Vinyl polymers-coated lorazepam particles for potential intrananal delivery

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ABSTRACT

A particle engineering method that adsorbs a microfine vinyl polymer coat to crystalline drug microparticles has been shown to be an effective way to control delivery. However, the means by which the functional performance of such microparticles is altered by the behaviour of the polymers in the microparticle coat remains unclear. The aim of this study was to determine the influence of vinyl polymer coating on the *in vitro* delivery characteristics of intranasal lorazepam microparticles. A series of four, similarly sized (ca. 10 µm), lorazepam-rich microparticles with different polymer coats were generated. The absorption of the polymer coats appeared to disrupt lorazepam solid state dimer formation in the microparticles, which manifested in a reduction in drug melting point. Mildly cohesive particles (aerodynamic diameter of 32 µm) that allowed rapid drug release (ca. 80% in 5 min) were generated when partially hydrolysed PVA dominated the microparticle coat, whilst fully hydrolysed PVA reduced particle cohesion and retarded drug release (ca. 15% release in 5 min). Infrared analysis showed that the properties of the microparticles were dictated by the strength of the hydrogen bonding in the polymer coat and not the strength of coat adsorption that was facilitated by hydrogen bond formation between the hydroxyl groups of the PVA and the hydroxyl group at position C3 of the lorazepam diazepine ring.

Keywords: lorazepam; intranasal delivery; coating; microparticles; poly(vinyl alcohol); PVP.

INTRODUCTION

Insomnia is commonly treated by oral administration of lorazepam, but the 1-2 hour delay to induce significant sedation is a major barrier to effective therapy. This delayed onset can often result in over dosing due to the patient taking another dose in an attempt to initiate the clinical effects. Intranasal administration of lorazepam could dramatically improve the immediacy of sedation. The large surface area of the nasal mucosa provides rapid absorption into the systemic circulation and the possibility of direct access to the central nervous system (Costantino et al., 2007). Nasal delivery also has the benefits of being non-invasive and avoiding 'first-pass' metabolism.

Attempts to reformulate lorazepam have been hindered by its lack of aqueous solubility (ca. 0.08 mg/ml) (Moffat et al., 2004) and poor chemical stability (Archontaki et al., 1999). Although dissolving this active agent in propylene and polyethylene glycol solutions appears to resolve the chemical stability issues, there have been reports of possible toxicity associated with these solubilisers (Laine et al., 1995; Cawley, 2001). Considering the physicochemical properties of lorazepam, formulating this drug in the form of a dry powder would appear to be a sensible approach, but a particulate based system would require efficient aerosolisation and rapid dissolution to ensure a superior clinical outcome to the oral dosage form. Applying an appropriate particle engineering method to generate a lorazapam rich microparticle is one potential means to achieve this.

It has been demonstrated that efficient particle engineering facilitates fine control over the size, density and morphology of a material which can be used to influence the behavior of a delivery system. For example, Bao and Zhao (2010) reported a membrane emulsification approach that

could produce uniform microparticles with controllable size. Edwards et al. (1997) utilized a spray drying method for the formation of low density porous poly (lactic acid-co-glycolic acid) particles containing insulin and testosterone. Chew and Chan (2001) modified the surface morphology of bovine serum albumin microparticles to generate 'corrugated particles'. Rehman et al. (2004) employed supercritical fluids to modify the crystallinity of terbutaline sulphate microparticles. Rogers et al. (2003) attempted to use a method of spray-freezing into liquid to manufacture novel amorphous danazol microparticles with improved dissolution characteristics. However, many of these methods show limited control over drug crystallinity and the generated particles often exhibit an extremely diverse morphology.

One approach that has the potential to overcome the aforementioned issues is the generation of microparticulate carriers using an engineering technique that facilitates biocompatible macromolecule adsorption. Vinyl polymers such as poly(vinyl alcohol) (PVA) and poly(vinyl pyrrolidone) (PVP) have previously been shown to modify microparticle behavior in inhaled formulations (Buttini et al., 2008a,b), whilst maintaining excellent control of physical stability in both the dry state and in suspension (Jones et al., 2006a,b). The biocompatible macromolecule coating process is facilitated when vinyl polymers are employed as the coating agents by their ability to spontaneously adsorb onto the surface of hydrophobic drugs in aqueous solutions (Buttini et al., 2008b). Coating using vinyl polymers is known to proceed in a multilayered manner as a result of the intra and intermolecular hydrogen bonding that occurs between the vinyl polymer chains (Buttini et al., 2008a,b).

The aim of this study was to investigate how an adsorbed vinyl polymer coat influenced the key delivery characteristics of intranasal lorazepam microparticles. In order to achieve this, a specific series of vinyl polymer-coated microparticles were generated. The method of Buttini et al.

(2008a,b) was manipulated in order to modify the nature of polymer adsorption whilst maintaining a constant final particle diameter. In total, a series of four test microparticles were generated using a mixture of PVA and PVP which was varied to generate a 'standard' particle, similar to that produced previously ($Lorz_{pva}$) (Buttini et al., 2008a); a particle that had a coat dominated by fully hydrolysed PVA, i.e. a coat with extensive intra-molecular hydrogen bonding ($HyLorz_{pva}$); a particle with a high viscosity coat ($HwLorz_{pvp}$) and a particle where a high proportion of PVP was employed ($HpLorz_{pvp}$) (Table 1). The interaction of the two polymers with each other (Fourier Transform Infrared (FT-IR) spectrometry analysis) and with the drug (differential scanning calorimetry), an assessment of particle cohesiveness (impaction assessment) and drug release (modified United States Pharmacopeia (USP) dissolution) was compared in an attempt to elucidate the influence of the polymer employed in the adsorption process upon the particle behavior.

MATERIALS AND METHODS

Materials

Lorazepam (Ph Eur) was supplied by Cambrex Profarmaco (Milano, Italy). Potassium dihydrogen orthophosphate and high performance liquid chromatography (HPLC) grade orthophosphoric acid, cylcohexane, water, methanol, ethanol and acetonitrile were all purchased from Fisher Scientific (Loughborough, UK). Formic acid, 1-chlorobutane, sodium chloride and sodium dodecyl sulfate (SDS) were supplied by Sigma-Aldrich Ltd. (Poole, UK). PVA 28-99 and PVA 23-88 were supplied by KSE (Troisdorf, Germany). PVP (Kollidon 17) and Solutol HS 15 were supplied by BASF (Wantage, UK). PVP (Kollidon 90) was supplied by ISP (Calvert City, USA) and ammonium solution 25% by BDH (Poole, UK).

