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Short-term changes in drug agglomeration within interactive mixtures following blending

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ABSTRACT

The objective was to investigate the nature and extent of short-term dynamic changes to dissolution within specific interactive mixtures following blending. Two micronized drugs, nitrazepam and flunitrazepam, were formulated into lactose-based interactive mixtures containing a micronized surfactant. The dissolution rate of the drugs decreased significantly over a period of days after preparation. The dissolution was modelled using a multi-exponential equation, allowing estimation of agglomeration and dissolution rate. From this model, decreasing dissolution rates were consistent with increasing agglomeration. Particle-sizing studies provided evidence of an increase in drug agglomerates over the same timescale. This is the first study to report short-term dissolution changes immediately following secondary processing. Several hypotheses are proposed for increases in agglomeration, which potentially relate to changes in surface charge, particle rearrangements, recrystallisation at surfaces and the role of moisture, although the role of mechanical processing on agglomerate behaviour remains poorly understood. The observations from this study may have wider implications, for dissolution and for other powder-based drug delivery systems which include interactive mixtures with fine powders. This study emphasizes the need for improved understanding if we are to implement a "Quality by Design" ethos to improve control and risk management over the performance and stability of these systems.

1. Introduction

Mechanical processing is an essential step in standard pharmaceutical powder blend formation (secondary processing) or particle size reduction (primary processing). The specific process required is usually intended to achieve a particular material structure, and relies on imparting energy and thus powder or particulate motion. This motion will include impacts, rubbing and the making and breaking of material contact points. Such processing is associated with various stresses and tribological effects, and it is believed to have the potential to induce short to medium term physical changes in certain pharmaceutical materials (York, 1983; Elamin et al., 1995). The materials of concern may be drugs or excipients. Chemical reactions may also occur in the solid state, but these are not the issue investigated here. Understanding both the intended as well as unintended outcomes is clearly highly desirable.

Mechanical processing can take the form of a milling or a blending step, whereby the variation in outcome is as a result of the level of energy and the manner and rate in which the energy is applied. A number of studies have previously reported the effect of mechanically induced changes in surface properties of powder following milling steps (Elamin et al., 1994; Newell et al., 2001; Brodka-Pfeiffer et al., 2003; Briggner et al., 1994). The primary process step of milling (size reduction) is generally regarded as being highly energetic, and often inducing for example, a few molecular layers of disorder at the surface of the newly formed size-reduced particles; it is believed that this disorder can result in instability of the powder. The severity of physical changes following milling are reported to be proportional to the energy applied (Briggner et al., 1994; Ticehurst et al., 2000). This instability can be manifested by temporarily enhanced solubility (Elamin et al., 1994) or changes in dispersibility as aerosols (Young and Price, 2004). Such processes are well known in industry; for example in the formulation of dry powder inhalers, where powder conditioning is reported in attempts to reduce physical instability (see publication in patent applications; WO 92/18110, WO 00/32165, WO 05/105043). As illustrated by these patent applications, within the public domain, literature tends to focus on the practicalities of measuring and alleviating this phenomenon, but, studies revealing detailed understanding of the processes are rare. In an example, induced surface amorphous regions identified using AFM has been reported (Young and Price, 2004). Furthermore, Bridson et al. (2007) pointed out that very little has been published on the effects of secondary mechanical activation (blending) on solid dosage forms. Blending can range in energy from high to low shear methods, but is generally regarded as an inherently lower energy process than

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milling. The movement of large particles associated with tumbling processes in blending interactive mixtures, can provide impacting and abrasive collisions, suitable for agglomerate breakdown (De Villiers, 1997). However, the physical changes (agglomerate rather than particle fracture) may be considered to be less severe in mixing as opposed to milling. Unlike milling, where the process is normally limited to one component, blends often encourage contacts of unlike surfaces.

Resulting physical changes may be anticipated to include disorder in the surface structure of particles, electrostatic charge build-up or change in bi-polar charging, agglomerate formation or particulate rearrangements or chemical contamination. All of these result from the tribology of making and breaking of contacts experienced by the particles. As with milling, these induced changes can be of significant interest and importance as they may provide an unacceptable degree of uncertainty, change and inconsistency in product behaviour, potentially leading to catastrophic product failure. Given the discussion above, changes may, for example, be manifest in the form of changes to drug dissolution, or in the aerosolisation of drug particles, or indeed other issues of physical formulation instabilities complicating the product development process. These changes have potentially severe implications for the product development steps of pharmaceutical powder-based products, notably for systems exploiting specific interactive mixtures such as oral or inhaled solids.

In this study, we have examined the dissolution changes in powders following blending. The implications of this study are relevant to other drug delivery forms. Published accounts and studies of secondary process tribology induced material physical changes on dissolution are conspicuous by their absence, despite their potential impact. During manufacture, raw materials and intermediate materials are frequently subject to conditioning periods, under strictly controlled conditions which are set in place with the intention to minimize the problems associated with such material changes. However, experience suggests that these procedures are often historically based, and rarely supported by detailed empirical evidence or mechanistic understanding. The recent regulatory initiatives in risk-based approaches to pharmaceutical quality, for example see "Pharmaceutical Quality for the 21st Century" (from FDA web site http://www.fda.gov/oc/cgmp/) accessed May 2008, and "Quality by Design" (information from FDA web site http://www.fda.gov/cder/Offices/ONDQA/presentations/peri_2007l.pdf) accessed May 2008 are intended to encourage a more knowledge-based approach to these issues, with the motivation that a greater degree of understanding will significantly reduce the risk that these issues may lead to catastrophic product production or development failures.

As argued above, despite the growing experience in the fields of manipulating micronized drug powders for drug delivery purposes, a deeper understanding is often limited (or remains unpublished) and knowledge is frequently empirical. Thus there remain many gaps in our appreciation and understanding of the factors which control the physical stability of products containing fine (micronized) powders or in the engineering of improved performance with such particles. As recognized in "Quality by Design" initiatives, there is clearly a benefit to examining the short-term physical changes in micronized drug-excipient mixtures associated with mechanical processing, in order to quantify impact, and then to improve understanding of the process mechanisms behind this behaviour and hence mitigate risks.

The current study examines a specific formulation platform with the aim of probing the nature and extent of short-term dynamic changes to dissolution within these specific interactive mixtures and considers the factors involved. The project involved the investigation of storage on dissolution over one week, under a range of relative humidity (RH) conditions, of an interactive mix of nitrazepam as a micronized model drug, and a lactose-based carrier incorporating a ternary micronized surfactant, sodium lauryl sulphate (sls). 10%, w/w, drug blends were used. The formulations were used to reproduce conditions similar to those of Liu and Stewart (1998, 2002), and designed to produce an interactive blend that was known to contain combinations of both isolated and agglomerated drug particles. Hence, 10% drug was appropriate to allow for multilayer drug coverage on the carrier so that drug agglomerates could be present. A surfactant (sls) was included at 1% following previous observations of enhanced dissolution rate through increased de-agglomeration and transient increases in drug solubility in the diffusion layer (Alway et al., 1996; Liu and Stewart, 1998, 2002). Following validation of this approach, and interest in the data derived, a second drug, micronized flunitrazepam was formulated following identical protocols and similarly tested.

