

Intranasal lorazepam delivery

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2 **Vinyl polymers-coated lorazepam particles for potential intranasal delivery**

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25 **ABSTRACT**

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

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

47 **INTRODUCTION**

48

49 Insomnia is commonly treated by oral administration of lorazepam, but the 1-2 hour delay to
50 induce significant sedation is a major barrier to effective therapy. This delayed onset can often
51 result in over dosing due to the patient taking another dose in an attempt to initiate the clinical
52 effects. Intranasal administration of lorazepam could dramatically improve the immediacy of
53 sedation. The large surface area of the nasal mucosa provides rapid absorption into the systemic
54 circulation and the possibility of direct access to the central nervous system (Costantino et al.,
55 2007). Nasal delivery also has the benefits of being non-invasive and avoiding ‘first-pass’
56 metabolism.

57

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

68

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

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

81

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

93

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

97 (2008a,b) was manipulated in order to modify the nature of polymer adsorption whilst
98 maintaining a constant final particle diameter. In total, a series of four test microparticles were
99 generated using a mixture of PVA and PVP which was varied to generate a 'standard' particle,
100 similar to that produced previously (Lorz_{pva}) (Buttini et al., 2008a); a particle that had a coat
101 dominated by fully hydrolysed PVA, i.e. a coat with extensive intra-molecular hydrogen bonding
102 (HyLorz_{pva}); a particle with a high viscosity coat (HwLorz_{pvp}) and a particle where a high
103 proportion of PVP was employed (HpLorz_{pvp}) (Table 1). The interaction of the two polymers with
104 each other (Fourier Transform Infrared (FT-IR) spectrometry analysis) and with the drug
105 (differential scanning calorimetry), an assessment of particle cohesiveness (impaction assessment)
106 and drug release (modified United States Pharmacopeia (USP) dissolution) was compared in an
107 attempt to elucidate the influence of the polymer employed in the adsorption process upon the
108 particle behavior.

109

110 **MATERIALS AND METHODS**

111

112 **Materials**

113

114 Lorazepam (Ph Eur) was supplied by Cambrex Profarmaco (Milano, Italy). Potassium dihydrogen
115 orthophosphate and high performance liquid chromatography (HPLC) grade orthophosphoric
116 acid, cyclohexane, water, methanol, ethanol and acetonitrile were all purchased from Fisher
117 Scientific (Loughborough, UK). Formic acid, 1-chlorobutane, sodium chloride and sodium
118 dodecyl sulfate (SDS) were supplied by Sigma-Aldrich Ltd. (Poole, UK). PVA 28-99 and PVA
119 23-88 were supplied by KSE (Troisdorf, Germany). PVP (Kollidon 17) and Solutol HS 15 were
120 supplied by BASF (Wantage, UK). PVP (Kollidon 90) was supplied by ISP (Calvert City, USA)
121 and ammonium solution 25% by BDH (Poole, UK).

122

123 **Microparticle Production**

124

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

136

137
$$\text{yield (\%)} = \frac{\text{Mass of particles post spray drying}}{\text{Initial solid mass in the suspension}} \times 100 \quad (\text{Eq. 1})$$

138

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

145

146 **Laser Diffraction Particle Size Analysis**

147

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.

157

158 **Thermogravimetric Analysis**

159

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 $10^{\circ}\text{C}\cdot\text{min}^{-1}$ from 25°C to 300°C
164 was used to determine the percentage of volatiles present in the spray-dried samples.

165

166 **Differential Scanning Calorimetry**

167

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.

171 Approximately 2 mg of each sample was analysed by placing it into hermetically sealed pans
172 (TA instruments, Crawley, UK) with a pinhole in the roof. Samples were heated with a
173 modulated heating method ($\pm 1^{\circ}\text{C}\cdot\text{min}^{-1}$) using a heating rate of $10^{\circ}\text{C}\cdot\text{min}^{-1}$ from 25°C to
174 300°C . Oxygen free nitrogen was used as the cell purge gas with a flow rate of $100\text{ ml}\cdot\text{min}^{-1}$.
175 The melting temperature of the sample was taken as the onset of the endothermic peak of the
176 resultant thermograms.