Microparticle Production

Approximately 1.0 g of lorazepam (6.73 µm) was weighed in to an amber flask (100 ml) which was then filled with an aqueous solution containing mixtures of PVA and PVP of various grades and concentrations to produce the four suspensions (Table 1). These suspensions were spray-dried using a Buchi 191 bench top spray drier (Buchi, Switzerland). During spray-drying the suspensions were held at ambient temperature (20 ± 2°C) with the exception of $HpLorz_{pvp}$ which was heated to 80°C and agitated by constant magnetic stirring to ensure adequate suspension stability during manufacture (Stuart Scientific, Stone, UK). The inlet temperature of the spray-drier apparatus was maintained at 180ºC, the nozzle air flow at 650 $ml.min^{-1}$, the atomisation flow at 70% and a feed rate at 3 $ml.min^{-1}$. The spray-dried particles were collected on wax paper and stored under desiccation until required for further analysis. The yield of the process was calculated (Eq. 1).

$$\text{yield (\%)} = \frac{\text{Mass of particles post spray drying}}{\text{Initial solid mass in the suspension}} \times 100 \qquad \text{(Eq. 1)}$$

The lorazepam content uniformity of the spray-dried microparticles was tested by HPLC. A 10 mg aliquot of each microparticle batch was added to 100 ml of an ethanol/water co-solvent mixture (1:1, v/v), the solution was diluted 1:10 (v/v) using the same solvent and the lorazepam content assayed by HPLC. Lorazepam content in each formulation was calculated by dividing the drug mass by the mass of the particles post spray-drying and the relative standard deviation was used as the indication of drug content uniformity (n = 6).

146 **Laser Diffraction Particle Size Analysis**

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148 The size (volume mean diameter, (VMD)) of the spray-dried lorazepam microparticles was
149 determined using laser diffraction (Mastersizer X, Malvern, UK). The lens used was 100 µm,
150 active beam length 14.3 mm and the sample unit was a MS-7 (magnetically stirred cell). A
151 concentrated sample of each formulation was suspended in lorazepam-saturated cyclohexane
152 before being sonicated in a water bath (Model F5100b; Decon Laboratories, UK) for 10 s to
153 ensure dispersion of any aggregates. The samples were added drop-wise into the MS-7 cell
154 containing drug-saturated cyclohexane with continuous magnetic stirring until an ideal
155 obscuration (10-30%). Measurement was repeated eight times for each sample and three samples
156 measured per batch using a randomised sampling procedure.

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158 **Thermogravimetric Analysis**

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160 The volatile content of the spray-dried microparticles was determined using a 2050
161 thermogravimetric analyser (TGA) (TA instruments, Crawley, UK). Samples of each
162 microparticle batch (approximately 10 mg) were assessed in individual open aluminium pans and
163 placed into the sampler of the TGA instrument. A heating rate of $10^{o}C.min^{-1}$ from $25^{o}C$ to $300^{o}C$
164 was used to determine the percentage of volatiles present in the spray-dried samples.

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166 **Differential Scanning Calorimetry**

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168 Thermal measurements were carried out using a 2920 modulated differential scanning
169 calorimetry (DSC) equipped with thermal solutions universal analysis software® (TA
170 Instruments, Crawley, UK). Prior to analysis the DSC was calibrated using an indium standard.

Approximately 2 mg of each sample was analysed by placing it into hermetically sealed pans (TA instruments, Crawley, UK) with a pinhole in the roof. Samples were heated with a modulated heating method (+/- $1^{o}C.min^{-1}$) using a heating rate of $10^{o}C.min^{-1}$ from $25^{o}C$ to $300^{o}C$. Oxygen free nitrogen was used as the cell purge gas with a flow rate of 100 $ml.min^{-1}$. The melting temperature of the sample was taken as the onset of the endothermic peak of the resultant thermograms.

Fourier Transform Infrared Spectroscopy

Absorption infrared spectra of the polymers, lorazepam and coated microparticles were recorded at room temperature using a Perkin-Elmer FTIR 1720X spectrometer (Perkin-Elmer, UK) fitted with a DurasamplIR attenuated reflectance (ATR) unit (SensIR Technologies, UK). The machine was calibrated with a polystyrene standard as per the manufacturer's instructions. The powders were pressed directly onto the ATR crystal using the sampling unit. Each sample was run at a 4 cm^{-1} resolution over the 400-4000 cm^{-1} range.

Particle Aerosolisation Assessment

The assessment of particle aerosolisation behaviour was performed using a Multi- Stage Liquid Impinger (MSLI) (Astra Draco, Lund, Sweden) and its design and working mechanism are detailed in Pharmacopeia (US Pharmacopeia XXXIII, 2010). A simple glass delivery device (made in house) was attached to the upper end of a stainless steel 90° induction port on top of the impinger. Ethanol (20 ml) was added to each stage of the MSLI and the rubber stoppers were inserted before the impinger was tilted to wet the stoppers and reduce electrostatic charge. A 2 mg sample was weighed directly into the delivery device. The vacuum pump was turned on (30

196 $L.min^{-1}$) while covering the end of the delivery device containing the powder to prevent ejection.
197 Uncovering the end of the device exposed the powder bed to the airflow which released the
198 material into the MSLI. The pump continued to draw air through the MSLI 10 s post dose release.
199 The particle size cut off points for each stage of the impinger were calculated as 18.39 µm at stage
200 1, 9.62 µm at stage 2, 4.38 µm at stage 3 and 2.40 µm at stage 4, any smaller material was collected
201 on a filter at the base of the impinger. The MSLI was carefully rotated and inverted, avoiding
202 transfer of solution between stages, in order to wash all internal surfaces of each stage of the
203 impinger. The metal throat was rinsed into Stage 1 and the connecting tube between each stage
204 was washed into each subsequent stage using ethanol. Each stage in turn was then emptied into a
205 50 ml volumetric flask and washed with approximately 20 ml of an ethanol/water co-solvent (1:1,
206 v/v). The rubber stoppers were also rinsed into the corresponding volumetric flask (50 ml) for each
207 stage. All samples were analysed by HPLC. Mass median aerodynamic diameters were calculated
208 by interpolation as per the European Pharmacopeia method (Anon, 2002).
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210 **Dissolution Testing**
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212 To determine the release rate of lorazepam, a modified USP dissolution method was developed
213 since it is practically difficult to keep the particles in the dissolution media when employing
214 traditional USP methods. The dissolution apparatus was performed in a water bath at 37ºC
215 (continually monitored using a temperature probe). The rotation speed of the apparatus was
216 calibrated and set at 100 rpm. The dissolution vessels were filled with 500 ml of water
217 containing SDS at either 0.2 or 0.5%, w/v (lorazepam solubility: 416.5 µg/ml at 0.2% and
218 1413.95 µg/ml at 0.5%). A known weight of lorazepam alone or in the form of the spray-dried
219 microparticles (ca. 2-5 mg of drug; sink conditions maintained in all experiments) was applied as
220 a thin layer to a piece of double sided PVA sellotape which was attached to a small metal disc.

