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De-agglomeration of micronized benzodiazepines in dissolution media measured by laser diffraction particle sizing

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Abstract

The objective of this research was to develop a method to characterize the degree of particle agglomeration using laser diffraction particle sizing, following the addition of benzodiazepine interactive mixtures to water. Interactive mixtures of diazepam, nitrazepam and oxazepam (up to 20% w/w) were prepared by mixing micronized benzodiazepines with lactose granules (250–355 μm). Micronized sodium lauryl sulfate and cetrimide (up to 5% w/w) were added to the benzodiazepine–lactose interactive mixes to produce ternary mixtures. Particle size distributions of benzodiazepines, after addition of the interactive mixtures to water, were determined using laser diffraction particle sizing. Bimodal distributions representing dispersed particles and agglomerates were observed initially after lactose carrier dissolution. Partial agglomerate to dispersed particle transition occurred during a 60-min observation period for all mixtures, reaching a constant level of agglomeration after this time. Interactive mixtures with higher benzodiazepine concentrations displayed transition profiles with higher levels of agglomeration. The presence of surfactant in interactive mixtures dramatically decreased agglomeration. Sodium lauryl sulfate was more effective than cetrimide in dispersing agglomerates. The shape of the transition curves during de-agglomeration demonstrated the presence of stable agglomerates that remained after the initial transition; these may be important in explaining dissolution and absorption rates.

Introduction

Dissolution studies of micronized drugs such as diazepam, nitrazepam, oxazepam and flunitrazepam in interactive mixtures have shown that increasing the drug concentrations in the interactive mixtures decreased the dissolution rate (Alway et al 1996; Supabphol & Stewart 1996a; Supabphol & Stewart 1996b; Stewart & Alway 1995). Drug agglomeration was observed during the dissolution of griseofulvin and oxazepam interactive mixtures (Westerberg & Nystrom 1993a, b; Liu & Stewart 1998). Modelling of the dissolution data showed that dissolution of drugs occurred from dispersed drug particles and agglomerates (Alway et al 1996). The initial percentage of dispersed particles and agglomerates, and the dissolution rate constants were derived by non-linear least squares parameter estimation algorithms using multi-exponential dissolution models. The incorporation of surfactants into interactive mixtures improved the dissolution rate of benzodiazepines by decreasing agglomeration and increasing the dissolution rate constants (Liu & Stewart 1998). The modelling outcomes have been supported by laser diffraction particle sizing that demonstrated the existence of both dispersed particles and agglomerates under non-sink conditions. The results presented in this manuscript were an integral part of a larger research program to test the hypothesis that, since dissolution will be maximized when the drug is fully dispersed, the state of agglomeration or dispersion of very poorly water-soluble drugs may be used to predict dissolution and bioavailability behaviour. In fact, an agglomeration profile might be a better in-vitro indicator than dissolution conducted in artificial surfactant or mixed solvent media. The purpose of the research reported in this paper was to develop a method to monitor the concentration of dispersed particles and agglomerates using laser diffraction when interactive mixtures containing poorly

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water soluble drugs were added to dissolution media, and to apply this method to study the effects of drug and surfactant concentration on the concentration of dispersed particles and agglomerates.

Materials and Methods

Materials

Diazepam, oxazepam and nitrazepam (provided by Alphapharm, Australia) micronized, with volume mean diameters of 7.8 μm, 7.1 μm and 3.1 μm, respectively, were the adherent model drugs. Sodium lauryl sulfate (sls; Sigma, St Louis, MO) and cetrimide (Sigma, St Louis, MO) were micronized and possessed projected area diameters less than 10 μm when examined using SEM. Micronization was achieved by fluid energy milling (Chrispro Jetmill model 75P compressed air 5.8 atm at 12.7 L s^{-1}) and stored at room temperature in a closed container. Lactose–povidone granules (250–355 μm) used as the carrier were prepared from 9 parts of lactose (Wyndalle, New Zealand) and 1 part of povidone (Kollidon 25, BASF, Germany) by wet granulation using 10% (w/w) povidone solution. The wet granules were tray dried in an incubator at 50 °C for 24 h and a 250–355 μm fraction was obtained by sieve classification using standard sieves (Labotechnics, Australia) and a sieve shaker (Fritsch, Australia).