2. Experimental

2.1. Materials

Micronized drugs (nitrazepam (d_v 6.9 μm) and flunitrazepam (d_v 4.6 μm)) from Alphapharm, Australia, were used as the model adherent drug in the interactive mixtures. These were micronized by fluid energy mill (Chrispro Jetmill 75P, UK). Volume mean diameter (d_v) particle size was obtained from a wet dispersion method on a Malvern Mastersizer S (Malvern Instruments, UK). The micronized drug was subsequently stored at room temperature over silica gel in a desiccator. Sodium lauryl sulphate (sls) (Sigma, Australia) was also used as a micronized powder. A model carrier was prepared for the specific purpose of this study following a validated wet granulation method (Stewart and Alway, 1995) comprising lactose (Wyndalle, NZ) and povidone (Kollidon® 25). A particle size fraction of 250–355 μm was obtained by sieve classification using a sieve shaker (Analysette, Fritsch, Germany) and analytical sieves (Labtechanics, Australia).

2.2. Methodology

2.2.1. Preparation of interactive mixtures

Micronized nitrazepam, micronized flunitrazepam, micronized sls and carrier were pre-weighed and equilibrated at 33% RH (in a sealed desiccator containing a layer of saturated magnesium chloride solution) in a refrigerated incubator (Thermoline RI-250, Australia) which was maintained at 25.0 °C for 3 days before preparing the interactive mixtures. A previously validated method for preparing uniform interactive mixtures, without any detected comminution of carriers, was used (Stewart and Alway, 1995; Alway et al., 1996) to produce the interactive mixture comprising 10% drug (nitrazepam or flunitrazepam), 1% sls and 89% carrier. Mixtures were formed by pre-blending micronized drug with micronized sls prior to placing between two layers of carrier in a glass tube. The tube was rotated several times to prevent any drug from adhering to the walls and agitated for 5 min (Supabphol and Stewart, 1996). Scanning electron microscopy (SEM) was used to observe the surface coverage of the carrier by the benzodiazepine particles.

2.2.2. Scanning electron microscope (SEM)

The nitrazepam interactive mixture was examined by SEM (Hitachi S-570, Japan). A sample of the interactive mixture was glued onto aluminium stubs and dried for 2 h at room temperature. After drying specimens, platinum coating (Balzers SCD-005 sputter coater, Japan) was conducted at a pressure of 50 kpa and a vacuum of 5×10^{-2} mb for 4 min. Examination of coated specimens was at 10 kv voltage and 0.50–10.00k magnification.

2.2.3. Spectrophotometric analysis

Spectrophotometric analyses were preformed using a scanning ultraviolet-visible spectrophotometer (Cecil 6000 series, model CE 6700, Cecil Instruments, England). Beer's Law calibration plots were obtained in absolute alcohol for the homogeneity assessment (nitrazepam, 308 nm, 2–25 mg%) and in distilled water for the dissolution studies (nitrazepam, 259 nm, 0.2–2.0 mg%; flunitrazepam, 252 nm, 0.2–2.5 mg%). At least four concentrations and four replicates were used for the calibration. 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 greater than 0.999 and precision was generally below 1% (except the lowest concentration in ethanol where the CV was 2.4%). The calibration in the dissolution medium was determined at 37 °C in the flow-through cells in the automated dissolution apparatus.

2.2.4. Dissolution of interactive mixtures

Dissolution studies were conducted on an automated dissolution apparatus consisting of a 6 vessel constant temperature water bath (Erweka DT6, Germany), multi-channel peristaltic pump (Watson Marlow Ltd., England) equipped with an on-line UV spectrophotometer (Cecil 6000, Model CE 6700, Cecil Instruments, England). Freshly distilled water (pH 6), degassed prior to use was equilibrated to 37.0 ± 0.5 °C. Distilled water was used as the dissolution medium since there was no significance difference between water and buffers (Stewart and Alway, 1995). Samples of interactive mixture (150 mg) were added to the dissolution apparatus in series and filtered samples were automatically assayed at 2 min intervals over 60 min. A USP/NF paddle method was used at a rotational speed of 100 rpm. The rotational speed of 100 rpm was selected to minimize the effects of extremes of speed: low speed may not provide adequate particle suspension, while higher speeds (>100 rpm) may artificially break up drug agglomerates, and detach drug particles (Supabphol and Stewart, 1996) and be unrealistic when compared with pharmaceutical testing standards (USP30-NF25, 2007).

2.2.5. Particle size distributions of interactive mixtures

Particle size distributions were determined using a validated laser diffraction technique (Malvern Mastersizer S, Malvern Instruments, England). Particle sizing was conducted under non-sink conditions to optimize obscuration. Powder samples were added to distilled water in the Small Volume Sample Dispersion Unit and the particles dispersed using a mechanical stirrer. A suitable amount (~75 mg) of the interactive mixture was added until the obscuration value was between 10% and 15%. The particle size distribution was determined after 1 min, at which stage the lactose carrier had dissolved, and so played no part in the size distributions with stable values recorded.

2.2.6. Storage conditions

Sealed desiccators containing a layer of saturated salt solution were used to obtain storage conditions of specific relative humidities. Saturated salt solutions of magnesium chloride, magnesium nitrate and potassium chloride provided RHs of approximately 33%, 53% and 85% respectively. The desiccators were stored in a refrigerated incubator (Thermoline RI-250, Australia) which was maintained at 25.0 ± 0.5 °C. The RH was confirmed using a thermohygrometer (Shinyei TRH-CZ, Japan) to within ±3.0%.

2.2.7. Powder dissolution modelling

The dissolution profile data were modelled by considering that the nitrazepam and flunitrazepam dissolution occurred from distributions of individually dispersed or aggregated particles which existed after dissolution of the carrier after the interactive mixtures were placed in the dissolution medium (Alway et al., 1996). Multi-exponential equations were used to fit the dissolution data, i.e.

$$C = C_d \exp(-tk_d) + C_{a1} \exp(-tk_{a1}) + C_{a2} \exp(-tk_{a2}) + \ldots$$

where C was the percent of undissolved particles at time t, C_d, C_{a1} and C_{a2} were the initial percent of dispersed (d) and aggregated particles (a1, a2, ...), respectively, and k_d, k_{a1} and k_{a2} were the dissolution rate constants for the dispersed (d) and aggregated particles (a1, a2, ...), min^{-1}, respectively. Combined data from replicate determinations were modelled using the nonlinear least squares curve fitting software package Sigmaplot® (Jandel Scientific 1994) which utilised the Marquardt–Levenberg algorithm (Levenberg, 1944; Marquardt, 1963) to determine the absolute true minima for the sum of squared deviations. Data were unweighted and truncated when the mean of the dependent variable was not significantly different from the final data point. Residuals were shown to be randomly distributed using the runs test (Bennett and Franklin, 1967). The modelling of dissolution data using this methodology has been previously described (Alway et al., 1996).