The metal disc was screwed directly on to the end of the stirring rods and inserted into the dissolution vessel. Samples (1 ml) were taken at a range of time points up to 60 min for HPLC assay and immediately replaced with the same volume of fresh, pre-warmed dissolution media.

Lorazepam Assay

A reverse-phase (RP) HPLC method was used to quantify lorazepam. The analysis was performed using a Waters HPLC system which consists of an autosampler (Waters 717 plus), a pump controller (Waters 600E), a dual lambda absorbance detector (Waters 2487) and Millennium software (Version 4.0). A Chromolith® performance RP-18e (100 x 4.6 mm) column with a Chromolith® RP-18e guard cartridge (5 x 4.6 mm) was used for the separation. The mobile phase consisted of 75% (v/v) water (pH 2.1, adjusted with orthophosphoric acid) and 25% (v/v) acetonitrile containing 35 mM potassium dihydrogen orthophosphate with a flow rate of 2 $ml.min^{-1}$. The sample injection volume was 10 µl and UV detection was at 220 nm. The resulting lorazepam retention time was 12 min. The needle wash was performed using a mixture of methanol and water (90:10, v/v). The method was validated in terms of system suitability, sensitivity (LOD = 0.157 µg/ml ± 0.094, LOQ = 0.477 µg/ml ± 0.285), linearity (> 0.999), accuracy (between 95-105%) and precision (intermediate precision between 0.95 and 1.05) and shown to be 'fit for purpose' for the analysis of lorazepam.

Statistical Analysis

All data were presented as mean ± standard deviation and statistical analysis was performed using SPSS version 16.0. A statistically significant difference was determined at a minimal level

of significance of 0.05. All data were checked in terms of normality (Kolmogorov-Smirnov test) and analysed using one-way analysis of variance (ANOVA) with a Tukey HSD post hoc test.

RESULTS

Particle Production and Characterisation

The spray-drying process successfully generated a series of flowable powders. The production yields ranged from 17-40%, which was considered typical of such a manufacture process (Buttini et al., 2008a; Sollohub and Cal, 2010). The median particle diameter of the unprocessed lorazepam was 6.73 ± 0.08 µm (Table 2). Adsorbing the polymer coats to the drug, independent of polymer type and content, significantly increased the particle size ($p < 0.05$). The microparticle coats in which PVP controlled the coat properties generated the largest particles ($HpLorz_{pvp}$ –10.74 ± 0.06; $HwLorz_{pvp}$ –11.96 ± 0.16 µm) (Table 2). Altering the PVA grade had no discernable effect on particle size ($HyLorz_{pva}$ – 9.45 ± 0.05; $Lorz_{pva}$ – 9.85 ± 0.42 µm, no significant difference, $p > 0.05$). Despite the relatively high variability (10-15%), the final lorazepam content of the polymer-coated microparticles appeared to be greatest when there was a high initial drug loads added to the feed stock prior to spray-drying. For example, when 59% w/w lorazepam was loaded onto the $Lorz_{pva}$ microparticle batch, 39 ± 14% lorazepam was retained after processing. In a similar manner the 93% w/w lorazepam that was present initially in the $HyLorz_{pva}$ batch generated microparticles containing 89 ±15% w/w lorazepam.

The absence of any weight loss in the TGA profiles of the microparticle samples (up to a temperature of 120ºC) indicated a lack of significant bound water (< 0.1 %). The absence of water uptake by that material was not unexpected as the crystalline drug was suspended in the

water and the drying conditions of the processing method were optimised previously (Buttini et al., 2008b). The DSC thermograms of the microparticle samples displayed two thermal events , (data not shown), one which was identified as the lorazepam melting peak at 159-170°C and a second which was identified as lorazepam/polymer degradation at >310°C (degradation confirmed by weight loss). The lorazepam alone had a melting onset temperature of *ca*. 190°C (82°C in Jug and Becirevic-Lacan, 2008) and this decreased as the polymer content of the microparticles increased. For example, $HyLorz_{pva}$, which had a lorazepam content of *ca*. 90%, displayed a melt at 170°C, whereas $Lorz_{pva}$ which had a lorazepam content of *ca*. 40% displayed at melt at 159°C. The reduction in melting point was mirrored by a corresponding reduction in melting enthalpy, for example lorazepam particles displayed a melt enthalpy of 245.7 J/g/ºC compared to the enthalpy of $HyLorz_{pva}$ and $Lorz_{pva}$ which had a melt enthalpy of 13 and 6 J/g/ºC, respectively.

The two grades of PVA displayed typical FTIR traces prior to adsorption onto the lorazepam. The OH peak for the partially hydrolysed material was recorded at 3301 cm^{-1} and the hydrogen bonded and non-hydrogen bonded C=O peaks were present at 1715 and 1735 cm^{-1}, respectively (Figure 1). The lack of significant acetate groups in the fully hydrolysed PVA was demonstrated by the absence of the C=O peaks. In addition, the fully hydrolysed PVA showed a more restricted OH environment, probably due to more extensive intermolecular hydrogen bonding, shown by a up field shift of the OH peak (to the lower wave number). A greater degree of structural constraint appeared also to be present in the OH groups of the polymer when the fully hydrolysed PVA was the major component of the lorazepam microparticle coat, the OH peak occurred at 3200 cm^{-1} for the $Lorz_{pva}$ microparticle (Figure 1), but *ca*. 3100 cm^{-1} for the $HyLorz_{pva}$ microparticle (again an up field shift, data not shown). The $HwLorz_{pvp}$ showed even more extensive hydrogen bonding compared to the PVA dominated coats with a OH peak at

3062 cm^{-1} (Figure 2), but the $HpLorz_{pvp}$ retained a low field absorbance of 3357 cm^{-1} (data not shown). The changes in the OH region were mirrored by the C=O spectral shifts, that is, the adsorption of the polymers resulted in a downfield displacement of the C=O peak, except for the $HpLorz_{pvp}$ microparticle which was the only system to record at C=O peak at *ca*. 1730 cm^{-1} (data not shown). Interestingly, the strong, sharp lorazepam absorbance bands recorded at 3356 and 3458 cm^{-1} for the hydroxyl group at position C3 of the diazepine ring that were present when the $HyLorz_{pva}$ and $HwLorz_{pvp}$ microparticles were analysed lost their distinction when both the $HpLorz_{pvp}$ and $Lorz_{pva}$ particles were assessed (Figures 1 and 2).