177

178 **Fourier Transform Infrared Spectroscopy**

179

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

186

187 **Particle Aerosolisation Assessment**

188

189 The assessment of particle aerosolisation behaviour was performed using a Multi- Stage Liquid
190 Impinger (MSLI) (Astra Draco, Lund, Sweden) and its design and working mechanism are
191 detailed in Pharmacopeia (US Pharmacopeia XXXIII, 2010). A simple glass delivery device
192 (made in house) was attached to the upper end of a stainless steel 90° induction port on top of the
193 impinger. Ethanol (20 ml) was added to each stage of the MSLI and the rubber stoppers were
194 inserted before the impinger was tilted to wet the stoppers and reduce electrostatic charge. A 2 mg
195 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).

209

210 **Dissolution Testing**

211

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 37°C
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.

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

224

225 **Lorazepam Assay**

226

227 A reverse-phase (RP) HPLC method was used to quantify lorazepam. The analysis was
228 performed using a Waters HPLC system which consists of an autosampler (Waters 717 plus), a
229 pump controller (Waters 600E), a dual lambda absorbance detector (Waters 2487) and
230 Millennium software (Version 4.0). A Chromolith[®] performance RP-18e (100 x 4.6 mm) column
231 with a Chromolith[®] RP-18e guard cartridge (5 x 4.6 mm) was used for the separation. The
232 mobile phase consisted of 75% (v/v) water (pH 2.1, adjusted with orthophosphoric acid) and
233 25% (v/v) acetonitrile containing 35 mM potassium dihydrogen orthophosphate with a flow rate
234 of 2 ml.min⁻¹. The sample injection volume was 10 µl and UV detection was at 220 nm. The
235 resulting lorazepam retention time was 12 min. The needle wash was performed using a mixture
236 of methanol and water (90:10, v/v). The method was validated in terms of system suitability,
237 sensitivity (LOD = 0.157 µg/ml ± 0.094, LOQ = 0.477 µg/ml ± 0.285), linearity (> 0.999),
238 accuracy (between 95-105%) and precision (intermediate precision between 0.95 and 1.05) and
239 shown to be 'fit for purpose' for the analysis of lorazepam.

240

241 **Statistical Analysis**

242

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

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

247

248 **RESULTS**

249

250 **Particle Production and Characterisation**

251

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

266

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

270 water and the drying conditions of the processing method were optimised previously (Buttini et
271 al., 2008b). The DSC thermograms of the microparticle samples displayed two thermal events ,
272 (data not shown), one which was identified as the lorazepam melting peak at 159-170°C and a
273 second which was identified as lorazepam/polymer degradation at >310°C (degradation
274 confirmed by weight loss). The lorazepam alone had a melting onset temperature of *ca.* 190°C
275 (82°C in Jug and Becirevic-Lacan, 2008) and this decreased as the polymer content of the
276 microparticles increased. For example, HyLor_{z_{pva}}, which had a lorazepam content of *ca.* 90%,
277 displayed a melt at 170°C, whereas Lor_{z_{pva}} which had a lorazepam content of *ca.* 40% displayed
278 at melt at 159°C. The reduction in melting point was mirrored by a corresponding reduction in
279 melting enthalpy, for example lorazepam particles displayed a melt enthalpy of 245.7 J/g°C
280 compared to the enthalpy of HyLor_{z_{pva}} and Lor_{z_{pva}} which had a melt enthalpy of 13 and 6 J/g°C,
281 respectively.

282
283 The two grades of PVA displayed typical FTIR traces prior to adsorption onto the lorazepam.
284 The OH peak for the partially hydrolysed material was recorded at 3301 cm⁻¹ and the hydrogen
285 bonded and non-hydrogen bonded C=O peaks were present at 1715 and 1735 cm⁻¹, respectively
286 (Figure 1). The lack of significant acetate groups in the fully hydrolysed PVA was demonstrated
287 by the absence of the C=O peaks. In addition, the fully hydrolysed PVA showed a more
288 restricted OH environment, probably due to more extensive intermolecular hydrogen bonding,
289 shown by a up field shift of the OH peak (to the lower wave number). A greater degree of
290 structural constraint appeared also to be present in the OH groups of the polymer when the fully
291 hydrolysed PVA was the major component of the lorazepam microparticle coat, the OH peak
292 occurred at 3200 cm⁻¹ for the Lor_{z_{pva}} microparticle (Figure 1), but *ca.* 3100 cm⁻¹ for the
293 HyLor_{z_{pva}} microparticle (again an up field shift, data not shown). The HwLor_{z_{pvp}} showed even
294 more extensive hydrogen bonding compared to the PVA dominated coats with a OH peak at