Particle size analysis

The particle size distributions of the micronized drugs were determined using laser diffraction (Malvern Mastersizer S, Malvern Instruments, UK). Dispersion was achieved using a sonicator to break up the agglomerates in the suspension of drug in sls aqueous solution (0.5 mg mL^{-1}). The particle size distributions of the drugs in the interactive mixtures were determined under non-sink conditions. A suitable amount of suspension or interactive mixture was added to 100 mL purified water at 20 °C in the Small Volume Sample Dispersion Unit Stirrer, and the speed was set to mid range and was constant for all the determinations. The obscuration of the sample was 10–15%. For each interactive mixture, distributions were determined at 2-min intervals for 1 h.

Verification and stability test of the Mastersizer S

Standard glass beads of approximately 100 μm (ATA Scientific, Australia) were used as the known particle sample size. Glass beads were added to the Small Volume Sample Unit until an obscuration of 15% was obtained. The particle size distribution was measured with five replicates for verification and with three replicates every 2 min for 1 h to determine stability of the instrument. The parameters of obscuration, equivalent volume mean diameter, the volume (v) median diameter and total glass beads volume concentrations (%vol) were obtained during 60 min. The mean values of these parameters showed little variability over 60 min with CVs in each run in the range of 0.12 to 1.46%. The particle size distributions of different concentrations of glass beads (0.50 g, 0.75 g and 1.00 g in 100 mL purified water) were measured every 2 min for 1 h using three replicates. The coefficients of variation of the mean volume concentration of the glass beads for the three samples were $\pm$ 3.08%. Also the three different volume concentrations of glass beads remained almost constant over 60 min with the CV for the 60-min run being not more than $\pm$ 0.41% and the CV for the three replicates being not more than $\pm$ 4.21%. A linear relationship existed between the amount of added particles (weight) and measured particle volume concentrations.

Interactive mixtures

Interactive mixtures were prepared using a previously validated method; micronized drug (and surfactant) was placed between two layers of carrier in a glass vial and shaken vigorously by hand for 5 min (Alway et al 1996). Homogeneity of all mixtures was determined by removing 20 $\times$ 100 mg samples, extracting into absolute alcohol (CSR, Australia), and assaying spectrophotometrically. Coefficients of variation of all mixtures used in this research were less than 2.8%.

Spectrophotometric analysis

Spectrophotometric analyses were performed using a scanning UV-vis spectrophotometer (Cecil 6000 series, model CE 6700, Cecil Instruments, UK). Beer's law calibration plots were obtained in absolute alcohol for the homogeneity studies (diazepam, 315 nm, 3.0–12.0 mg%; nitrazepam, 309 nm, 0.5–2.0 mg%; oxazepam, 316 nm, 2.0–8.0 mg%). Linear regression analysis indicated no significant deviations from linearity and no significant deviations from a zero intercept. Absorbance of the lactose carrier was insignificant in the dissolution studies, and was small (< 0.05) and accounted for in the homogeneity determinations. Correlation coefficients were 1.000. The precision and accuracy were determined at three concentrations (low, medium and high). The coefficients of variation were less than 0.38% and the assays remained within 99.6 to 102.2%.

Statistical analysis

Linear regression analysis was undertaken for the spectrophotometric analysis calibration plots using at least four concentrations and four replicates. The de-agglomeration profiles were compared using a paired t-test with a level of significance being 0.05.

Results and Discussion

Change in size distribution of interactive mixtures in distilled water over time

When the diazepam(10%)–lactose binary and diazepam(10%)–sls(1%)–lactose ternary interactive mixtures

were added to the distilled water at a concentration giving non-sink conditions, the lactose carrier dissolved rapidly leaving fully dispersed particles and agglomerates of diazepam. Dissolution of diazepam also occurred to yield a saturated solution.