Particle Aerosolisation

The lorazepam recovery from all the aerosolisation assessments was in the range of 95 – 115% and this illustrated that the aerosolisation test system was fit for purpose. The deposition of the particles when the uncoated lorazepam was aerosolised into the impactor appeared to be evenly distributed across the 4 collection stages. This is typical behaviour for a polydisperse microparticle with a particle diameter of approximately 7 µm that did not significantly aggregate upon aerosolisation (Figure 3). The lack of significant aggregation of the uncoated lorazepam was reflected in the mass median aerodynamic diameter (MMAD) of *ca*. 6 µm, i.e. a value that was similar to the volume median diameter (*ca*. 7 µm) for the same material. Although MMAD takes into account density this was assumed to be relatively constant and close to 1 g/cm^3 across the powders as the drug core was suspended in the feed stock in all the systems. Scanning electron micropscopy (SEM) analysis showed that the particles had a smooth external surface without pores supported this (data not shown, morphology identical to that reported in (Jones et al., 2006a). $HyLorz_{pva}$, $HwLorz_{pvp}$ and $HpLorz_{pvp}$ behaved in a similar manner to the uncoated lorazepam whereby the particle MMAD was similar to their volume mean diameter (VMD) and

this indicated that the particles that were aerosolised into the impactor were not aggregated. The $Lora_{pva}$ microparticle delivered approximately 4 times more lorazepam (significantly higher than the uncoated lorazepam, $p < 0.05$) into the first stage of the impactor and this resulted in an MMAD of 32 µm. This deposition pattern was indicative of significant aggregation post aerosolisation which is typically observed from a cohesive powder. Between 30-40% of the total dose was retained by the delivery device. This was consistent across the 4 powders and was a function of the efficiency of the device to fluidise the powder bed.

Dissolution Testing

Measuring the dissolution rate for the microparticles was extremely difficult because the microfine polymer coat aided particle wetting and thus the rate at which the drug was released so rapid. It was not physically possible to take enough samples within a very short time course when the steady state release was established. Therefore, both non-micellar (0.2%, w/v) and miclellar (0.5%, w/v) SDS solutions were employed as the dissolution media to qualitatively evaluate and rank the dissolution rate of different particle systems. Using 0.2% and 0.5% SDS, the only rate that could be measured with any degree of certainty was the H_yLorz_{pva} which released the drug at a zero order release constant of 0.014 in 0.2% SDS (0-60 min) and 0.046 in 0.5% SDS (0-15 min). This trend, that is a more rapid dissolution rate in the dissolution media that contained 0.5% SDS, was evident throughout the dissolution results to the extent that the dissolution profiles in both media provided an identical rank order in terms of dissolution speed (Figure 4 and 5). In the absence of reliable rate measurements, the extent of drug release in 5 min (D_{5min}) provided an appropriate index with which to compare the lorazepam release rate from the microparticles. The D_{5min} for uncoated lorazepam in the media containing 0.2% SDS was 53% and for the media in 0.5% SDS it was 75%. Upon addition of a microparticle coat

containing fully hydrolysed PVA the dissolution rate of the drug was reduced (D_{5min} 14% and 33% for 0.2% and 0.5% SDS, respectively) whilst each of the other polymer coats improved the rapid drug dissolution. For example the $HpLorz_{pvp}$ coat increased D_{5min} from 53% to 90% in the medium with 0.2% SDS (Figure 4).

DISCUSSION

The particle size of a formulation presented to the nasal cavity plays an important role in the deposition and clearance of the drug that it releases. Therefore developing a dry powder for intranasal administration that displays adequate chemical stability, flow, aerosolisation characteristics and drug release can be a time consuming and costly process. There is, as with pulmonary delivery, no clear consensus among researchers as to what is the 'ideal' particle size for a nasal delivery. However, previous work has suggested that a powder with a high proportion of particles with a size of less than 1 μm has the potential to cause undesired toxicity due to lower respiratory tract deposition (Chien and Chang, 1987; Hinchcliffe and Illum, 1999). Particles with size of above 10 μm are thought to almost exclusively deposit in the nasal cavity when inhaled directly into the nose (depending on the method by which they are aerosolised) although most of the marketed locally acting formulations dose a population of particles that is closer to 100 μm (Chien and Chang, 1987; Hinchcliffe and Illum, 1999). Considering that rapid drug dissolution was desirable in this work, a size of between 5-10 μm, under normal administration conditions, was considered the preferred size for lorazepam (Lansley and Martin, 2001; Sinko, 2006).

Producing an intermit mixture of drug and excipients in a small microparticle is technically challenging using equipment that is capable of large scale production. Particle size was

considered as the critical parameter to keep consistent across the powders if the fundamental interactions and drug release from the particles were to be investigated. In the current study by varying the grade of polymer, quantity of polymer and coating thickness a series of particles with similar diameters were generated and this allowed the particle surface to be modified whilst retaining a relatively consistent particle surface area. Large differences in particle surface area or disaggregation can often dominate both dissolution and cohesion and hide underlying composition effects. It therefore must be accepted that it was not feasible to control particle coating thickness (which was related to lorazepam content) or incrementally change excipient composition across these studies. Any effect that this design had on result interpretation is clarified at each stage of the subsequent discussion.

Buttini et al. (2008b) found that vinyl polymer adsorption was a multi-layered process that allowed high loads of polymers to be captured on a microparticle surface. The consequence of the high polymer loading capacity is that the initial drug-polymer ratios are roughly maintained during spray-drying. The lorazepam microparticles generated in this work demonstrated an identical trend which was considered indicative of a similar multilayer absorption process occurring. Although the extent of adsorption is known to be a consequence of the substrate and polymer-surface interactions, the adsorption process was not quantified in this work as it had been in the previous study (Buttini et al., 2008b). The focus was to investigate the molecular interactions and functional effects of the polymer coat on the delivery characteristics.