295 3062 cm^{-1} (Figure 2), but the HpLorz_{pvp} retained a low field absorbance of 3357 cm^{-1} (data not
296 shown). The changes in the OH region were mirrored by the C=O spectral shifts, that is, the
297 adsorption of the polymers resulted in a downfield displacement of the C=O peak, except for the
298 HpLorz_{pvp} microparticle which was the only system to record at C=O peak at *ca.* 1730 cm^{-1} (data
299 not shown). Interestingly, the strong, sharp lorazepam absorbance bands recorded at 3356 and
300 3458 cm^{-1} for the hydroxyl group at position C3 of the diazepine ring that were present when the
301 HyLorz_{pva} and HwLorz_{pvp} microparticles were analysed lost their distinction when both the
302 HpLorz_{pvp} and Lorz_{pva} particles were assessed (Figures 1 and 2).

303

304 **Particle Aerosolisation**

305

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

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

327

328 **Dissolution Testing**

329

330 Measuring the dissolution rate for the microparticles was extremely difficult because the
 331 microfine polymer coat aided particle wetting and thus the rate at which the drug was released so
 332 rapid. It was not physically possible to take enough samples within a very short time course
 333 when the steady state release was established. Therefore, both non-micellar (0.2%, w/v) and
 334 micellar (0.5%, w/v) SDS solutions were employed as the dissolution media to qualitatively
 335 evaluate and rank the dissolution rate of different particle systems. Using 0.2% and 0.5% SDS,
 336 the only rate that could be measured with any degree of certainty was the H_yLor_{Z_{pva}} which
 337 released the drug at a zero order release constant of 0.014 in 0.2% SDS (0-60 min) and 0.046 in
 338 0.5% SDS (0-15 min). This trend, that is a more rapid dissolution rate in the dissolution media
 339 that contained 0.5% SDS, was evident throughout the dissolution results to the extent that the
 340 dissolution profiles in both media provided an identical rank order in terms of dissolution speed
 341 (Figure 4 and 5). In the absence of reliable rate measurements, the extent of drug release in 5
 342 min ($D_{5\text{min}}$) provided an appropriate index with which to compare the lorazepam release rate
 343 from the microparticles. The $D_{5\text{min}}$ for uncoated lorazepam in the media containing 0.2% SDS
 344 was 53% and for the media in 0.5% SDS it was 75%. Upon addition of a microparticle coat

345 containing fully hydrolysed PVA the dissolution rate of the drug was reduced ($D_{5\text{min}}$ 14% and
346 33% for 0.2% and 0.5% SDS, respectively) whilst each of the other polymer coats improved the
347 rapid drug dissolution. For example the HpLorz_{pvp} coat increased $D_{5\text{min}}$ from 53% to 90% in the
348 medium with 0.2% SDS (Figure 4).

349

350 **DISCUSSION**

351

352 The particle size of a formulation presented to the nasal cavity plays an important role in the
353 deposition and clearance of the drug that it releases. Therefore developing a dry powder for
354 intranasal administration that displays adequate chemical stability, flow, aerosolisation
355 characteristics and drug release can be a time consuming and costly process. There is, as with
356 pulmonary delivery, no clear consensus among researchers as to what is the ‘ideal’ particle size
357 for a nasal delivery. However, previous work has suggested that a powder with a high proportion
358 of particles with a size of less than 1 μm has the potential to cause undesired toxicity due to
359 lower respiratory tract deposition (Chien and Chang, 1987; Hinchcliffe and Illum, 1999).
360 Particles with size of above 10 μm are thought to almost exclusively deposit in the nasal cavity
361 when inhaled directly into the nose (depending on the method by which they are aerosolised)
362 although most of the marketed locally acting formulations dose a population of particles that is
363 closer to 100 μm (Chien and Chang, 1987; Hinchcliffe and Illum, 1999). Considering that rapid
364 drug dissolution was desirable in this work, a size of between 5-10 μm , under normal
365 administration conditions, was considered the preferred size for lorazepam (Lansley and Martin,
366 2001; Sinko, 2006).