3. Results

3.1. Nitrazepam interactive mixture

Twenty random samples (approximately 150 mg each) of the fresh interactive mixture and the mixture after one week storage at 33% RH and 85% RH were assayed for nitrazepam. All mixtures had coefficients of variation (CV) of less than 2%, indicating acceptable homogeneity. The samples were taken systematically from fixed areas of the mix to avoid mixture disturbance likely with random

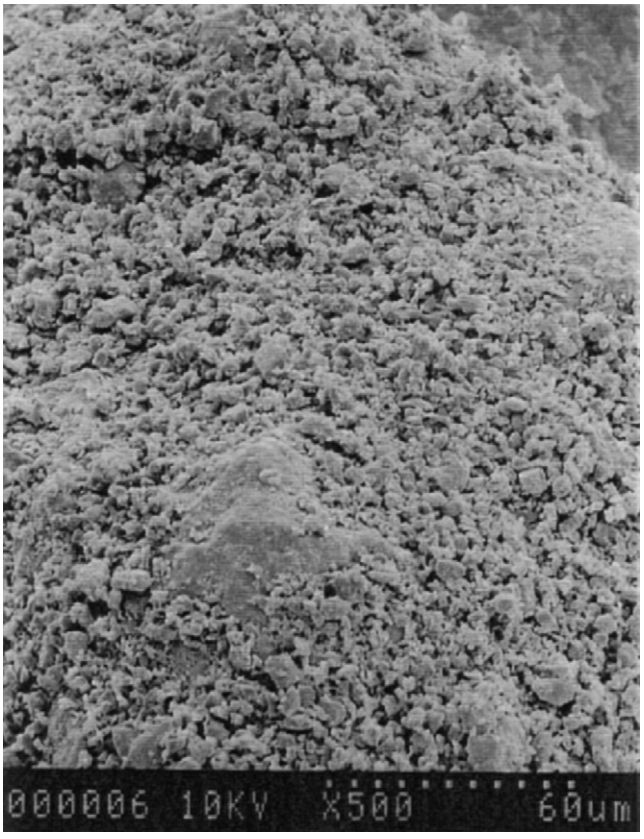

Fig. 1. Scanning electron micrograph of freshly prepared 10% nitrazepam-sls-lactose-povidone interactive mixture (×500).

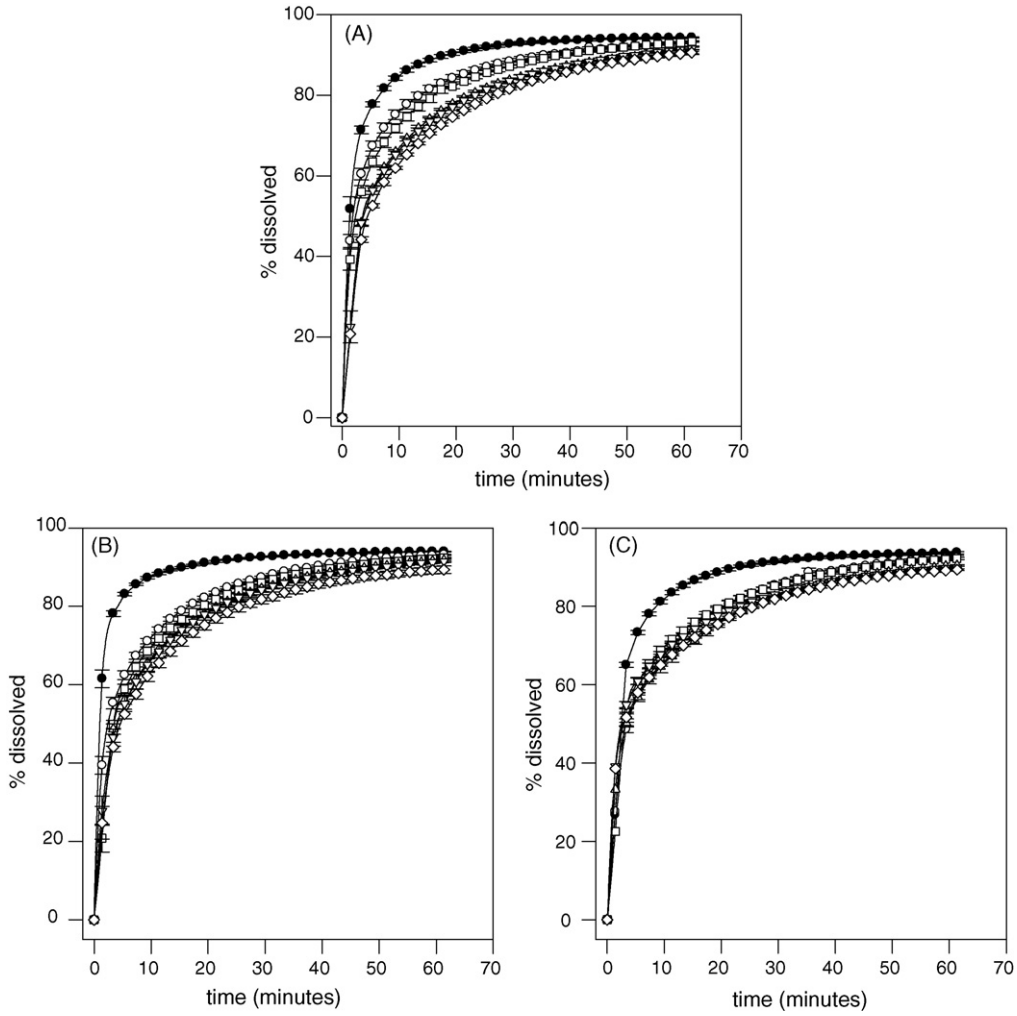

Fig. 2. Dissolution profiles of 10% nitrazepam interactive mixtures at the following storage times: (●) time zero and after, (○) 6, (□) 24, (△) 48, (▽) 96 (120 – 53% RH) and (◊) 168 h of storage. (A) 33% RH (25 °C), (B) 53% RH (25 °C) and (C) 85% RH (25 °C) (Error bars represent standard error of mean {$n = 6$}).

sampling. There was a statistically significant difference in mean nitrazepam content of interactive mixtures before and after storage, with p-values less than 0.01 (85% RH; normality test failed with $p < 0.0001$, Mann–Whitney Rank Sum Test used, $\alpha = 0.05$) as determined at the 5% level using a two tailed paired t-test. The significant changes seen in mean nitrazepam content could be due to a number of factors including sampling variance, moisture uptake in samples (3–5%, 85% RH) and/or decay in electrostatic adhesion forces resulting in slight constituent segregation with nitrazepam particle detachment from the carrier surface. However CVs were ≤2% indicating acceptable homogeneity after one week and these findings were consistent with those of Bryan et al. (1979).

Scanning electron micrographs of the interactive nitrazepam mixture indicated multi-layer drug coverage with formation of some nitrazepam agglomerates on the carrier surface (Fig. 1), indicating structures previously discussed by De Villiers (1997). After one week of storage at low RH, no apparent visual differences in surface coverage were observed. Samples stored at high RH could not be scanned due to problems in sealing the SEM vacuum chamber caused by samples with high moisture content.

3.2. Chemical stability of nitrazepam interactive mixture

Prior to starting the study, the chemical stability of the nitrazepam in the interactive mixture was confirmed before and after storage at 33% RH and 85% RH using a validated stability indicating HPLC assay to analyse the nitrazepam and possible degradation products. Further, both Thermogravimetric Analysis and Differential Scanning Calorimetry studies were conducted on the micronized nitrazepam before and after storage at 33% RH and 85% RH to successfully confirm that hydrates did not form during storage and that polymorphic transitions did not occur (Andreou, 1998).

