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RESEARCH PAPER

# Use of Sodium Chloride to Facilitate Reduction of Particle Size of Dexamethasone During Ball Milling

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*Key Words:* Ball milling; Polysorbate 20; Dexamethasone; Sodium chloride.

## INTRODUCTION

Ball milling is frequently used to reduce the particle size of hydrophobic drugs. The milling slurry typically is between 5–100 times more concentrated with respect to the active drug. Sodium chloride is frequently used as a tonicity adjusting agent. A typical formulation may contain hydrophobic drug, surfactant, sodium chloride, and other ingredients. It was of interest to determine the effect of the milling slurry composition on the particle size of the suspended drug. A reduction in particle size could potentially translate into a more robust manufacturing process, as well as improved bioavailability of the suspended active.

## MATERIALS AND METHODS

### Materials

Materials were weighed on either Sartorius (model no. B3100s) or Ohaus (explorer) balances. Ball milling was carried out on a lab scale roller mill (U.S. Stoneware, Mahwah, NJ) using 500 mL Nalgene® LDPE bottles with screw caps. Solutions were stored in Pyrex® glass bottles with screw caps. The suspended particles were sized using a Microtrac X100 particle size counter. A Perkin-Elmer (Pyris 1) differential scanning calorimeter was used to measure melting temperature. Surface tension measurements were performed using a Kruss du Nouy tensiometer. A Lightnin Mixer (model no. L1UO8F) was used for mixing the suspensions. A corning 250 mL filter system with a 0.22 μm cellulose acetate membrane was used to filter solutions used as particle carriers. The chemicals used in the study were polysorbate 20, polysorbate 80, benzalkonium chloride (as a 10% solution in water), sodium chloride, dexamethasone and mannitol; all either USP or NF grade. Purified water was used throughout the study.

### Methods

Unless otherwise indicated, either 5.0 g or 22.0 g of surfactant (polysorbate 20 or polysorbate 80) was added into a tared 500 mL Nalgene® bottle. For experiments that included benzalkonium chloride (BAC) in the milling slurry, 4 g of BAC solution was added to the contents. Either purified water or a

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***Table 1.*** Composition of milling slurry.

| Composition | Control (I) | Modified control (II) | Control + excess polysorbate 20 (III) | Control + BAC (IV) | Control + saturated sodium chloride (V) | Control + saturated mannitol (Va) | Control + sodium chloride (VI) |
|---|---|---|---|---|---|---|---|
| Polysorbate 80 | | 2.5% | | | | | |
| Polysorbate 20 | 2.5% | | 11% | 2.5% | 2.5% | 2.5% | 2.5% |
| Dexamethasone | 5% | 5% | 5% | 5% | 5% | 5% | 5% |
| BAC | | | | 0.2% | | | |
| Excess undissolved sodium chloride | | | | | | | P |
| Saturated sodium chloride solution | | | | | P | | |
| Saturated mannitol solution | | | | | | P | |
| Volume mean diameter, $D_v$ (μm) of milled dexamethasone, ($D_{10\%}$, $D_{90\%}$) | 1.9 (0.6, 3.5) | 1.1 (0.4, 2.0) | 1.9 (0.5, 3.0) | 2.1 (0.7, 3.9) | 1.1 (0.4, 2.0) | 1.9 (0.6, 3.2) | 1.1 (0.4, 1.9) |

P, Present.

saturated solution of sodium chloride or a saturated solution of mannitol was then added to make quantity sufficient (QS) to 200 g. When purified water was used, either 0, 5, 10, or 85 g of sodium chloride was added to the QS contents. Dexamethasone (10 g) was added, and the bottle was swirled gently to wet the powder completely. A known amount of milling beads was added. The bottle was capped and placed horizontally on the roller mill. The contents were milled for a minimum of 12 hr. The contents of slurry composition I were also milled for 72 hr. Table 1 indicates the composition of the milling slurries for the various experiments.

Saturated solutions of dexamethasone or mannitol were prepared by separately suspending an excess of dexamethasone or mannitol, respectively, in water. The suspensions were stirred at 500 rpm for 2 hr. They were then separately filtered through a 0.2 μm filter and stored in separate Pyrex® bottles.

