspensions was determined by solution calorimetry as recently described by our research group.[18] Briefly, approximately 100 mg of nanosuspension was filled in a glass-crushing ampoule that was subsequently positioned in a 100 ml glass vessel filled with the respective dissolution media for naproxen or cinnarizine. The measurement started when the capsule was broken. As outlined by Kayaert *et al*. the temperature increase (cinnarizine, exothermic) or decrease (naproxen, endothermic) as a consequence of the dissolution of the nanocrystals was recorded as a function of time and subsequently transformed to a classical dissolution curve (percentage dissolved as a function of time).[18] Experiments were carried out in duplicate at 25°C.

Concentration determination using HPLC

Concentration determination was performed using a Waters HPLC system (Milford, USA) consisting of a Waters 1525 binary HPLC pump set at 1 ml/min flow rate, a Waters 717plus Autosampler set at 10 µl injection volume (and 50 µl in case of dissolution of unmilled cinnarizine) and a Waters 2487 Dual Lambda Absorbance detector. A Merck KGaA Lichrospher 60 RP–select B column (Darmstadt, Germany) was used. Thirty per cent (v/v) of 25 mM sodium acetate (pH 3.5) with 0.02 M of SLS and 70% (v/v) methanol were used in case of naproxen as a mobile phase; the mobile phase for cinnarizine consisted of 68% (v/v) of methanol and 32% (v/v) of the same buffer. The detector was set at 331 nm for naproxen and 250 nm for cinnarizine. Data were analysed using Breeze software Version 3.30 (also Waters).

Surface analysis using scanning electron microscopy

Scanning electron microscopy (SEM) analysis of the coated beads was carried out using a Phillips XL30 SEM-FEG (Philips, Eindhoven, The Netherlands) equipped with an Schottky field-emission electron gun. A beam of 12 kV was used and detection was performed using a conventional

Table 1 Particle size data for the different nanosuspensions

Code	After milling		After 1 month		Ok	Stable?
	D50 (μm)	D90 (μm)	D50 (μm)	D90 (μm)		
NapHPMC5	0.37	0.53	0.38	0.62	x	+
NapHPMC10	0.39	0.57	0.40	0.62	x	+
NapHPMC20	0.38	0.55	0.40	0.71	x	+
NapHPMC35	0.40	0.67	0.43	0.81	x	+
NapHPMC50	0.38	0.53	0.41	0.73	x	+
NapTPGS5	0.54	**2.86**	0.55	**1.87**		
NapTPGS10	0.42	0.72	0.45	0.75	x	+
NapTPGS20	0.41	0.65	0.40	0.62	x	+
CinHPMC10	**1.26**	**6.03**	**3.00**	**43.87**		
CinHPMC20	0.66	**3.03**	0.87	**2.78**		
CinHPMC50	0.48	1.43	0.70	**2.89**	**x**	
CinTPGS5	**3.29**	**7.46**	**5.74**	**119.79**		
CinTPGS10	0.49	1.09	0.47	1.01	x	+
CinTPGS20	0.43	0.87	0.41	0.77	x	+

Suspensions marked with a cross (x) are considered nanosuspensions. Suspension marked with a + are stable. Values in bold do not fulfil the criteria for a nanosuspension (having a D(v,50) lower than 1 μm and a D(v,90) lower than 1.5 μm).

Everhart–Thornley secondary electron detector. All samples were gold coated using a sputtering device (Balzers Union, Liechtenstein).

Statistical methods

An unpaired *t*-test ($P = 0.05$) was used to determine if the difference in concentration for the different formulations was significant at each time point. The dissolution curves for naproxen were compared with each other and those for cinnarizine also. Each time point was determined in triplicate.

Results and Discussion

Effect of stabilizer concentration on nanocrystal size

In the pharmaceutical community a suspension is considered 'nano' when the crystals have a mean diameter below 1 μm.[7] In a first part of this study the influence of the stabilizers on the crystal size was investigated to obtain suitable nanosuspensions. The different combinations of drug and stabilizers are shown in Table 1. For each of these samples the D(v,50) and D(v,90) are given immediately after milling and after 1 month of storage at room temperature (RT). The samples marked with a cross (x) are samples that were considered nanosuspensions if the D(v,50) is lower than 1 μm and a D(v,90) lower than 1.5 μm, this criterion was set a *priori* based on what is common for pharmaceutical research.