For the binary interactive mixture of diazepam (10%) and lactose, a tri-modal particle size distribution, with modes approximately 400 μm, 50 μm and 7 μm representing undissolved particles of the carrier, diazepam agglomerates and dispersed diazepam, respectively, were evident 4 s after the mixture was added to the water. Laser diffraction provided the volume size distributions and thus the carrier particle volume contributed significantly to the overall distribution. After 2 min, the carrier particles had dissolved (Liu & Stewart 1998). The resulting bimodal distribution with modes approximately 50 μm and 7 μm represented agglomerates and dispersed diazepam particles, respectively. These dispersed particle and agglomerate distributions had been identified in previous dissolution modelling studies undertaken in our laboratory (Alway et al 1996; Liu & Stewart 1998). Over a 60-min period, the area under the agglomerate distribution (approximately 50 μm) became smaller while the area under the dispersed particle distribution (approximately 7 μm) became larger, demonstrating a transition from agglomerates to dispersed particles.

The same type of behaviour was seen when ternary mixtures of diazepam (10%)–sls (1%)–lactose were added to distilled water. However, there were some specific differences with dispersed particle and agglomerate distributions with modes at 4.8 μm and 34 μm, respectively, and a higher initial concentration of dispersed particles due to the presence of the surfactant in the interactive mixture enhancing dispersion. These results were consistent with previous dissolution data modelling studies, which showed that the initial concentration of dispersed particles in ternary mixtures was higher than that of binary mixtures (Alway et al 1996; Supabphol & Stewart 1996a; Liu & Stewart 1998). Surfactant present in the interactive mixture enhanced the rate of agglomerate transition by aiding in particle wetting and dispersion.

Monitoring the degree of particle dispersion

Use can be made of the bimodal distribution described above to monitor the degree of particle agglomeration. If A_{dp} represents the area under the disperse particle distribution and C_{tpv} is the total volume concentration obtained from the particle size analysis ($C_{tpv} = -20\ln(1 - Ob)/3b\Sigma(ViQi/di)$, where Ob is the obscuration, b is the beam length, Vi is the percentage relative volume in class i with mean class diameter of di, and Qi is the extinction efficiency at size class di which depends on the particle optical properties (Malvern Instruments 1996)), then the dispersed particle volume concentration can be calculated.

$$C_{dpv} = C_{tpv} * A_{dp} \quad (1)$$

If A_{ap} represents the area under the agglomerate distribution and C_{tpv} is the total volume concentration, then the agglomerate particle volume concentration can be determined from equation 2

$$C_{apv} = C_{tpv} * A_{ap} = C_{tpv} - C_{dpv} \quad (2)$$

For the particles remaining suspended in the medium, the percentage of dispersed particles (% dispersed) and the percentage of agglomerated particles (% agglomerated) are:

$$\%\ \text{dispersed} = C_{dpv}/C_{tpv} * 100 \quad (3)$$

$$\%\ \text{agglomerated} = C_{apv}/C_{tpv} * 100 \quad (4)$$

The percentage of dispersed or agglomerated particles can be used to compare changes in the state of particle dispersion with time.

De-agglomeration of diazepam(10%)–lactose binary and diazepam(10%)–sls(1%)–lactose ternary mixtures

The agglomerate-particle transition for the 10% diazepam binary and ternary mixtures is shown in Figure 1. There was a significant difference between the binary and ternary profiles (paired *t*-test). The percentage of agglomerated particles decreased from 62.9% to 34.5% over 60 min. The total particle volume concentrations (not shown) of binary mixtures changed from 0.0138% after 2 min to 0.0087% after 1 h, demonstrating the dissolution of some diazepam and all the carrier particles. The agglomerated particle volume concentration (not shown) decreased from 0.0087 to 0.0030%.

For the ternary diazepam–sls mixture, the initial concentration of agglomerates was lower than that of the binary mixture due to the influence of surfactant in wetting and dispersing the agglomerates (Figure 1). The percentage of agglomerates further decreased over the 60-min observation period from 15.6 to 7.8%. The total particle volume concentrations (not shown) changed from 0.0089% after 2 min to 0.0073% after 1 h, due to diazepam and carrier dissolution. The agglomerated particle volume concentration (not shown) decreased from 0.00138 to 0.00057%. Although the same amount of binary and ternary mixtures was used, the total particle volume concentration of binary mixtures was slightly higher than that of the ternary mixtures. The reason could be associated with sampling but it was more likely to be caused by rapid wetting of diazepam due to the sls present in the ternary mixtures resulting in more rapid initial dissolution of particles over the 60 min or with transient increased solubility of the diazepam in the agglomerate environment due to high local concentrations of sls producing a supersaturated solution. The surfactant concentration in the dissolution medium after dispersion in the bulk was well below the critical micelle concentration.