3.3. Dissolution of nitrazepam interactive mixture over one week

The changes in nitrazepam dissolution from the interactive mixture were investigated, with samples taken at storage times (0, 6, 24, 48, 96 and 168 h) from environmental conditions (33%, 53% and 85% RH at 25 °C). Fig. 2A–C shows the dissolution profiles of the 10% nitrazepam-sls-carrier mixture. Substantial changes in the rate of dissolution were seen over this period. Kruskal–Wallis one-way ANOVA confirmed statistically significant differences ($p < 0.0001$) in the median percent dissolved after 6 h storage for all RHs, and throughout all time periods up to and including one week.

The mixture stored at 33% RH indicated a steady progressive drop in nitrazepam dissolution rate over a one week period (Fig. 2A), while the dissolution data for mixtures stored at higher RHs suggested the rate had already made its greatest change after 6 h, and subsequent change was minimal (Fig. 2B and C). This observation indicated increasing humidity enhanced the process of change, and this was consistent with accelerated recrystallisation of any surface

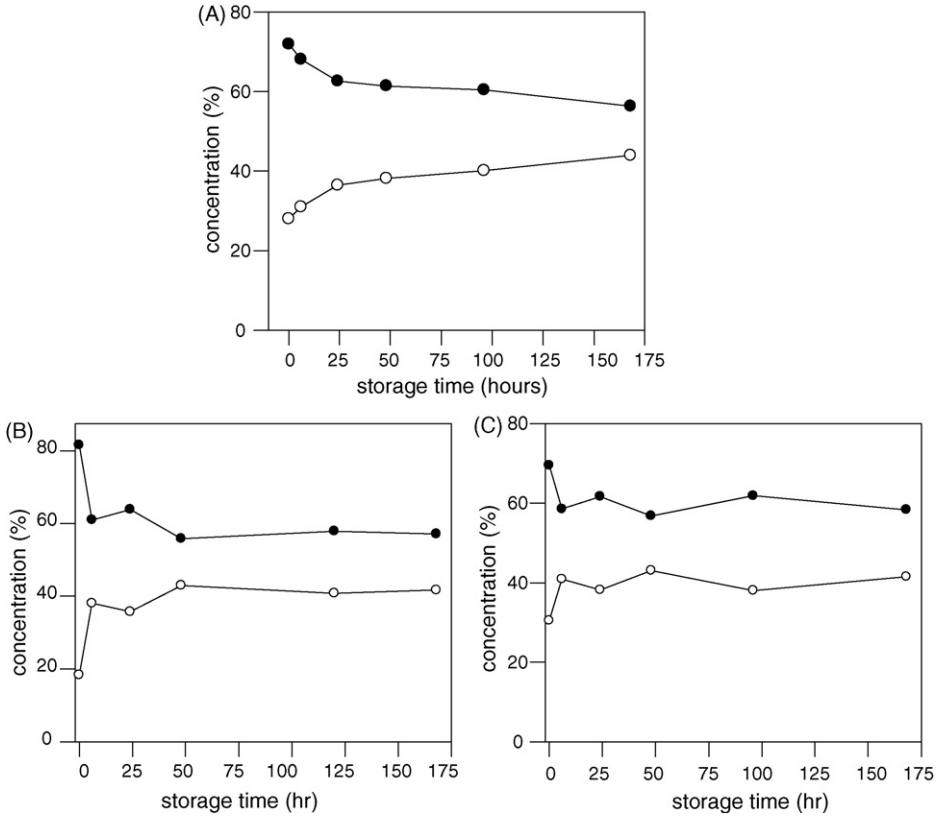

Fig. 3. Estimated initial percent concentration of agglomerated and dispersed nitrazepam mixtures: Ca (○) and Cd (●). (A) 33% RH (25 °C), (B) 53% RH (25 °C) and (C) 85% RH (25 °C).

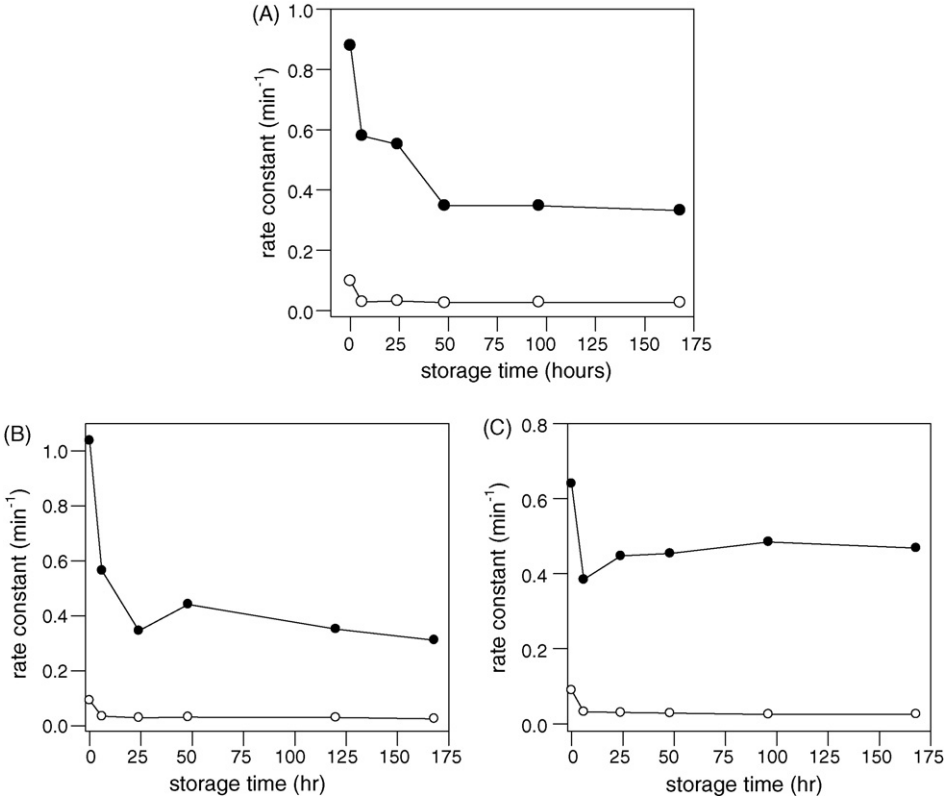

Fig. 4. Estimated dissolution rate constants for nitrazepam mixtures: Ka (○) and Kd (●). (A) 33% RH (25 °C), (B) 53% RH (25 °C) and (C) 85% RH (25 °C).

disordered domains in the presence of enhanced levels of moisture acting as a plasticizer.

3.4. Dissolution modelling

The data modelling demonstrated that the bi-exponential model of the form;

$$C = C_d \exp -k_d t + C_a \exp -k_a t$$

provided the best fit and the parameters of initial concentrations of dispersed particles and agglomerates and their respective rate constants were estimated.