As expected from the study of Van Eerdenbrugh *et al.*,[17] naproxen is very easily stabilized as a concentration of 5% of HPMC or 10% of TPGS is sufficient to stabilize the nanosuspension. After 1 month only a very small increase in particle size is observed, proving that even a significant delay between milling and coating is allowable in this specific case.

For cinnarizine more stabilizer was needed to obtain a nanosuspension, this is due to its more hydrophobic surface.[17] In case of TPGS a good nanosuspension was obtained with 10% of TPGS. HPMC, on the other hand, is not as effective as TPGS and 50% was needed to obtain satisfying results. Important to notice is that the sample with 50% HPMC was no longer considered as a nanosuspension according to our above-mentioned criterion (<1.5 μm D(v,90)) when stored at RT for 1 month. TPGS nanosuspensions were stable for at least 1 month.

Table 2 Coating efficiency of the bead formulations without additional HPMC ($n = 6$)

Code	Drug load (%) (SD)
NapHPMC20	62.1 (3.3)
NapHPMC35	89.4 (6.1)
NapHPMC50	95.4 (2.0)
NapTPGS10	65.2 (2.0)
NapTPGS20	82.1 (3.5)
CinHPMC20	82.9 (0.2)
CinHPMC50	99.0 (4.8)
CinTPGS10	57.3 (6.3)
CinTPGS20	69.6 (8.9)

Drug load was calculated towards the core and not the total mass.

Effect of stabilizer type and concentration on coating efficiency

After identifying the appropriate concentrations of stabilizers to obtain a nanosuspension, the suspensions were all coated with the same parameters to test whether the suspensions could be coated as such. Table 2 lists all samples tested and shows the coating efficiency (drug load divided by theoretical drug load multiplied by 100). The drug load is calculated towards the sugar core and not to the whole formulation; in this way it is possible to compare the different formulations. Calculating the drug load with respect to total mass would make it difficult, as the amount of stabilizer is not the same in all formulations.

A clear trend was observed in both HPMC and TPGS stabilized nanosuspensions, as more stabilizer resulted in a higher drug load (Table 2). These data show that there is a

critical amount of stabilizer needed to make the drug crystals adhere to the sugar core. Clearly, TPGS and HPMC have a double function during this process: they should prevent agglomeration of the nanocrystals and they have to act as a coating agent to stick the nanocrystals to the sugar core. The coating process was considered successful if the coating efficiency was 90% or more (this was deemed sufficient for the main purpose of this study, investigating the effect of physicochemical properties of the drug on the fast release properties after this particular type of drying). For TPGS sufficient coating efficiency could not be reached with the nanosuspensions. The conclusion at this point of the study was that nanosuspensions with 35 or 50% of HPMC could be coated without further treatment, allowing us to study the dissolution of polymer-stabilized and coated formulations for both drugs. The 35% HPMC coating was included after considering the standard deviation but only formulations with 50% were used for the dissolution experiments. To broaden the scope of the article and further prove the broad applicability of the layering process, we decided to further work on the surfactant-stabilized nanosuspensions. Surfactant-based nanosuspensions will be needed for drugs with more hydrophobic surfaces.[17] By further optimizing the TPGS coatings it was subsequently possible to study their dissolution behaviour, giving additional information on the critical parameters of the layering process.

Modified TPGS nanosuspensions to increase drug loading

Two options were possible to increase the coating efficiency of TPGS stabilized nanosuspensions according to the previous data. The first one is to increase the amount of TPGS as an upward trend was detected (Table 2). But as TPGS itself is a bioactive compound, we decided not to investigate this option. The second option is to add HPMC – known for its excellent film-forming properties and giving excellent results in the first part of this study – to a TPGS-stabilized nanosuspension to improve its coating performance.

With NapTPGS10 suspensions a good coating efficiency was obtained when adding 40% of HPMC, as shown in Table 3. Cinnarizine nanosuspensions stabilized with 10% TPGS could not be coated with sufficient efficiency when adding 40% of HPMC. Suspensions with 20% TPGS, on the other hand, could be coated easily when 40% of HPMC was added. These results prove that coating on inert beads is broadly applicable to all nanosuspensions regardless of the stabilizer used, with some minor alterations to the formulation. Further optimization of the process itself in terms of maximal drug layering or ideal core size was beyond the scope of this study.