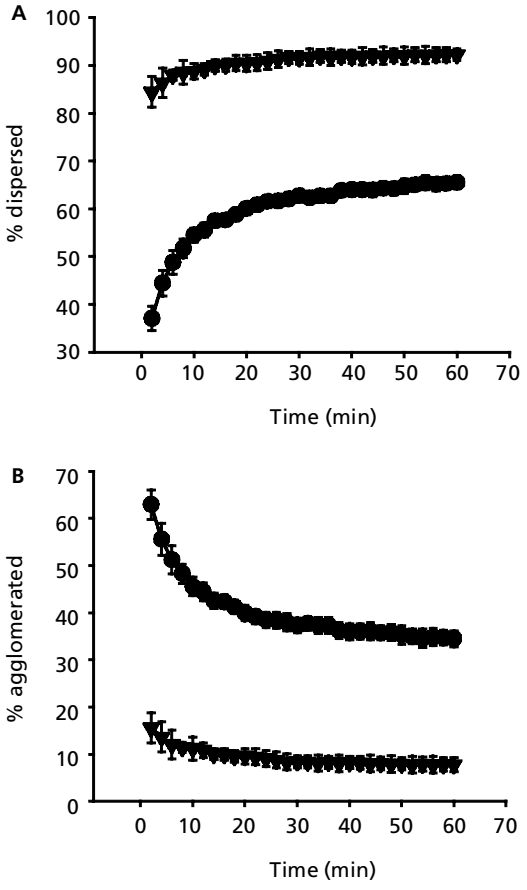


Figure 1 De-agglomeration of diazepam(10%)–lactose binary (●) and diazepam(10%)–sls (1%)–lactose ternary (▼) interactive mixtures with time in 100 mL purified water at 20 °C showing the % dispersed (A; mean (s.d.)) and % agglomerated (B; mean (s.d.)).

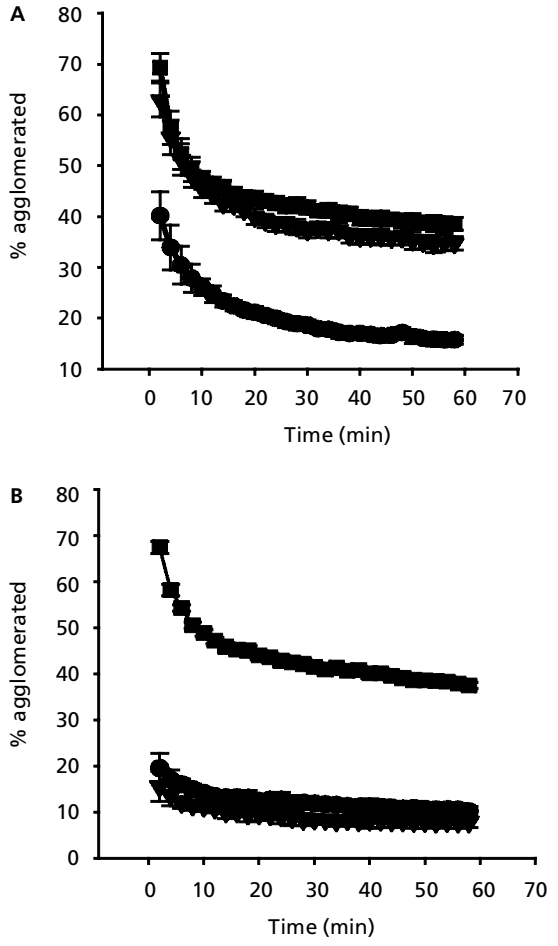


Figure 2 Influence of diazepam concentration on the % agglomerated (mean (s.d.)) over time for diazepam–lactose binary (A) and diazepam–sls(1%)–lactose ternary (B) interactive mixtures in 100 mL purified water at 20 °C (● 5% diazepam mixtures; ▼ 10% diazepam mixtures; ■ 20% diazepam mixtures).