A saturated solution of sodium chloride was prepared by suspending an excess of sodium chloride in water. The suspension was stirred at 500 rpm for 2 hr. It was then allowed to settle. The decanted supernatant was used for experiment V (see Table 1). When a saturated solution of sodium chloride was used as a particle carrier fluid, it was filtered through a 0.2 μm filter and stored in a Pyrex® bottle.

Suspensions of dexamethasone in water with either polysorbate 80 or polysorbate 20 were also prepared that were sized without ball milling. For these experiments, 0.25 g of polysorbate 80 or polysorbate 20 was weighed in 100 mL beakers. The contents were QS to 10 g with either water or a saturated solution of sodium chloride. Then, 0.5 g dexamethasone was added to the beaker. The contents were stirred for 1 hr and sized. In these set of experiments, the particle carrier was either a saturated solution of dexamethasone or a saturated solution of sodium chloride. The compositions of the various slurries are shown in Table 2.

The intraday variability of milling experiments and particle sizing was assessed by repeating experiments with control (I) and control + sodium chloride (VI).

Particles were sized using a Microtrac model X100 with Microtrac version 7.02 software using a saturated solution of dexamethasone as the particle carrier fluid. The particle and the carrier fluid refractive indices were assumed to be 1.59 and 1.33, respectively. The carrier fluid refractive index was 1.45 when a saturated solution of sodium chloride

***Table 2.*** Unmilled slurry experiments.

| | VII | VIII | IX | X |
|---|---|---|---|---|
| Dexamethasone | 5% | 5% | 5% | 5% |
| Polysorbate 80 | | | 2.5% | 2.5% |
| Polysorbate 20 | 2.5% | 2.5% | | |
| Saturated solution of sodium chloride for QS | | P | | P |
| Water for QS | P | | P | |
| Volume mean diameter, $D_v$ (μm) of suspended dexamethasone | 4.6[a] | 3.3 | 2.8 | 2.8 |

P, Present.
[a]6.4 μm when a saturated solution of sodium chloride was used as a particle carrier.

was used. The particle shape was assumed to be irregular, and the particles were assumed to be transparent. The flow rate was 20 mL/sec. Channels were set from 0.122 to 124.5 μm in a geometric root 4 progression. The number of channels was 40, and the residuals were enabled. The percent transmission ranged from 90 to 96%, and the loading factor ranged from 0.01 to 0.05. The counting time was set at 30 sec, with an average of 3 runs reported.

Differential scanning calorimetry and x-ray powder diffraction was conducted on the milled dexamethasone (slurry compositions I and VI) to ensure that any differences in the particle size of the milled dexamethasone were not due to polymorphic change. The dexamethasone was separated from the milled slurry by filtration through a 0.2 μm filter. The cake on the filter was washed with ~300 mL of water. The cake was air-dried at room temperature for 24 hr and submitted for analysis. Differential scanning calorimetry was carried out from ambient room temperature to 300°C at a ramp rate of 10°C/min under a stream of nitrogen (carrier gas flow rate of 20 mL $min^{-1}$). X-ray powder diffractograms were obtained using monochromatic $CuK_\alpha$ radiation. The samples were deposited on an off-axis cut quartz plate.

Surface tension measurements were performed using a Kruss du Nouy Tensiometer at 21.5°C. The tensiometer was equipped with a platinum ring of 6 cm circumference. The instrument was calibrated using a 200 mg weight. An average of three measurements is reported. The variation was not more than ±0.5 dynes $cm^{-1}$. For the surface tension measurements, 2.5% w/w solutions of polysorbate 20 in either water or saturated sodium chloride solution were prepared. In addition, a 2.5% w/w solution of polysorbate 80 in water was also prepared.

All the chemicals used in the study met USP/NF specifications. The same lots of dexamethasone, polysorbate 20, and polysorbate 80 were used throughout.