Table 3 Coating efficiency of formulations containing additional HPMC ($n = 6$)

Code	Drug load (%) (SD)
NapTPGS10HPMC40	92.2 (3.0)
CinTPGS10HPMC40	78.8 (1.2)
CinTPGS20HPMC30	85.6 (3.1)
CinTPGS20HPMC40	101.8 (2.0)

Drug load was calculated towards the core and not the total mass.

To find the reason for the low drug loads (as revealed by HPLC analysis) the coatings were visualized using SEM. From the pictures in Figure 1 (with each picture representative for the entire batch), it is possible to distinguish successful and failed coatings from each other. Figure 1a shows an uncoated bead, the surface of this type of bead is rough. In Figure 1b and 1c beads coated with CinTPGS20HPMC40 and NapHPMC50 are shown. These coated beads have a much smoother surface. In Figure 1d a bead coated with CinTPGS10HPMC40 is shown and a defect in the coating can clearly be seen. These defects are the result of coatings that do not stick well to the sugar beads and are the cause of low drug load. Further investigation of the coatings by SEM shows that the nanosuspensions are nicely layered onto the cores, suggesting that the association of stabilizer and core is retained. In Figure 1e the edge of a broken coating of CinTPGS10HPMC40 is depicted and in Figure 1f a deliberately made hole in a NapTPGS10HPMC40 coating. In Figure 1f the rough surface of the bead, the smooth layer of the coating and the individual crystals in the edge of the coating are clearly detectable.

Dissolution characteristics of the coated beads

Previous work by Van Eerdenbrugh *et al*.[19] showed that drying could change the dissolution characteristics of nanosuspensions. To ensure that bead layering is not only an efficient drying technique but also retains the high dissolution rate of the nanocrystals, a dissolution study was performed using the successful formulations in terms of drug load and unmilled powder as a comparison. The dissolution curve of the coarse naproxen powder and of two bead formulations is shown in Figure 2. A statistically significant increase in dissolution rate for both bead formulations is observed when compared to the coarse powder. No significant differences between both bead formulations could be found for all time points and both reach ca. 100% dissolution in less than 5 min. It is important to keep in mind that the dissolution time of the bead formulations could be less than 5 min as the original nanosuspensions dissolve in less than 30 s. The dissolution of the nanosuspensions was measured by solution calorimetry because the filtration set-up is to slow to analyse timepoints below 5 min.[18] As a conclusion we can state that for naproxen drying nanosuspensions by coating them on an inert carrier retains the fast dissolution.

Slightly different results were obtained for cinnarizine. From Figure 3 it is clear that an increase in dissolution rate is obtained with the nanocrystals coated on the beads if compared to the coarse cinnarizine powder (dissolution of ca. 100% after 15 min compared to only 11% after 1 h). The dissolution of the bead formulations proved to be significantly faster at all time points than that of the coarse powder. When comparing the bead formulations with each other a statically significant difference could only be observed for the first time point, where the HPMC formulation is slightly faster. Knowing that small errors in the sampling time (about 1 min) are likely and because of the fast dissolution with big changes in concentration, the authors do not consider this difference to