Effect of benzodiazepine concentration on de-agglomeration

The particle size distributions of 400 mg 5%, 200 mg 10% and 100 mg 20% of diazepam mixtures, 200 mg 4%, 100 mg 8% and 50 mg 16% of nitrazepam mixtures and 200 mg 3%, 100 mg 6% and 50 mg 12% of oxazepam mixtures were measured over 60 min after addition to water. The percentage of agglomerates was calculated and de-agglomeration profiles are shown in Figures 2–4 for all mixtures. Agglomeration profiles for all concentration of binary and ternary mixtures for the three drugs were significantly different (paired *t*-test). The results demonstrated that, in general, the percentage of agglomerates was higher in mixtures with high drug loading for all drugs in both binary and ternary mixtures. For example, the percentage of agglomerates for 5% diazepam binary mixtures was much lower than that of 10% or 20% mixtures, while the percentage of agglomerates of 5% diazepam ternary mixture was similar to that of 10% mixture and both were much lower than that of the 20% mixtures. The increase in dispersion in the 5% and 10% ternary mixtures of diazepam was probably due to the higher ratios of sls to diazepam and the subsequent increased capability of the surfactant in the agglomerate to cause dispersion. A similar effect was seen with the oxazepam mixtures, although, while the percentage of aggregates of 4% nitrazepam binary or ternary mixtures was much lower than that of 8% and 16% mixtures, little difference was seen between the binary and ternary mixtures.

The de-agglomeration profiles demonstrated benzodiazepine concentration dependence on the degree of particle dispersion in water as the dissolution medium. The shape of the de-agglomeration profiles revealed some difference in behaviour between the three benzodiazepines used in this study. Diazepam and oxazepam produced de-agglomeration profiles where the initial and terminating degree of agglomeration were in rank order of drug concentration; however, all nitrazepam mixtures, although showing that the initial degree of agglomeration was concentration dependent, demonstrated profiles with a common degree

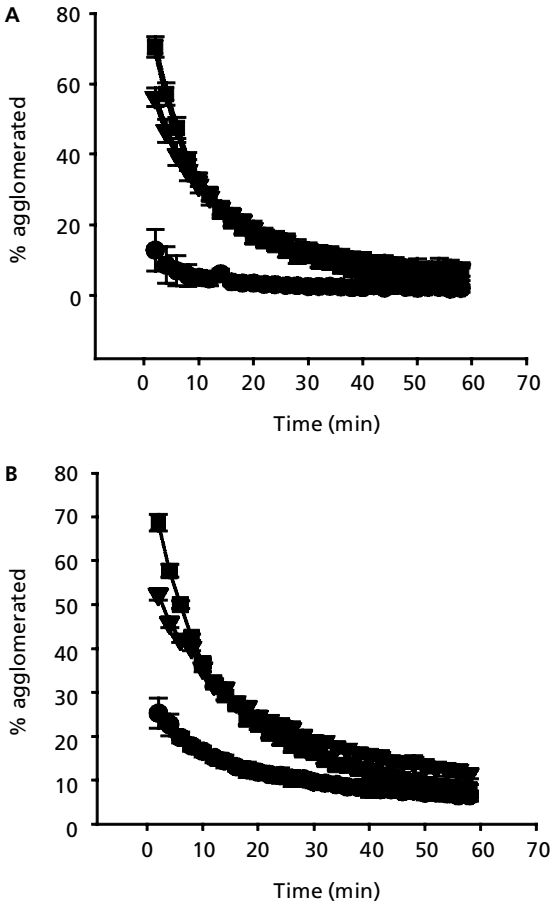


Figure 3 Influence of nitrazepam concentration on the % agglomerated (mean (s.d.)) over time for nitrazepam–lactose binary (A) and nitrazepam–sls(1%)–lactose ternary (B) interactive mixtures in 100 mL purified water at 20 °C (● 4% nitrazepam mixtures; ▼ 8% nitrazepam mixtures; ■ 16% nitrazepam mixtures).