Fig. 3 represents the estimated changes in initial concentration of dispersed and agglomerated nitrazepam (C_d and C_a) in these interactive mixtures under each storage condition. Fig. 4 shows the estimated changes in dissolution rate constants of dispersed and agglomerated nitrazepam (k_d and k_a) under each storage condition. The estimated initial concentration of nitrazepam agglomerates appeared consistent around 20–28%. The general trend, consistent with the changes in the dissolution profiles observed in Fig. 2, was an increase in nitrazepam agglomerate initial concentration to between 42% and 44% with a corresponding decrease in the concentration of dispersed particles. This change was comparatively rapid at the higher RHs. The dissolution rate constant of the agglomerates remained relatively constant over the storage period; however, there was a decrease in the dissolution rate constant for the dispersed particles with storage time Fig. 4. The rate of decrease was more rapid at higher RHs. Thus, the decrease in dissolution rate of nitrazepam over storage time appeared from this modelling to be attributable to an increase in state of particle agglomeration. At 33%,

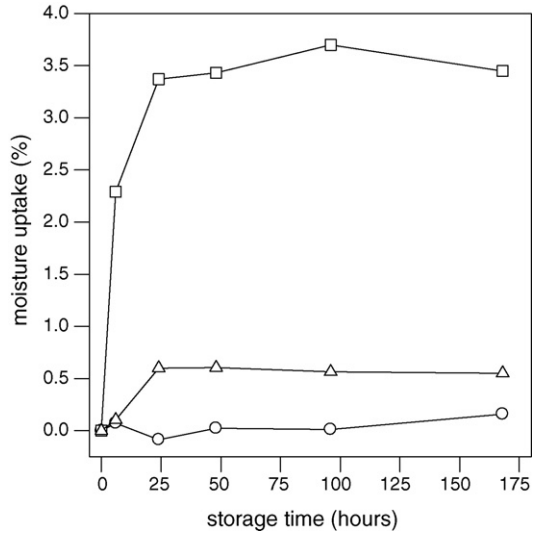

Fig. 5. Percent moisture uptake after storage at (○) 33% RH (25 °C), (△) 53% RH (25 °C) and (□) 85% RH (25 °C).

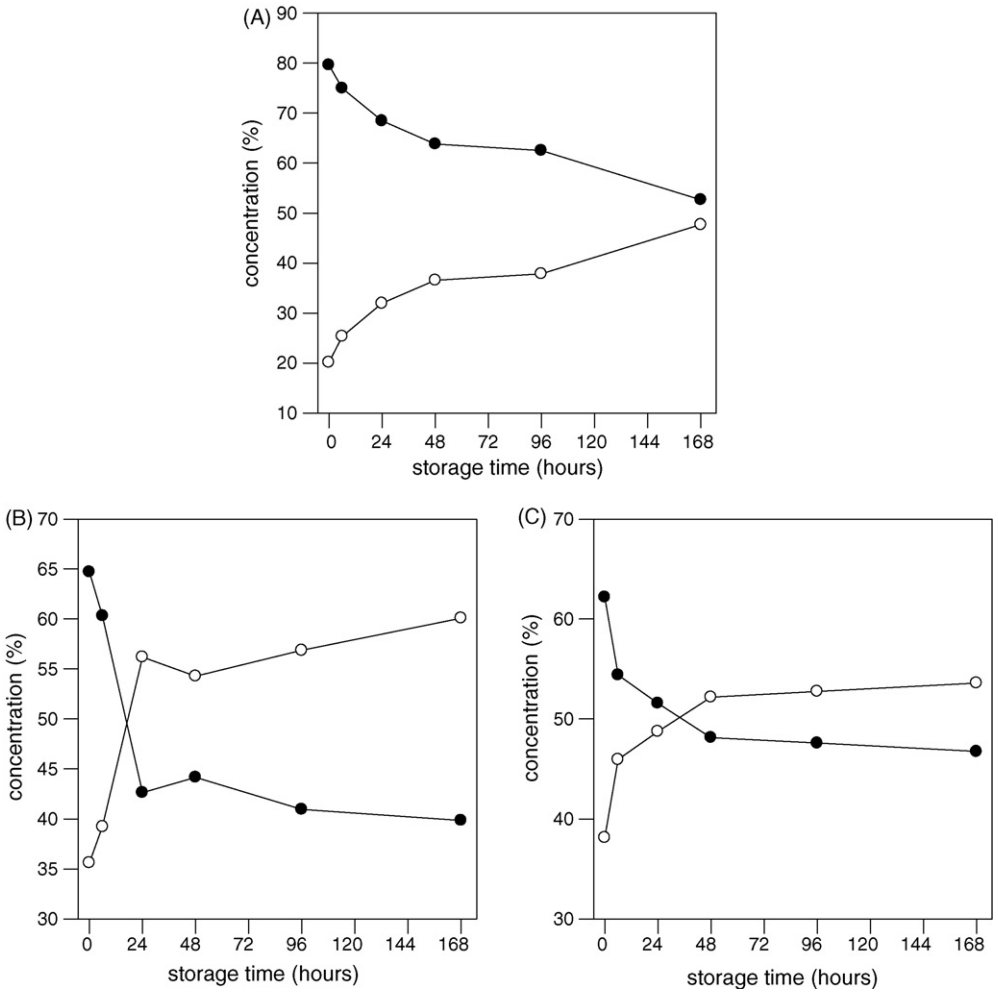

Fig. 6. Estimated initial percent concentration of agglomerated and dispersed flunitrazepam mixtures: Ca (○) and Cd (●). (A) 33% RH (25 °C), (B) 53%RH (25 °C) and (C) 85%RH (25 °C).