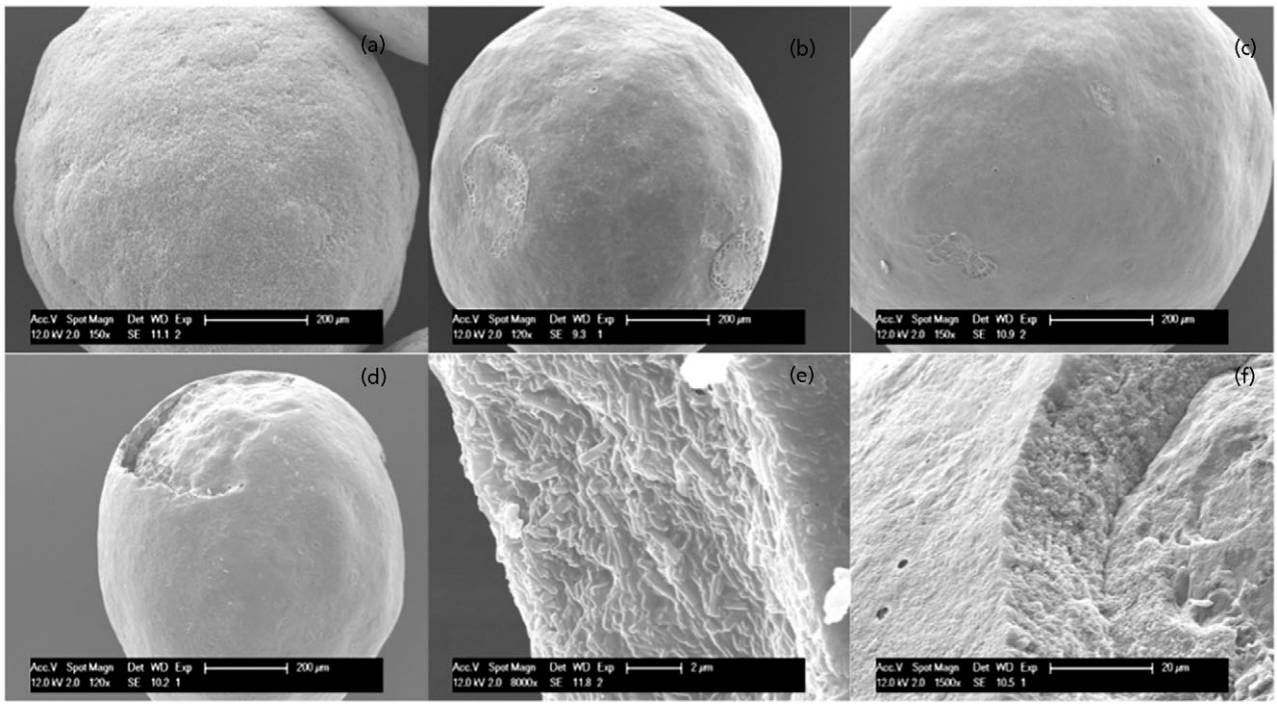

Figure 1 SEM pictures of different beads or bead formulations: (a) an uncoated bead; (b) a bead coated with CinTPGS20HPMC40; (c) a bead coated with NapHPMC50; (d) a bead coated with CinTPGS10HPMC40; (e) the edge of a broken CinTPGS10HPMC40 coating; (f) the edge of a NapTPGS10HPMC40 coating.

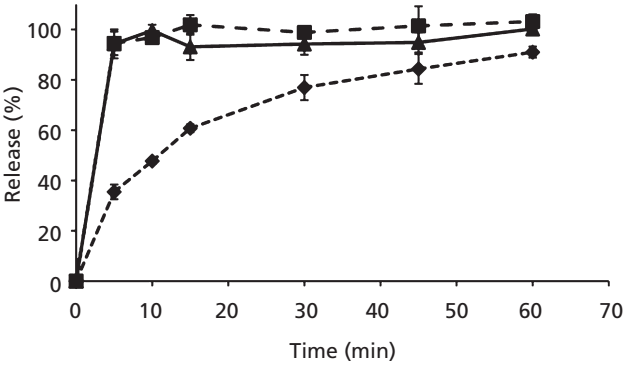

Figure 2 Dissolution curves of naproxen ($n = 3$): ◆, the dissolution curve of unmilled naproxen powder; ■, the dissolution curve of naproxen nanosuspension stabilized and coated with 50% HPMC; ▲, naproxen nanosuspension stabilized with 10% TPGS and coated with an extra 40% HPMC.

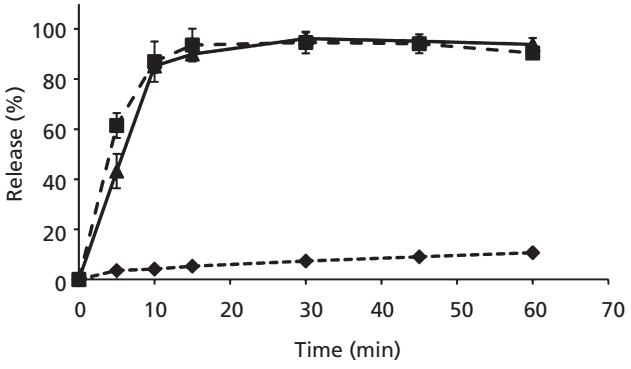

Figure 3 Dissolution curves of cinnarizine ($n = 3$): ◆, the dissolution curve of unmilled cinnarizine powder; ■, the dissolution curve of cinnarizine nanosuspension stabilized and coated with 50% HPMC; ▲, cinnarizine nanosuspension stabilized with 20% TPGS and coated with an extra 40% HPMC.