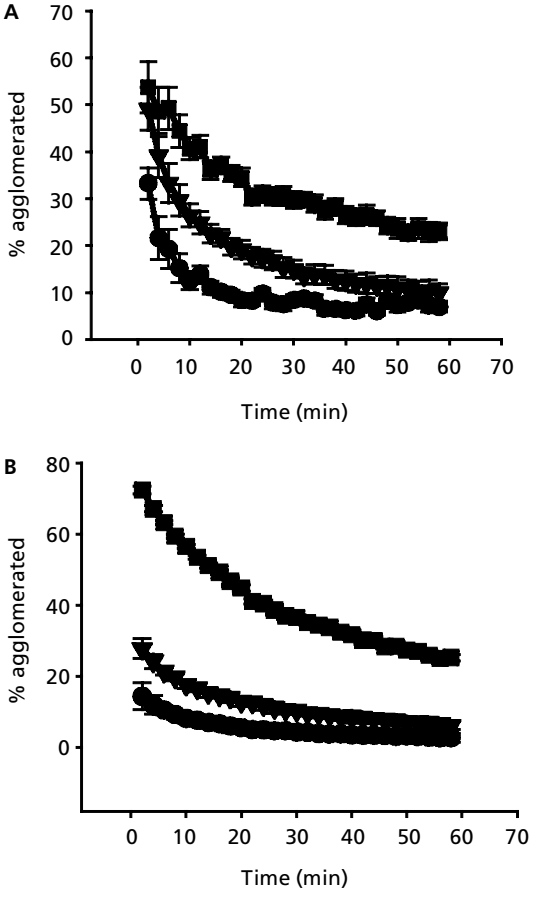


Figure 4 Influence of oxazepam concentration on the % agglomerated (mean (s.d.)) over time for oxazepam–lactose binary (A) and oxazepam–sls(1%)–lactose ternary (B) interactive mixtures in 100 mL purified water at 20 °C (● 3% oxazepam mixtures; ▼ 6% oxazepam mixtures; ■ 12% oxazepam mixtures).

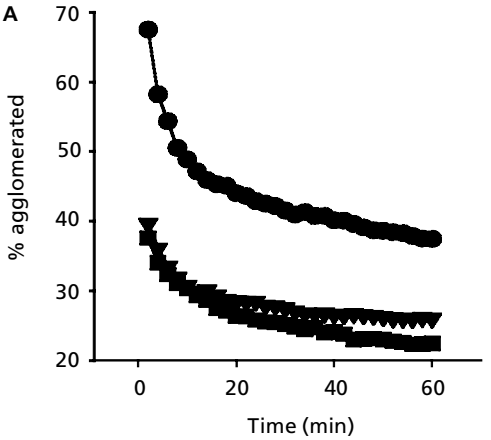
of agglomeration after 60 min. As the agglomerates were formed during the preparation of the mixture, the difference in behaviour could reflect differing mechanisms of agglomeration on the carrier surface perhaps related to the drug's physical properties, surface adhesion and packing.

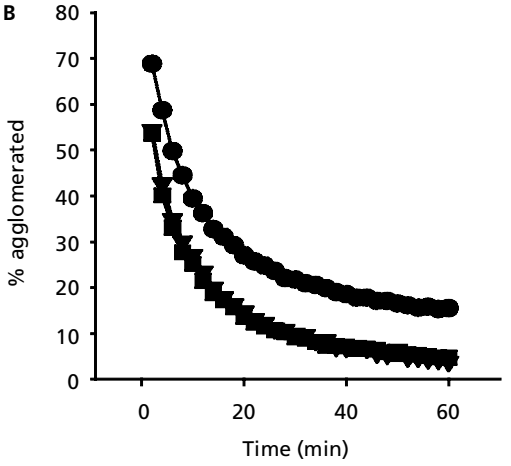
Effect of sls concentration on de-agglomeration