## RESULTS

Figure 1 shows the effect of milling slurry composition on the particle size of dexamethasone. The volume mean particle diameters for the various slurry compositions are shown in Table 1. Slurry compositions II, V, and VI yielded the smallest volume mean diameter ($D_v$) of 1.1 μm. A $D_v$ of 1.9 μm was obtained with slurry compositions I and III, whereas slurry composition IV had a $D_v$ of 2.1 μm. There was no further reduction in $D_v$ when slurry composition I was milled for 72 hr. The particle size distributions obtained with slurry compositions II, V, and VI were unimodal, whereas those obtained with slurry compositions I, III, IV, and Va were bimodal. Table 1, therefore, also shows $D_{10\%}$ and the $D_{90\%}$ for these distributions.

The intraday variability in $D_v$ for two slurry compositions, I and II, was ≤3.2% for the two slurry compositions studied. This effectively means that the experimental method should be capable of distinguishing between volume mean diameters 60 nm apart.

The particle size of dexamethasone obtained without milling for slurry compositions VII through X was measured. The $D_v$ of nonmilled dexamethasone with 2.5% polysorbate 80 (composition IX) and 2.5% polysorbate 20 (composition VII) was 2.8 μm and 4.6 μm, respectively. When a saturated solution of sodium chloride was used for QS, there was no appreciable change in the $D_v$ of the polysorbate 80 formulation, whereas the $D_v$ of the polysorbate 20 formulation decreased from 4.6 μm to 3.3 μm.

The effect of increasing the concentration of sodium chloride in the milling slurry (composition I) on the particle size was measured. It can be seen that the $D_v$ decreases from 1.9 μm for a composition with no salt to 1.1 μm for a slurry composition

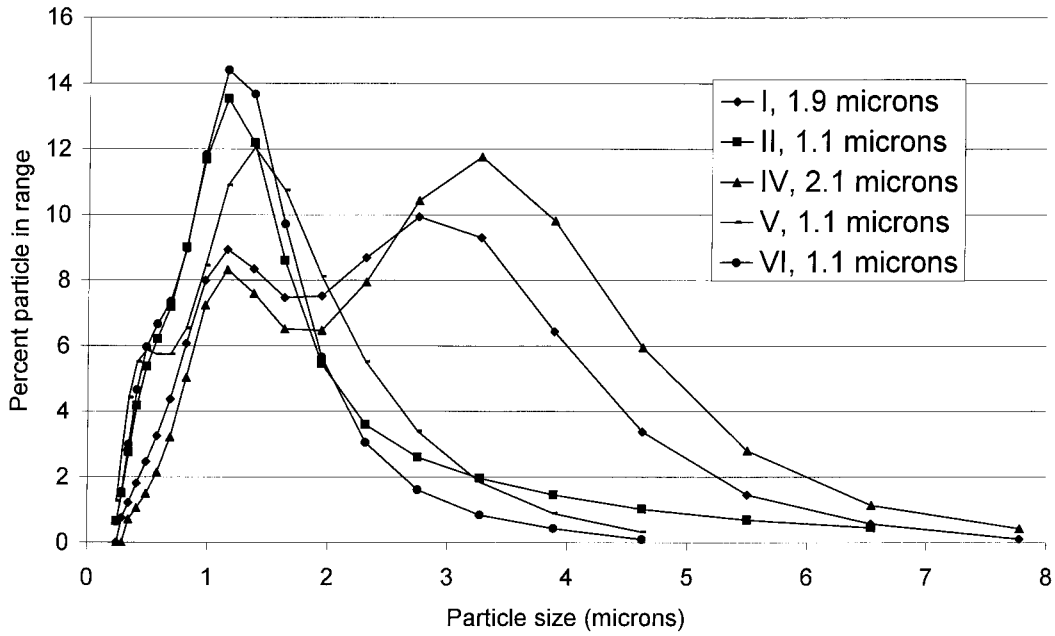


***Figure 1.*** Effect of composition of milling slurry on particle size.

containing a saturated solution of sodium chloride. The intermediate $D_v$ for sodium chloride concentrations of 0.43 and 0.85 molar were 1.6 and 1.4 μm, respectively.

Thermograms were obtained using slurry compositions I and VI. The raw material and the milled steroid had one endotherm each at 267.4°C and 261.3°C, respectively. The melting point of dexamethasone[9] is reported to be 262°C–271°C. In addition, the x-ray diffractograms obtained using the previously described slurry compositions were identical.