have a physical meaning. When comparing the original nanosuspensions of cinnarizine to the bead formulations and considering that the nanosuspenions dissolve within 30 s regardless of stabilizer type or small differences in size (measured by solution calorimetry), it is clear that the dissolution rate is reduced after coating in the case of cinnarizine. The increase in dissolution time can be due to agglomeration of the nanocrystals while being released from the coating. Alternatively, the dissolution could also be slowed down because the polymer that forms the coating needs to dissolve first to release the drug nanocrystals. The latter explanation is less likely, as this was not observed with naproxen nanocrystals under comparable conditions. In order to confirm nanocrystal reagglomeration following release from the beads, laser diffraction experiments were carried out.

Nanocrystal reagglomeration after release from the beads is observed in the case of cinnarizine (Figure 4). Figure 4b shows two particle size distribution curves: the first one is that of the uncoated beads without ultrasonication and the second one with ultrasonication, proving that ultrasonication has very little influence on the measurement in case of uncoated beads. This is important as ultrasonication will be used to obtain information on the coated nanoparticles. In Figure 4c the

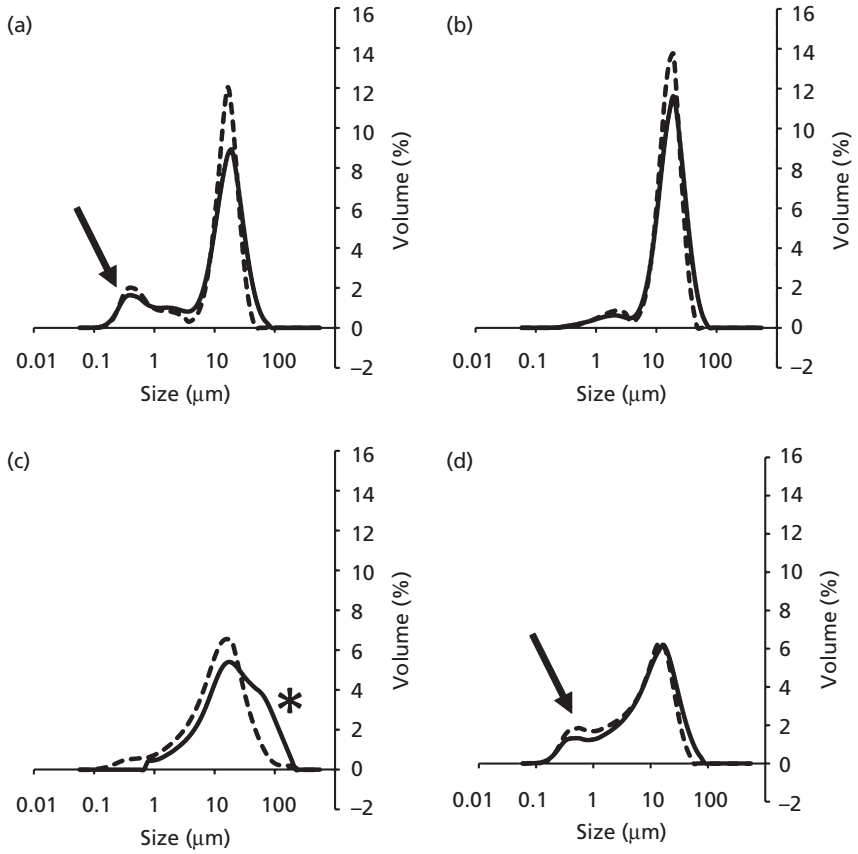

Figure 4 Laser diffraction curves of uncoated beads and the bead formulations: (a) curves with ultrasonication (dotted line) and without ultrasonication (solid line) of the naproxen formulation with 50% HPMC; (b) curves for the uncoated beads; (c) curves for the cinnarizine formulation with HPMC 50%; (d) curves for cinnarizine stabilized with 20% TPGS and coated with 40% extra HPMC; the arrows point to the fraction smaller than 1 μm. In (c) the asterisk points to agglomerated nanocrystals with a size bigger than the undissolved starch. These agglomerates are broken up by ultrasonication.