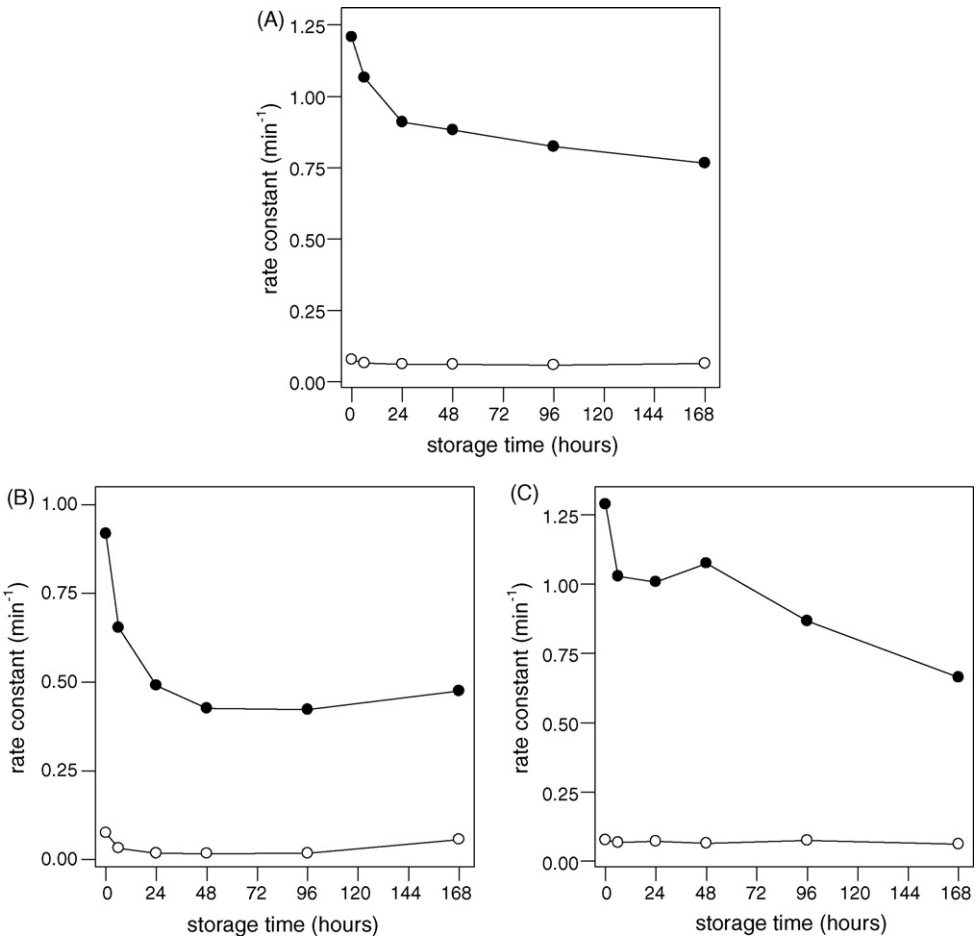

Fig. 7. Estimated dissolution rate constants for flunitrazepam mixtures: Ka (◯) and Kd (●). (A) 33% RH (25 °C), (B) 53% RH (25 °C) and (C) 85% RH (25 °C).

53% and 85% RH the moisture uptake was <0.1%, ~0.6% and ~3.5% after one week of storage respectively, and moisture equilibrium was reached after about 24 h in most cases (Fig. 5).

3.5. Flunitrazepam interactive mixture

Flunitrazepam was used as a second model compound to investigate if the drug dissolution changes after storage were drug (nitrazepam) specific. Flunitrazepam was selected since it belonged to the benzodiazepine series but had different physico-chemical properties (melting point, dissociation constant, solubility profile and distribution constant) to nitrazepam. Formulations were prepared and dissolution tests conducted using identical procedures to those for nitrazepam. The initial homogeneity and chemical stability of the flunitrazepam blends were not tested directly, however dissolution data did not indicate poor content uniformity, and this was deemed to be satisfactory for the purpose of these studies.

3.6. Dissolution of flunitrazepam interactive mixture over one week

As before, the dissolution profiles of the 10% flunitrazepam, 1% sls and 89% carrier interactive mixture were determined at 0, 6, 24, 48, 96 and 168 h after storage under 33%, 53%, and 85% RH and 25 °C. The dissolution profiles are not shown as the outcomes of the dissolution modelling will be shown in Figs. 6 and 7, but had a very similar dissolution pattern of behaviour to those of nitrazepam in Fig. 2. Kruskal–Wallis one-way ANOVA on ranks, $\alpha = 0.05$ (one-way ANOVA, normality test failed, $p < 0.0001$) was undertaken on the percent dissolved values. There was a statistically significant difference (33% RH $p < 0.02$; 53% RH $p < 0.002$; 85% RH $p < 0.005$) in the median percent dissolved data after 6 h of storage and throughout all time periods up to and including after one week (168 h). Dunn's test for multiple comparisons versus the control group (time zero) was applied ($\alpha = 0.05$).

The dissolution data were best fitted by a bi-exponential model. Fig. 6 represents the estimated initial concentration of dispersed particles and agglomerated particles for the interactive mixture. Increasing storage times led to an increase in agglomerate concentrations at all relative humidities (33%, 53%, 85% RH). The rate of change in initial agglomerate concentration was slower at the 33% RH. The maximum level of agglomerates was greatest at the 53% and 85% RH. These results are essentially similar to the behaviour seen with the nitrazepam-sls-lactose interactive mixtures. Fig. 7 represents the estimated dissolution rate constants for the dispersed and aggregated particles of the interactive mixture. Over all relative humidity conditions (33%, 53% and 85% RH) the dissolution rate constant for the dispersed drug particles decreased with longer storage times. The decreasing k_d values were reflected in a decrease in the drug dissolution profiles of the interactive mixture with storage time and were congruent with the changes seen in the k_d values of nitrazepam-sls-lactose interactive mixture. The k_d of freshly prepared flunitrazepam-sls-lactose interactive mixtures varied from 0.90 to 1.25 min^{-1} and decreased to approximately one half of the time zero rate constants after 168 h (Fig. 7). The significant drug dissolution changes seen in both nitrazapam-sls-lactose and flunitrazepam-sls-lactose interactive mixtures occurred at all three relative humidity conditions, and thus these changes were

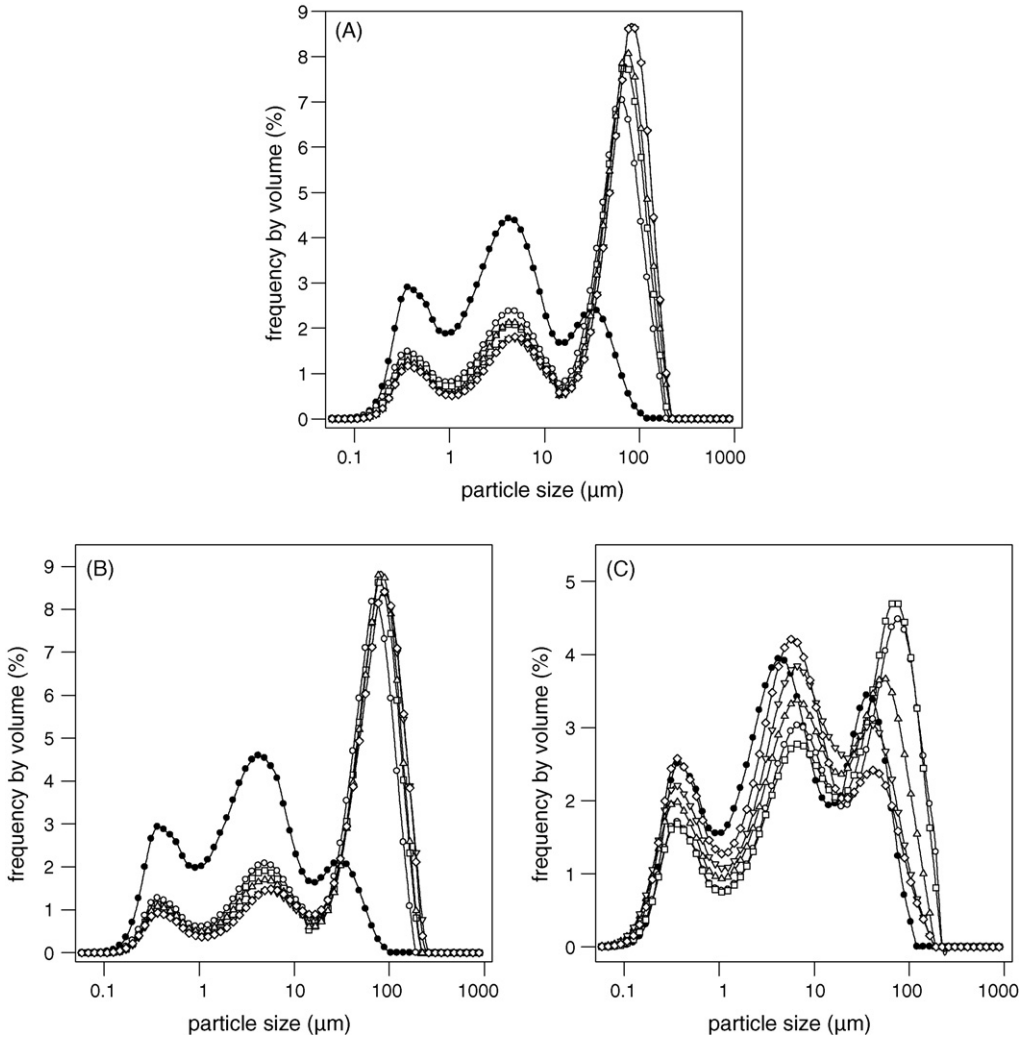

Fig. 8. Particle size distribution of 10% nitrazepam interactive mixtures at: (●) time zero and after, (○) 6, (□) 24, (△) 48, (▽) 96 and (◊) 168 h of storage. (A) 33% RH (25 °C), (B) 53% RH (25 °C) and (C) 85% RH (25 °C).