The surface tensions of water, 2.5% w/w polysorbate 20 in water, 2.5% w/w polysorbate 20 in a saturated solution of sodium chloride, and 2.5% w/w polysorbate 80 in water, were 69.0, 38.0, 35.5 and 41.5 dynes $cm^{-1}$, respectively.

## DISCUSSION

Addition of excess salt to the milling slurry followed by ball milling resulted in a $D_v$ of 1.1 μm (milling composition VI). In this case, excess solid salt is present in the milling slurry above its solubility limit . It was originally thought that the cubic lattice crystal of salt may permit crystal-to-crystal grinding and abrasion in addition to the attrition provided by the beads, thereby permitting a larger decrease in $D_v$ than that achievable using polysorbate 20 alone. However, subsequent experiments with saturated and even subsaturated salt solutions yielded a substantial decrease in $D_v$.

In milling slurries V and VI, the aqueous medium is saturated with sodium chloride. In addition, in slurry VI, there is excess solid sodium chloride present as a solid phase. It can be seen that the lowest particle size of the suspended steroid is obtained when these milling compositions are used. Polysorbate 80 and mannitol were included in the study in an attempt to elucidate the mechanism by which addition of salt to polysorbate 20 would facilitate a reduction of particle size after milling.

The experiments with nonmilled dexamethasone were carried out for two reasons. The first was to determine if milling was required to reduce the particle size of slurry prepared with polysorbate 20 and a saturated solution of sodium chloride. The second was to determine if the lower particle size obtained after milling dexamethasone with a saturated solution of sodium chloride was not an artifact of the measurement itself. As can be seen from slurry compositions VII and VIII, there was a reduction in $D_v$ when a saturated solution of sodium chloride was used instead of water in the unmilled

slurry. However, the reduction was not significant, compared with the $D_v$ obtained after ball milling the slurry (V and VI). Also, when a saturated solution of sodium chloride was used as the particle fluid carrier, the $D_v$ obtained was higher than that obtained with either VII or VIII.

The polysorbates are polyoxyethylene sorbitan fatty acid esters. Polysorbate 80 and polysorbate 20 are polyoxyethylene derivatives of monooleate and monolaurate esters, with molecular weights of 1,310 and 1,128 g/mol, respectively. The hydrophilic-lipophilic balance for polysorbate 80 and polysorbate 20 is 15.0 and 16.7, respectively.[1] There are several possible mechanisms by which the addition of salt to the milling slurry would be able to decrease the particle size of the milled hydrophobic steroid. These include the following:

1. *A decrease in the critical micellar concentration (CMC) of the surfactant*: The addition of salts decreases the CMC of nonionic surfactants.[7] This is especially true of cations like sodium because of its inability to form complexes with the ether oxygen linkages of the polyoxyethylene moieties.[11] The CMC of polysorbate 80[2,3] and polysorbate 20[4] is reported as 0.01 and 0.05 mM. The operating concentration of surfactants in the milling experiments was 20.4 mM for polysorbate 20 (except milling slurry III) and 19.1 mM for polysorbate 80. Therefore, the concentration of polysorbate 80 and polysorbate 20 is 1,910 and 408 times more then their respective CMC. A total polysorbate 20 concentration of 11% represents a 1,950-fold excess above its CMC. This excess is similar to that found for polysorbate 80 at a concentration of 2.5%. If the dexamethasone particle size was predominantly determined by the number of micelles of surfactant and, assuming that the aggregation number for the two surfactants is of the same order of magnitude,[12] one would expect similar milled diameters at polysorbate 20 and polysorbate 80 concentrations of 11, and 2.5%, respectively. Because this is not true (compare milled dexamethasone diameters using compositions II and III), it would appear that, even if the CMC of polysorbate 20 is decreased in the presence of salt, this effect alone may not explain the reduction of milled particle size.
2. *A decrease in molecular diffusion or Ostwald ripening*: It is known that larger particles will grow at the expense of smaller particles in a polydisperse suspension or emulsion. This phenomenon is termed Oswald ripening.[5,6] It occurs because the vapor pressure (or escaping tendency) of smaller particles is larger than that of larger particles, as predicted by the Kelvin[13] equation. The smaller particles therefore dissolve and crystallize on the larger particles in an attempt to lower the thermodynamic free energy of the system. The solubility of dexamethasone in water[9] at 25°C is reported as $10^{-4}$ g/mL. It may be estimated[14] that, in a hypothetical mixture of 1 μm and 10 μm crystals, the time for the dissolution of the 1 μm crystals may be of the order of minutes, when no crystal dissolution or growth inhibitors are present. The addition of surfactant can considerably slow down this process because of loss of available sites for deposition. The addition of both sodium chloride and mannitol to the aqueous phase may also retard this process by decreasing the intrinsic solubility of dexamethasone in the salt or mannitol-saturated medium. However, as can be seen from Table 1, milling slurry composition Va yields a $D_v$ of 1.9 μm. This is not significantly different from the control slurry composition I. Therefore, it would appear that Ostwald ripening is a negligible contributor to diffusive crystal growth during ball milling.
3. *Crystal-to-crystal grinding and abrasion in addition to the attrition provided by the beads*: Experiments performed with a saturated solution of sodium chloride (V) yield a volume mean diameter equal to those performed with excess sodium chloride (VI). Thus, it would appear that the effect of crystal-to-crystal grinding and abrasion provided by the sodium chloride crystals is negligible.
4. *Effecting a change in phase of the surfactant*: For a binary system, the phase changes for a nonionic surfactant are thermotropic in nature and include the Krafft point[8] and the cloud point. The Krafft point is the temperature at which the solubility of the surfactant equals its CMC. For nonionic surfactants, the solubility usually does not drop below the CMC before the freezing point of water is reached[10] (i.e., their Krafft points are near the vicinity of 0°C). It is unlikely