Thermal analysis was used to determine the lorazepam response to polymer adsorption. The shift in the melting point of the drug that had been coated by the polymers suggested that there was a strong interaction between vinyl polymers and the lorazepam. Of the three known lorazepam polymorphs, the crystalline melt observed in this work indicated the presence of the most stable

form, which is known to form a dimer in the solid state (Jug and Becirevic-Lacan M, 2008). The high lorazepam melt enthalpy (245 J/g) was similar to the high value reported previously (ca. 190 J/g) (Jug and Becirevic-Lacan M, 2008) and therefore the dimer formation theory associated with this high value was applicable in this work. If this was assumed to be the case, the melting point shift, when the polymer coat was added to the lorazepam, was most probably a result of the polymers disrupting the drug dimer hydrogen bond formation. The alternative hypothesis, i.e., that the drug may convert to a less stable polymorph or an amorphous material, would appear unlikely given the lack of any re-crystallisation transitions in the thermal profiles and the lack of water absorption by the powders. The possibility of dimer formation made the calculation of the powder crystallinity complex. In this work the production method, i.e. spray-drying from a drug suspension, the relatively low solubility of the drug and the surface adsorption of the polymers meant that it would be very unlikely that any amorphous material generated would be incorporated into the surface of the particles and thus confound the conclusions from the subsequent particle assessment. Therefore more in-depth solid state analysis was not conducted.

The presence of two vinyl polymers and the lorazepam in the four coated microparticle systems made precise infrared spectral assignments difficult. However, it was clear from the recorded traces that the type, nature and extent of hydrogen bonding in the materials used in this work was critical to the nature of polymer coat. The most extensive polymer adsorption was associated with a loss in the sharp lorazepam absorbance bands for the hydroxyl group at position C3 of the lorazepam diazepine ring, the most extensive changes in the thermal profiles and the appearance of a new C=O peak down field which represented the release of the constraints imposed upon this functionality by the polymer hydrogen bonding. These results suggest the partially hydrolysed PVA adsorbs to the lorazepam surface by hydrogen bonding with the C3 of the lorazepam diazepine ring (Figure 6). This adsorption process releases the

constraints on the acetate group of the partially hydrolysed PVA as it is removed from close association with the alcohol groups which form strong hydrogen bonds. This acetate group is known to disrupt the alcohol hydrogen bonding and therefore affect the orientation of this group towards the particle surface theoretically allowing the PVA alcohols to form new hydrogen bonds as described previously (Jones et al., 2005). Fully hydrolysed PVA retains a greater structural rigidity upon adsorption according to the FTIR spectra, which is not unexpected as the few acetates that are present have little disruption effect on the alcohol groups in PVA. Employing fully hydrolysed PVA as the main component in the microparticle coat resulted in a less disruption of the lorazepam dimers which suggests weaker polymer adsorption. In terms of the effect of PVP on adsorption, low MW is preferred as the high MW would increase the solution viscosity hindering the migration of polymer to the drug surface. In addition, the stronger interaction between PVP and highly hydrolysed PVA in solution would also deter the adsorption process (Jones, et al., 2005). This was supported by the missing lorazepam characteristic peak at 3356 and 3458 cm^{-1} in $Lorz_{pva}$ and $HpLorz_{pvp}$ and the fact that both showed higher polymer contents compared to $HyLorz_{pva}$ and $HwLorz_{pvp}$.

The most widely employed approach to formulating dry powder aerosol delivery systems for pulmonary drug delivery is to combine the drug particles with coarse carrier such as lactose which can aid powder flow, aersolisation and act as a bulking agent. Upon delivery, the adhesion forces between drug particles and the carrier must be overcome to enable the efficient aerosolisation of the former. However, the interactions between the drug particles and course carrier, e.g. the adhesive and cohesive forces between the components of the systems, may heavily disturb the aerosolisation of drug particles (Murnane et al., 2009; Young et al., 2009). In addition, the presence of a third component, ‘fine lactose’ particles, which always accompany lactose carrier in commercially sourced material, make the aerosolisation behaviour of the drug particles more

complicated and difficult to control (Jones and Price, 2006). In powders intended for nasal delivery a carrier such as lactose is typically not employed. The coated microparticles produced in this work were flowable, a carrier was not needed and this simplified the formulation approach and made it suitable for nasal delivery.

The MSLI apparatus that is specified both in United States Pharmacopeia and European Pharmacopeia is widely accepted and utilized to evaluate the aerosolisation of drug power formulations via the determination of particle size distribution. The particles collected before stage 3 can be considered to theoretically deposit in the nasal cavity, however simply adding up the amount deposited in the nose provides little mechanistic information with regard to the material's aerosolisation properties or cohesion. A much more useful index that can be drawn out from the deposition data is the MMAD. The comparison of MMAD with the original VMD of the material prior to aerosolisation has previously been reported to provide an insight into the dominant microparticle forces that influence a powder upon aerosolisation (Buttini et al., 2008a). In this work such a comparison showed that only one microparticle system appeared to be cohesive after fluidisation by airflow, $Lorz_{pva}$, which was the microparticle coat which appeared to interact most strongly with the surface of the lorazepam. This suggests that the breaking of the PVA hydrogen bonds during the adsorption process has two effects: 1.) it increases the ability to form new interactions with other excipients or solvents and 2.) it increases the ability to form particle-particle bonds resulting in increased cohesion. It is acknowledged that particle aerosolisation is not only influenced by the excipient properties (Minne et al., 2008), but also the particle size (Donovan and Huang, 1998), density (Ting et al., 1992), morphology (Crowder et al., 2002) and surface roughness (Tang et al., 2004), however in this work these were all approximately equivalent.

Due to deleterious influence of high MW PVP on adsorption, the dissolution profile of $HwLorz_{pvp}$ was not examined. Ideally the *in vitro* dissolution test of the coated lorazepam particles should be performed using the artificial nasal fluids (ANF) that contain mucins, a family of high molecular weight, heavily glycosylated proteins (Baumann et al., 2009). However, as the chemical stability of lorazepam in the ANF was unknown and the presence of glycosylated proteins would increase the complexity of the HPLC analysis an SDS loaded aqueous solution was used as the mimetic media in this work. The critical micelle concentration (CMC) of SDS at 37^{o}C has been reported to be ca. 0.25% (w/v) (Goddard and Benson, 1957) and this resulted in two concentrations of the SDS being used in the work, 0.2 and 0.5% (w/v), that is, one system which formed SDS micelles and one that did not. Although the presence of micelles did speed up the dissolution they did so evenly across the microparticles and hence the media employed to test the drug release did not have a significant effect on result interpretation. Generating an artificial surfactant based fluid in which to test drug release was not ideal and probably limited the capacity of the release assay to discriminate between the powders tested. However, the benefits of testing the drug release under sink conditions where drug solubility, which was impossible to finely control, did not confound the results was considered more important than mimicking in vivo conditions. It is important that the differences in release across the powders, although appear to be relatively small are interpreted with this experimental design consideration in mind as such differences increase dramatically as the surfactant concentration is lowered.