curves of CinHPMC50 are given, measured with and without ultrasonication. The curve measured without ultrasonication clearly shows agglomeration as no second population (next to the uncoated beads) can be observed in the nanometer range. A second proof is that by ultrasonication of the suspension the particle size decreases (curve shifts to the left), suggesting a break up of agglomerates and proving the presence of nanoparticles next to the residual material of the beads. Careful observation determined that the crystals released from the beads of CinTPGS20HPMC40 are also agglomerated but to a far lesser extent, as a significant amount of crystals is smaller than 1 μm (Figure 4d). These data indicate that adding TPGS helps to protect the nanocrystals against agglomeration after release from the beads. A possible explanation why this was not observed in the dissolution experiments can be found in the vigorous stirring during particle size measurement as compared to the gentle mixing while performing dissolution experiments. The vigorous stirring breaks up the agglomerates of the TPGS-stabilized suspension but not those of the HPMC-stabilized suspension. For naproxen formulations agglomeration was not observed (Figure 4a).

One could argue about the use of demineralized water instead of the dissolution media to study agglomeration phenomena with laser diffraction (LD). Due to the vigorous stirring during LD, it is not possible to use large amounts of SLS since foaming occurs, which disturbs the measurement. The presence of SLS is, however, not needed during such short experiments because the original (uncoated) nanosuspensions are not agglomerating during a measurement in water. The presence of material from uncoated beads proved not to influence the agglomeration of the original nanosuspensions.

Laser diffraction data together with the dissolution data prove that the hydrophobicity of the surface of nanocrystals influences the release properties after drying using bead coating. The easily stabilized naproxen nanosuspensions result in bead formulations that release nanocrystals without agglomeration. Cinnarizine, however, was much more difficult to stabilize as a nanosuspension due to the more hydrophobic surface of the crystals. These results prove that the ability to generate stable nanosuspensions is no guarantee for obtaining bead formulations with fast release and without agglomeration. The laser diffraction experiments performed on cinnarizine suggest that adding more of the surface active compound TPGS can overcome the agglomeration problem after release from the beads even if this excess stabilizer is not needed to create initially stable nanosuspensions. If (re)agglomeration is a problem we suggest using higher amounts of surfactant.

Overall the dissolution experiments prove that formulation as nanocrystals is a successful technique and that drying the suspensions by coating on a sugar bead only reduces the effectiveness of the formulation to some extent for cinnarizine. The fact that cinnarizine release was slightly decreased due to agglomeration after drying was also observed by Van Eerdenbrugh *et al*.[19] When comparing the results for the bead formulations with the spray-drying results obtained in that study, the observations are similar. Cinnarizine agglomerates and naproxen does not. This indicates that regardless of the drying technique, drying of compounds with hydrophobic surfaces is not straightforward and is definitely related to drug surface hydrophobicity. Further research should focus on ways to reduce agglomeration, as this will probably be useful for all drying techniques.

Conclusion

In this study we identified several parameters that have a significant influence on the result of bead layering of nanosuspensions as a drying technique. Having the right amount of coating agent to stick the nanocrystals to the sugar cores is a prerequisite. We also observed that a successful coating is no guarantee for adequate nanocrystal and drug release since this is seriously influenced by drug surface hydrophobicity. Very good results were obtained for naproxen with nearly complete drug release after less than 5 min, which is more than threefold faster than the coarse powder and comparable to the nanosuspension itself. Complete release of cinnarizine, however, was only obtained after 15 min. Compared to the coarse powder this is a huge increase as the powder only reached about 11% release after 1 h. The difference between cinnarizine and naproxen can be attributed to reagglomeration of the nanocrystals when released from the beads in the case of cinnarizine (because of the hydrophobic surface). The results also show that TPGS stabilizes the nanocrystals better than HPMC when released from the coating. This result suggests the use of high(er) concentrations of surfactant when reagglomeration after drying is a problem; even if this is not needed for the stabilization of the original nanosuspension. This immediately makes clear that a successful and stable nanosuspension will not necessarily give a successful bead formulation after drying.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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