that the presence of salt causes a drastic change in the Krafft point.

5. *Causing a change in polymorphic form of the suspended steroid*: Differential scanning calorimetry and x-ray powder diffraction scans demonstrate that there is no change in the polymorphic form of dexamethasone when milled without or with salt (compositions I and VI, respectively).
6. *Lowering the surface tension of the surfactant*: The surface tension of polysorbate 20 in the presence of salt is not substantially lower than that without salt. In addition, the surface tension of a 2.5% w/w aqueous solution of polysorbate 80 is higher than that of a 2.5% solution (with or without salt) of polysorbate 20. Therefore, a reduction in surface tension is not an adequate explanation for the reduction in particle size.

Barry and Eleini[15] studied the solubilizing efficiency of a series of cetyl polyoxyethylene esters for a range of steroids. They found that the solubilizing efficiency decreased as the polyoxyethylene chain length was increased. Conversely, the solubilizing power of polysorbate 20 for a wide range of steroids has been studied.[16] This uptake ranged from $10^{-4}$ mol steroid (17α-ethynyl testosterone) to $10^{-1}$ mol steroid (17α-ethynylestradiol-17β) per mol of polysorbate 20. This large difference in solubilizing power was interpreted as the effect induced by the structural change when going from a ketone group of the estrone to the more hydrophilic hydroxy group of the diol. Lundberg et al.[17] measured the uptake of three steroids in tetradecyl trimethyl ammonium bromide. Addition of 0.2 M sodium chloride decreased the solubilization of testosterone and progesterone, and increased that of estrone. This was interpreted to mean that the first two steroids were solubilized in the polyoxyethylene layer of the micelle, whereas the estrone was solubilized in the hydrocarbon core.

Because the addition of sodium chloride to polysorbate 20 decreases the particle size of the suspended dexamethasone, it appears that dexamethasone may be solubilized in the hydrocarbon core of the polysorbate micelle. The 16-carbon oleic acid component polysorbate 80 is more hydrophobic than the 12-carbon lauric acid component of polysorbate 20. Therefore, it may not be unreasonable to assume that polysorbate 80 has a greater solubilizing power (smaller particle size) for dexamethasone than polysorbate 20.

## CONCLUSIONS

Inclusion of sodium chloride in the milling slurry containing dexamethasone and polysorbate 20 significantly decreases the $D_v$ of the milled steroid. The reduction in $D_v$ is directly proportional to the amount of sodium chloride added. The mechanism(s) or the combination of mechanisms by which sodium chloride affects this size reduction could not be clearly elucidated from this study.

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