not due to the formation of hydrates on storage. If the formation of hydrates was the reason for these drug dissolution changes on storage then significance changes would not have occurred at low relative humidity.

3.7. Agglomerate and dispersed particle size measurement

In addition to the dissolution studies, particle size distributions of the nitrazepam and flunitrazepam powder mixtures were determined during the storage period. The purpose of this particle size analysis was to look for evidence of the proposed shifts in populations of the individually dispersed drug particles and the agglomerated drug particles, i.e. as indicated from the changes in dissolution and then interpreted by the bi-exponential modelling analysis. Three modes were noted in each size distribution. The smallest, occurring at median size of approximately 0.2 μm, may reflect a number of submicron particles present but is suspected to be an artifact from the system algorithm and problems with optimising refractive index values, and so is recognized but not addressed as distinct from the dispersed primary drug particles here. The second at a median size of about 6 μm (along with the mode at 0.2 μm) is considered to represent the majority of dispersed primary drug particles, and the third mode, between 20 and 100 μm, is proposed to represent the agglomerated drug entities.

Fig. 8 represents the change in particle size distribution of freshly prepared and stored nitrazepam-sls-lactose interactive mixtures after storage at 33%, 53% and 85% RH. The distribution (mean d_v ~100 μm) was attributed to drug agglomerates. The distributions in Fig. 8 were consistent with the modelling outcomes of the dissolution data indicating that the dissolution occurred from both dispersed nitrazepam particles and nitrazepam agglomerates. The area under the particle size distribution for the lower two modes was indicative of initial concentrations of dispersed particles of nitrazepam determined during modelling while the area under the highest mode was indicative of initial concentration of agglomerated particles. The median particle sizes of the lower two modes was indicative of the dissolution rate constants for dispersed particles while the median particle sizes of the highest modes was indicative of the agglomerate dissolution rate constants.

At 33% and 53% RH the agglomerate concentration increased rapidly after 6 h and reached equilibrium after about 24 h. These results were also encouragingly consistent with the modelling data which indicated rapid increase in the drug agglomerate concentration after 6 h. For example, at 33% RH, the initial change in nitrazepam size distribution was clearly seen with a marked increasing concentration of agglomerates and a marked movement of the agglomerate median diameter to larger sizes. After 6 h, the agglomerate concentration continued to increase with a corresponding decrease in the dispersed particle concentration. In

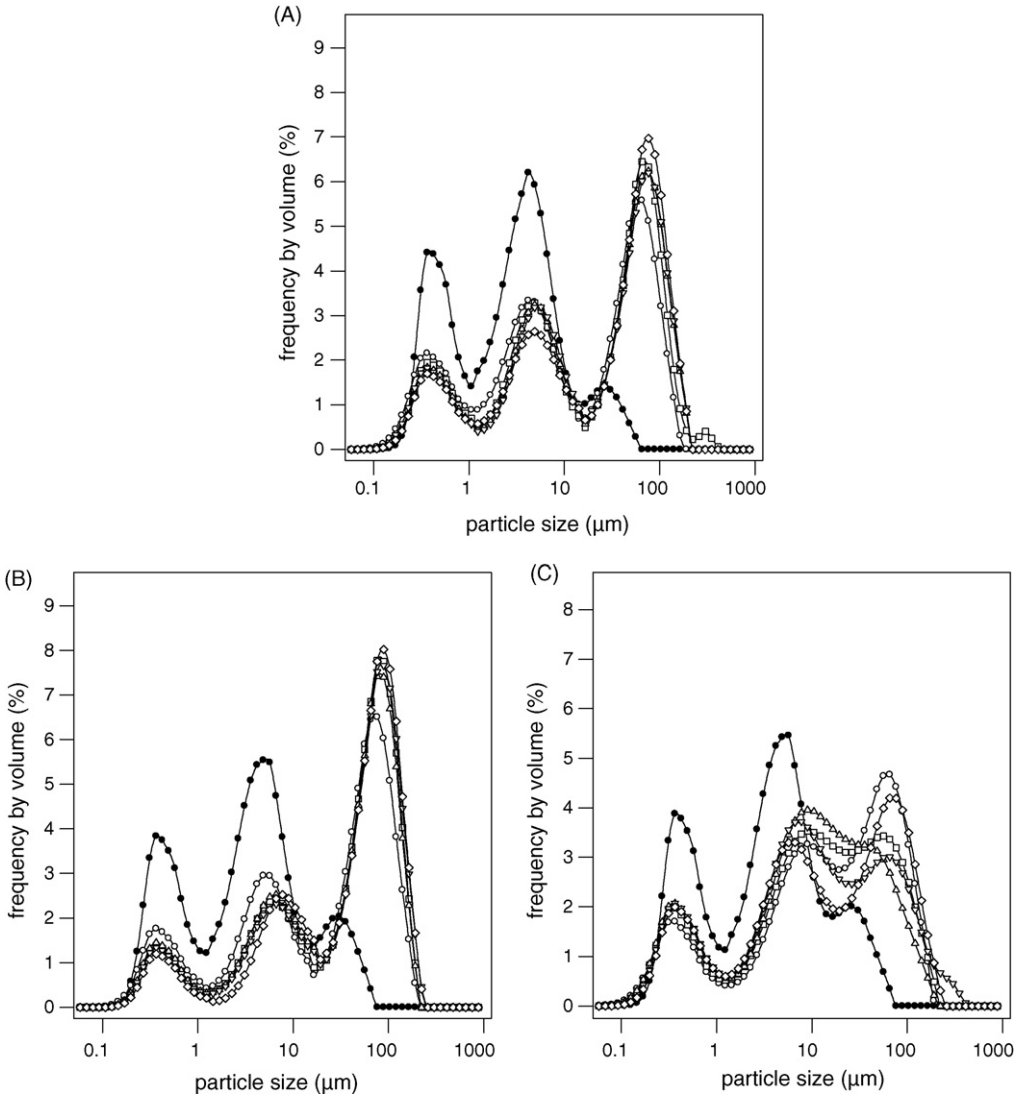

Fig. 9. Particle size distribution of 10% flunitrazepam interactive mixtures at: (●) time zero and after, (○) 6, (□) 24, (△) 48, (▽) 96 and (◊) 168 h of storage. (A) 33% RH (25 °C), (B) 53% RH (25 °C) and (C) 85% RH (25 °C).

addition, there was a small increase in the median of the dispersed particle distribution over the storage period. Both of these results were consistent with the increase in initial agglomerate concentrations and the decrease in the dissolution rate constants of the dispersed particles seen in the modelling results in Figs. 3 and 4. Similar results were seen for the particle size distributions for the mixture stored at 55% RH. At 85% RH there was an initial increase in the agglomerate mode up to 48 h. However, this agglomerate mode appeared to decrease thereafter, possibly indicating a two stage mechanism due to the high moisture content.