The superior release of the lorazepam from the microparticles which according the FTIR and DSC studies underwent most structural re-orientation during the adsorption process was not surprising. Nor was the fact that the highly hydrolysed PVA polymer which retained the greatest extent of intra-molecular hydrogen bonding released the lorazepam at the slowest rate. This

behaviour suggests that the vinyl interactions dominate particle properties and like in previous work the decreased cohesion between particles and increased wetting, as a result of the greater conformation freedom of the polymers on the surface of the drug, led to the dissolution profiles that were generated.

CONCLUSION

This study demonstrated that the delivery characteristics of lorazepam from microparticles coated with vinyl macromolecules (PVA and PVP) was probably controlled by a complex equilibrium of hydrogen bonding. Manipulating the grade and blend of the polymers allowed coats to be formed around the crystalline lorazepam microparticles with differing degrees of intra-molecular mobility. Using a vinyl polymer which displayed extensive hydrogen bonding produced the least cohesive microparticle because the alcohol functional groups were in the main restricted by their innate intra and intermolecular interactions. The adsorption process did not liberate significant numbers of the alcohol functional groups to improve the capability of the drug to interact with the dissolution solvent when fully hydrolysed PVA was used to coat the drug a delay to the drug release was observed. Breaking the vinyl polymer hydrogen bond network through the introduction of more acetate groups and a more intensive adsorption process increased the adhesivity of the polymer in the coat and lead to more extensive solvent interactions thus rapid drug release.

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List of Tables and Figures

Table 1. Lorazepam suspensions used for the microparticle engineering process. In each suspension 1gram of lorazepam was suspended in 100 ml of water to which the polymers were added; PVA and PVP represent poly(vinyl alcohol) and poly(vinyl pyrrolidone), respectively; the data of PVA hydrolysis was from the products MSDS (material safety data sheets). K17 and K 90 correspond to a molecular weight of *ca*. 12,000 and 1300,000, respectively.

Formulation	PVA (g), (hydrolysis, %)	PVP (g) (grade)
$HyLorz_{pva}$	0.06, (99)	0.01 (K17)
$Lorz_{pva}$	0.6, (88)	0.1 (K17)
$HwLorz_{pvp}$	0.06, (88)	0.01 (K90)
$HpLorz_{pvp}$	0.06, (88)	0.1 (K17)

607

608 **Table 2**. The characterisation of spray-dried lorazepam microparticles coated with
609 macromolecules. Data are presented as particle size, drug content, melting point (T_m)
610 and enthalpy (ΔH_f) (mean ± one standard deviation where appropriate, n = 3-6)
611

Formulation	Size (μm)	Lorazepam (%)	T_m (ºC)	ΔH_f (J/g)
Lorazepam	6.73 ± 0.08	100	189.8	245.7
$HyLorz_{pva}$	9.45 ± 0.05	89.28 ±15.05	170.2	13.0
$HwLorz_{pvp}$	11.96 ± 0.16	51.72 ±12.64	169.3	13.4
$HpLorz_{pvp}$	10.74 ± 0.06	47.04 ± 0.91	156.9	4.8
$Lorz_{pva}$	9.85 ± 0.42	38.79 ± 14.00	159.0	6.2

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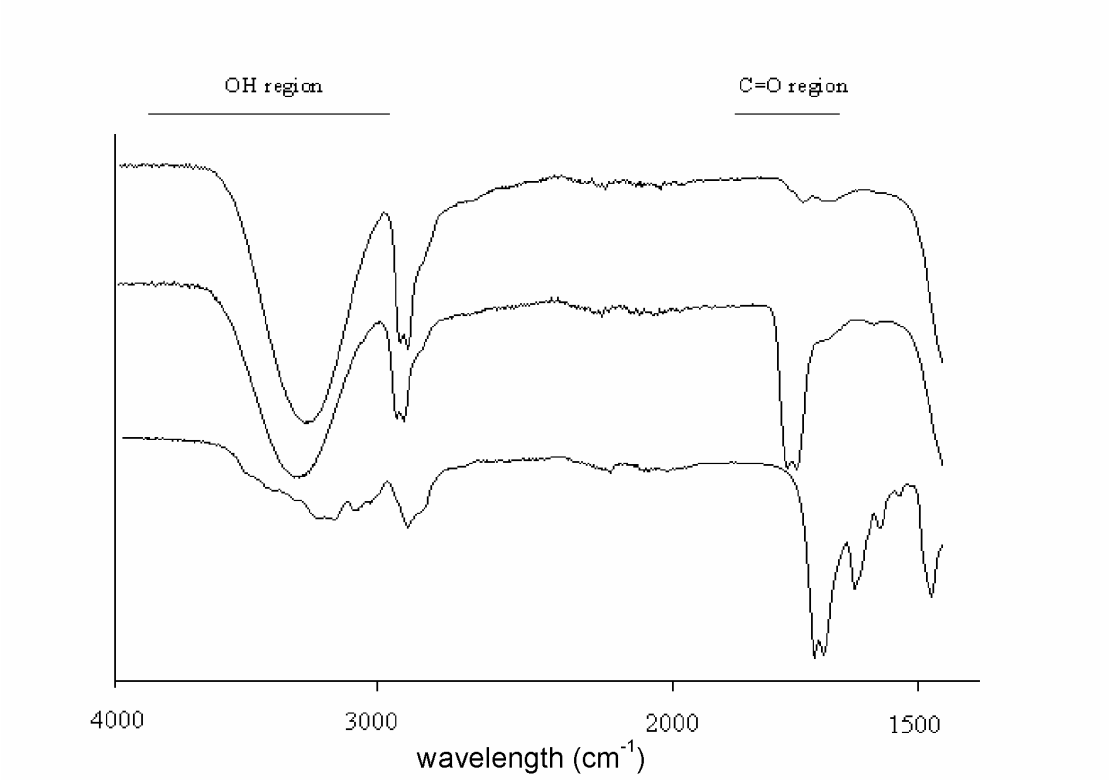
617 **Figure 1.** A Fourier infrared spectroscopy trace of the fully hydrolysed poly(vinyl
618 alcohol) (PVA) (top) the partially hydrolysed PVA (middle) and lorazepam with a coat
619 composed of partially hydrolysed PVA blended with poly(vinyl pyrrolidone) ($Lorz_{pva}$).