The dispersion behaviour of 100 mg 20% diazepam, 50 mg 16% nitrazepam and 50 mg 12% oxazepam ternary interactive mixtures with 1%, 3% and 5% sls was determined (Figure 5). The de-agglomeration profiles demonstrated less dispersion for the 1% sls mixture, while the 3% and 5% mixtures were similar; however, there were significant differences between the three sls concentrations for each drug (paired *t*-test). Observation of the profiles showed differences in the rate of de-agglomeration between the drugs; however, the major influence of sls was on the initial degree of agglomeration and not on the rate of de-agglomeration. The major influence of sls would be in the initial stages within the mixed drug–surfactant agglomerate environment where transient high concentrations of sls caused increased initial de-agglomeration (Stewart & Liu 2002). Once the sls had dissolved, it would be unlikely to affect the de-agglomeration process as it was no longer associated with agglomerates and its concentration in the water was too small to influence dispersion (Liu & Stewart 1998).

Comparison of sls and cetrimide on de-agglomeration

The dispersion of 200 mg 10% diazepam, 100 mg 8% nitrazepam and 100 mg 6% oxazepam interactive mixtures with 1% cetrimide was determined and compared with same amount of drug with 1% sls (Figure 6). The initial percentage of agglomerates in the benzodiazepine mixtures with cetrimide was lower than in mixtures with sls. This was particularly evident for diazepam and oxazepam mixtures. Observation of the de-agglomeration profiles confirmed that sls was more effective than cetrimide at the same concentration in causing de-agglomeration,





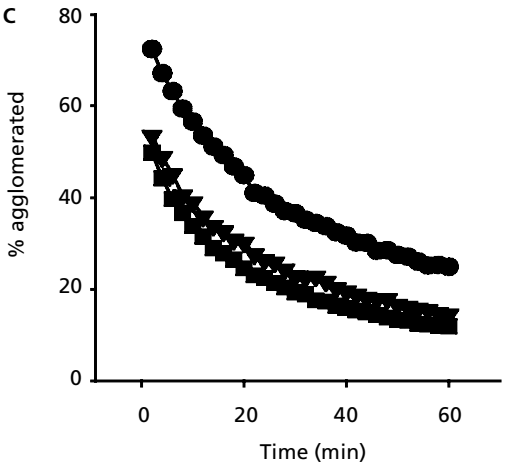
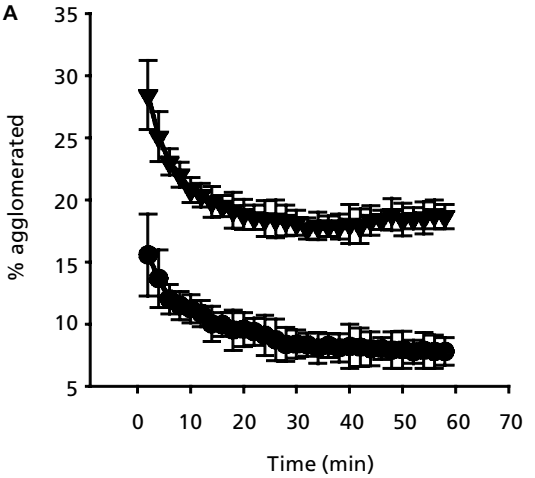
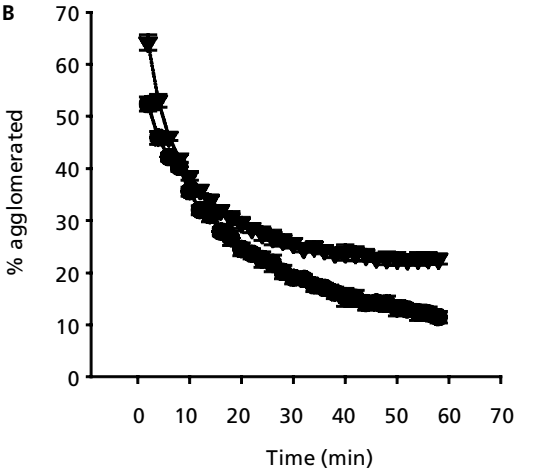


Figure 5 Influence of sls concentration on the % agglomerated (mean (s.d.)) over time for benzodiazepine–sls–lactose ternary interactive mixtures in 100 mL purified water at 20 °C (A, 20% diazepam; B, 16% nitrazepam; C, 12% oxazepam (● 1% sls; ▼ 3% sls; ■ 5% sls).