The particle size distributions of freshly prepared and stored flunitrazepam-sls-lactose interactive mixtures were determined in the dissolution medium after the lactose carrier had dissolved (Fig. 9). The results were very similar to those seen for nitrazepam in Fig. 8. The drug agglomerate distribution increased rapidly after 6 h of storage at 33% and 53% RH and was consistent with the modelling data which demonstrated an increasing agglomerate concentration on storage. At 85% RH there was an initial increase in agglomerate distribution after the first 6 h but which again decreased thereafter.

The relationship between the changes in the particle size distributions and the modelling outcomes at 85% RH were less compelling, and appeared more complex than those for mixtures stored at 33% and 55% RH in both drug cases, and the mechanisms involved are not immediately apparent. The difference in behaviour at 85% RH may be explained by the loss of electrostatic cohesive forces between agglomerated drug particles due to the high moisture environment leading to decreasing agglomerate distribution. However, the marked shift in median particle sizes, especially for the agglomerates, meant that different agglomerate distributions were being compared during the modelling process.

A shift in the mode of the particle size distributions to the right was observed with increasing storage time at all RHs (Fig. 9). These size distribution changes are qualitatively consistent with the conclusions derived from the dissolution data and the bi-exponential modelling.

4. Discussion

4.1. Possible mechanisms for the agglomeration changes observed

This study shows apparent changes in the dissolution profiles for interactive mixtures on short-term (one week) storage following secondary processing (blending). Interactive systems comprising nitrazepam-sls-carrier produced significant reduction in drug dissolution rate after storage. The significant drug dissolution changes

in the flunitrazepam-sls-lactose interactive mixtures seen on storage confirmed that these changes were not specific to nitrazepam. This finding indicates that other micronized pharmaceutical drugs, following incorporation into interactive mixtures, may undergo drug dissolution changes on subsequent storage and care should be exercised in interpreting dissolution data for these systems. Given that no changes in polymorph or hydration were detected during storage, an alternative explanation for these changes is required.

The bi-exponential modelling outcomes were consistent with the hypothesis that increased drug agglomeration had occurred and explained the reduction in dissolution rate. This was then further supported by the particle sizing analysis of the powder mixtures. So our modelling and particle sizing analysis suggests that these changes are due to relative changes in inter-particle adhesion, and consequent agglomerate composition and formation over the period of short-term storage.

It is not clear what degree and form of particle or material mobility is required to manifest these changes, i.e. is it possible these are as a result of gross particle mobility on the carrier surface during storage, or more slight movements due to relaxation and subtle changes of inter-particulate contacts, or even solid state mobility of molecules at contact points, as associated with amorphous–crystalline transitions. Few previous studies have indicated such changes. Despite this, anecdotal reports of industry experience suggest that agglomerate formation coupled with concurrent reduction in measured surface area following milling are not uncommon (personal communication: Brigner, L.E., 2008, in discussion of contents of Brigner, L.E. in Proceedings of Arden House European Conference, AAPS, London, 2008).

It is well known that adhesive forces during mechanical preparation of powders arise from a range of electrostatic, non-electrical van der Waals and from capillary based forces. Electrostatic forces can play a significant role in blends resulting from bi-polar triboelectrical charging. Their effects are particularly important over longer ranges, and are renown for being relatively short-term in nature, especially where materials or raised humidity allow efficient charge decay in powder beds. Rapid leakage of charge is commonly attributed to absorbed moisture films and humidity. The accelerated changes are observed in our interactive systems at higher humidities. Consequently, it is logical to propose that triboelectrical bi-polar charging is a possible contributor to the observed changes, and could drive relative particle movements.

Van der Waals forces, which tend to exert a greater influence over very short (nano-scale) distances can be modified by the presence of ternary agents (Begat et al., 2005). This is indeed the case when surfactant molecules provide a physical block to contact between solid surfaces. The effect can be an apparent reduction in the strong short-range van der Waals forces, thus allowing more facile subsequent rearrangement. It is likely that in these interactive mixtures, the drug particles will not be well coated with sls, but possess sporadic partial areas of the surfactant on its surface. Hence, initial rearrangement of these particles is postulated, from contacts interrupted by sls, to clean drug contacts. Moisture ingress may be involved in facilitating recrystallisation of disorder at the surface of each particle, where the disorder is generated by the tribological action. Such could also involve formation of solid bridges between particles, hence drawing them more strongly together as agglomerates. However, the role of sls is not clear if this view is taken.

Finally, capillary forces can become dominant forces of adhesion between particles, once sufficient moisture is present for these to be established, which is suggested by the moisture increases beyond 24 h on storage.

Consequently, during the storage of these interactive mixtures, numerous hypothesised mechanisms can be proposed to account for adhesion force changes in the drug-sls structures as a function of humidity over time.

We have previously raised interesting parallels in the behaviour of agglomerates in both oral and inhaled products (Allahham and Stewart, 2007): such may be explained by the nature of interparticulate forces and hence agglomerate structure and the agglomerate mechanical strength which controls dispersion behaviour. The previously proposed changes in adhesion of fine drug particles present in inhalation interactive mixtures (Young and Price, 2004) may in part be due to related mechanism(s) responsible for our observed changes here. Hence, the results of this study may also have relevance to inhaled dosage forms, and emphasize the need to study and understand such short-term changes in fine particle agglomeration.

5. Conclusions

This study has revealed that the dissolution rate of fine drug particles mechanically formulated into interactive mixtures with carrier and additives can decrease significantly on short-term storage of the order of days. These changes are accelerated in the presence of raised humidity.

We understand this is the first study to report such short-term dissolution changes immediately following secondary mechanical processing. Our modelling of the dissolution data, together with the particle sizing data indicate that dissolution changes result from increasing drug agglomeration. The observations may have wider implications, for dissolution and for other powder-based drug delivery systems where de-agglomeration is important, which include fine powders and/or interactive mixtures, such as oral and inhaled forms.

Extending our previous knowledge base, we have also attempted to develop potential hypotheses which are consistent with these changes. The mechanisms for these observed changes are not well understood. Several hypotheses are proposed, which relate to changes in electrostatic bi-polar surface charge, to subtle rearrangement in particle orientations driven by van der Waals forces, to recrystallisation at surfaces and to the role of moisture. These hypotheses indicate that surfactant may play a role, and further studies are underway to progress our knowledge of the effect of presence and concentration of surfactant.

The role of fine particle agglomeration and agglomerate behaviour remains poorly understood, despite its key role in oral and inhaled drug delivery forms and beyond. This study emphasizes the need for enhanced understanding if we are to improve long-term control and risk management over the performance and physical stability of these systems.

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