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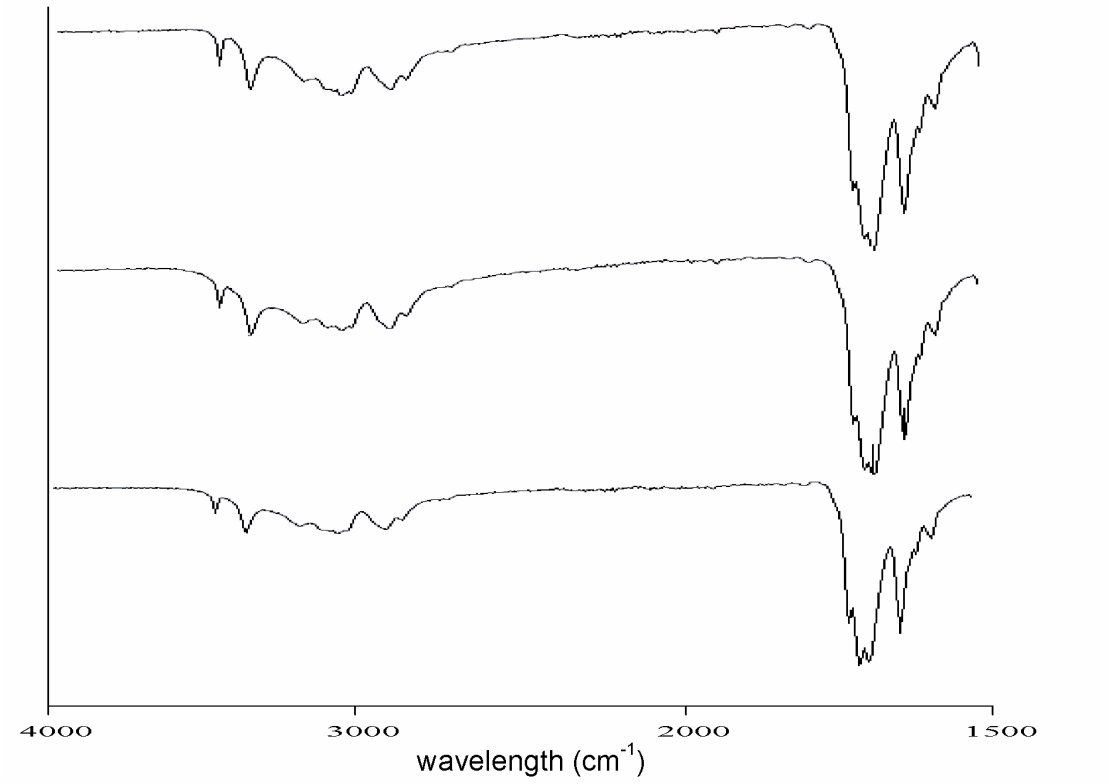
622

623 **Figure 2.** A Fourier infrared spectroscopy trace of the lorazepam (top), lorazepam with
624 a thin coat composed of partially hydrolysed PVA blended with poly(vinyl
625 pyrrolidone) (middle $HyLorz_{pva}$) and lorazepam with a thin coat composed of partially
626 hydrolysed PVA blended with high molecular weight poly(vinyl pyrrolidone)
627 ($HwLorz_{pvp}$).



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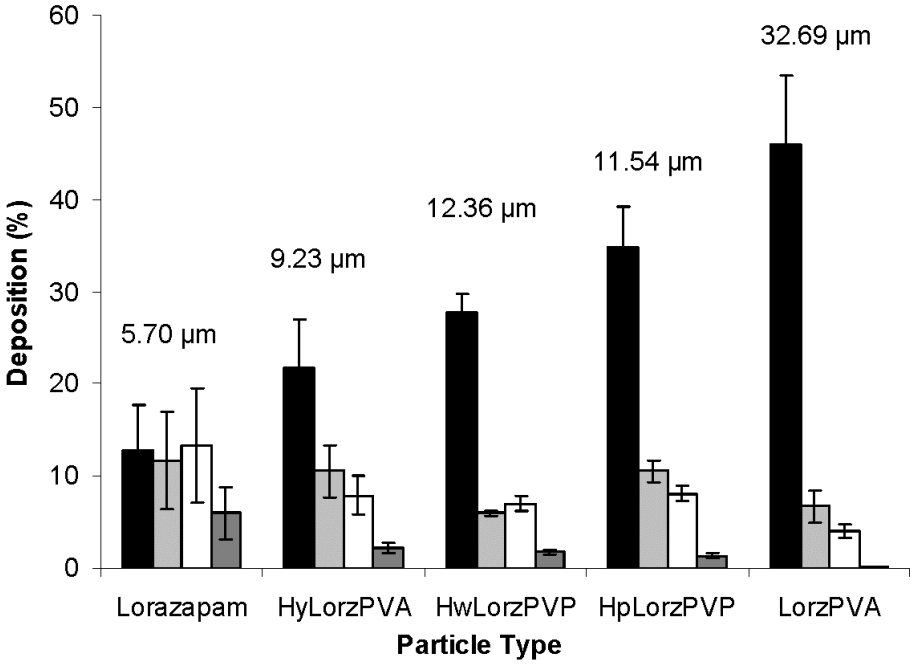
631 **Figure 3.** Aerosolisation of lorazepam and lorazepam coated microparticles into the
632 Multi-stage Liquid Impinger (MSLI) at the airflow rate of 30 ($L.min^{-1}$). Black bar is
633 stage 1 (cut off diameter 18.39 µm); light grey is stage 2 (cut-off diameter 9.62 µm);
634 white is stage 3 (cut-off diameter 4.38 µm) and dark grey is stage 4 (cut-off diameter
635 2.40 µm). The mass median aerodynamic diameter is displayed above each
636 formulation in µm. Data are presented as mean ± standard deviation (SD) (n =3-6).



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640 **Figure 4**. Dissolution profiles of lorazepam from various formulations using 0.2%
641 (w/v) sodium dodecyl sulfate (SDS) aqueous solution as the dissolution media.
642 Lorazepam alone (■) was used as control, $HyLorz_{pva}$ (▲) and $HpLorz_{pvp}$ (Δ) and
643 $Lorz_{pva}$ (○), particle composition details can be found in Table 2 (data expressed as
644 mean ± standard deviation, n = 4-6).