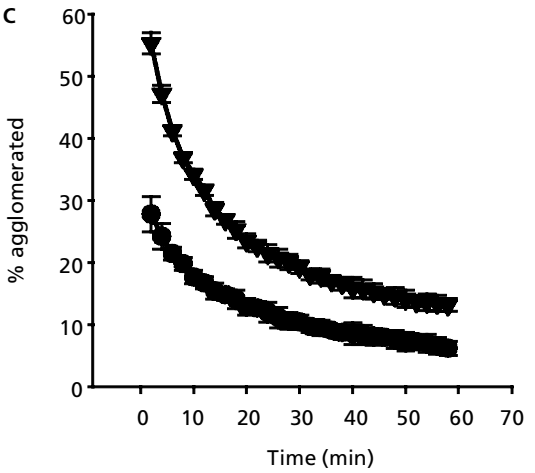


Figure 6 Influence of type of surfactant on the % agglomerated (mean (s.d.)) over time of benzodiazepine–surfactant(1%)–lactose interactive mixtures with sls (●) and cetrimide (▼) in 100 mL purified water at 20 °C (A, 10% diazepam; B, 8% nitrazepam; C, 6% oxazepam).

which was consistent with previous results from dissolution studies conducted under sink conditions (Liu & Stewart 1998).

Relationship with dissolution

The results obtained in this study were consistent with previous studies relating to the dissolution of drugs in interactive mixtures, even though the dissolution studies were performed under sink conditions (Liu & Stewart 1998; Supabphol & Stewart 1996a; Alway et al 1996, Stewart & Liu 2002). It is known that agglomerates form on the carrier surface during the preparation of the mixture, especially as the drug concentration in the interactive mixture increases (Westerberg & Nystrom 1993a, b; Liu & Stewart 1998). When the interactive mixture was placed in dissolution media, the carrier dissolved and the media contained agglomerated and dispersed drugs. The previous studies have interpreted dissolution behaviour using initial concentration of agglomerates. For example, increased drug concentration resulted in a greater concentration of agglomerates in the dissolution media with resulting concentration dependent dissolution behaviour and the presence of surfactant caused greater initial de-agglomeration due to the dissolved surfactant present in the agglomerate. In this study, we see an added complexity in the dissolution process i.e. an agglomerate to dispersed particle transition occurring in all the mixtures tested. The dissolution rate was not only dependent on the initial concentration of the agglomerates, but also depended on the extent and rate of transition. No doubt the kinetic parameters will be dependent on the in-vitro conditions, e.g. stirring speed, apparatus design, and temperature. However, maintaining constant conditions allowed transitions to be compared in the same manner with in-vitro dissolution testing. The methodology described in this research may be useful in formulation design and development to optimize drug and excipient selection to achieve appropriate dissolution profiles.

Conclusion

Particle size distributions of interactive mixtures in water using laser diffraction allowed the state of dispersion or agglomeration of several benzodiazepines to be determined. De-agglomeration of the benzodiazepines occurred over the 60-min observation period; the de-agglomeration curve approached a constant level of agglomeration after approximately 60 min. The extent of the agglomeration during the 60-min period was dependent on the benzodiazepine concentration in the interactive mixture, the type of benzodiazepine and the concentration and type of the surfactant incorporated into the interactive mixture. Higher benzodiazepine concentrations increased agglomeration, while increased surfactant concentration decreased agglomeration. While agglomeration has been observed during the dissolution of micronized drugs in interactive mixtures, the de-agglomeration transition profiles observed in this study have not been reported. Such transition profiles may be important in interpreting dissolution of drugs in general, since the transition rate and residual concentration of agglomerates will influence the dissolution process. Agglomeration characteristics will be related to the drug's physical and chemical properties and to formulation and processing strategies. An understanding of the influence of these properties on de-agglomeration profiles may provide a tool for optimizing excipient selection and processing conditions. In addition, provided that de-agglomeration profiles can be determined for more complex drug formulations, the de-agglomeration profile may provide a useful adjunct to dissolution in predicting the bioavailability of dissolution limited, poorly water soluble drugs.

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