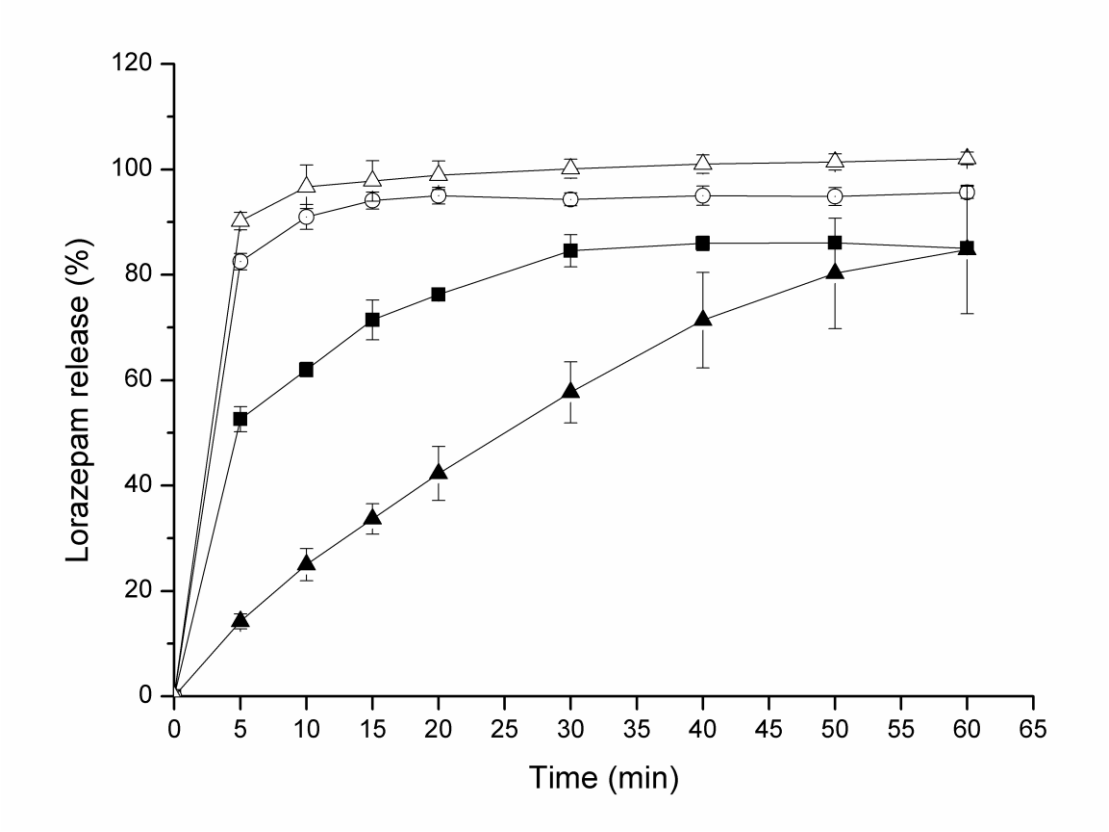

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Figure 5. Dissolution profiles of lorazepam from various formulations using 0.5% (w/v) sodium dodecyl sulfate (SDS) aqueous solution as the dissolution media. Lorazepam alone (■) was used as control, $HyLorz_{pva}$ (▲) and $HpLorz_{pvp}$ (Δ) and $Lorz_{pva}$ (○), particle composition details can be found in Table 2 (data expressed as mean ± standard deviation, n = 4-6).

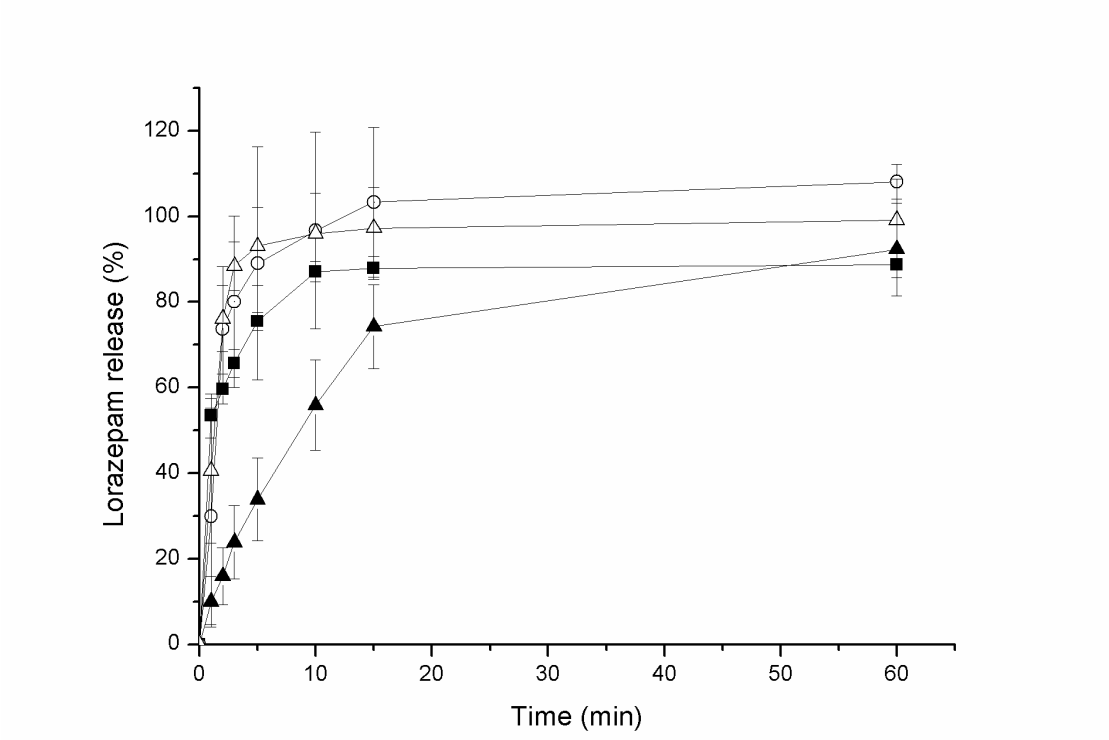

Figure 6. The mode of hydrogen bonding between lorazepam and partially hydrolysed poly(vinyl alcohol) (PVA).