367

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

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

380

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

390

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

395 form, which is known to form a dimer in the solid state (Jug and Becirevic-Lacan M, 2008). The
396 high lorazepam melt enthalpy (245 J/g) was similar to the high value reported previously (ca.
397 190 J/g) (Jug and Becirevic-Lacan M, 2008) and therefore the dimer formation theory associated
398 with this high value was applicable in this work. If this was assumed to be the case, the melting
399 point shift, when the polymer coat was added to the lorazepam, was most probably a result of the
400 polymers disrupting the drug dimer hydrogen bond formation. The alternative hypothesis, i.e.,
401 that the drug may convert to a less stable polymorph or an amorphous material, would appear
402 unlikely given the lack of any re-crystallisation transitions in the thermal profiles and the lack of
403 water absorption by the powders. The possibility of dimer formation made the calculation of the
404 powder crystallinity complex. In this work the production method, i.e. spray-drying from a drug
405 suspension, the relatively low solubility of the drug and the surface adsorption of the polymers
406 meant that it would be very unlikely that any amorphous material generated would be
407 incorporated into the surface of the particles and thus confound the conclusions from the
408 subsequent particle assessment. Therefore more in-depth solid state analysis was not conducted.

409

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

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

435

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

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

449

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

469

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

490

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

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

499

500 **CONCLUSION**

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

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597 List of Tables and Figures

598 **Table 1.** Lorazepam suspensions used for the microparticle engineering process. In
 599 each suspension 1gram of lorazepam was suspended in 100 ml of water to which the
 600 polymers were added; PVA and PVP represent poly(vinyl alcohol) and poly(vinyl
 601 pyrrolidone), respectively; the data of PVA hydrolysis was from the products MSDS
 602 (material safety data sheets). K17 and K 90 correspond to a molecular weight of *ca.*
 603 12,000 and 1300,000, respectively.

604

Formulation	PVA (g), (hydrolysis, %)	PVP (g) (grade)
HyLor _z _{pva}	0.06, (99)	0.01 (K17)
Lor _z _{pva}	0.6, (88)	0.1 (K17)
HwLor _z _{pvp}	0.06, (88)	0.01 (K90)
HpLor _z _{pvp}	0.06, (88)	0.1 (K17)

605

606

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 \pm one standard deviation where appropriate, n = 3-6)

611

Formulation	Size (μm)	Lorazepam (%)	T_m ($^{\circ}\text{C}$)	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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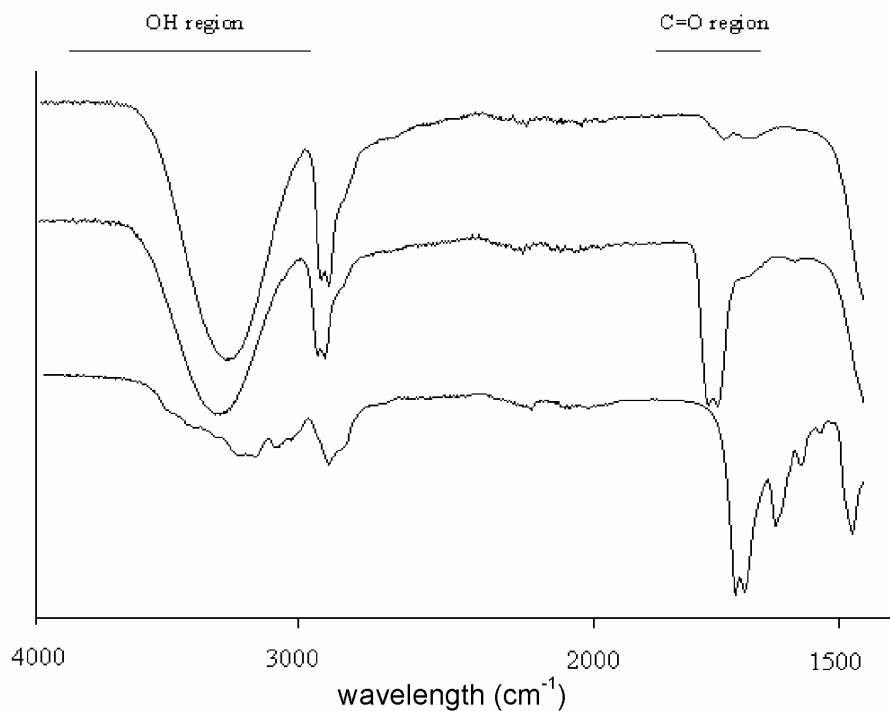
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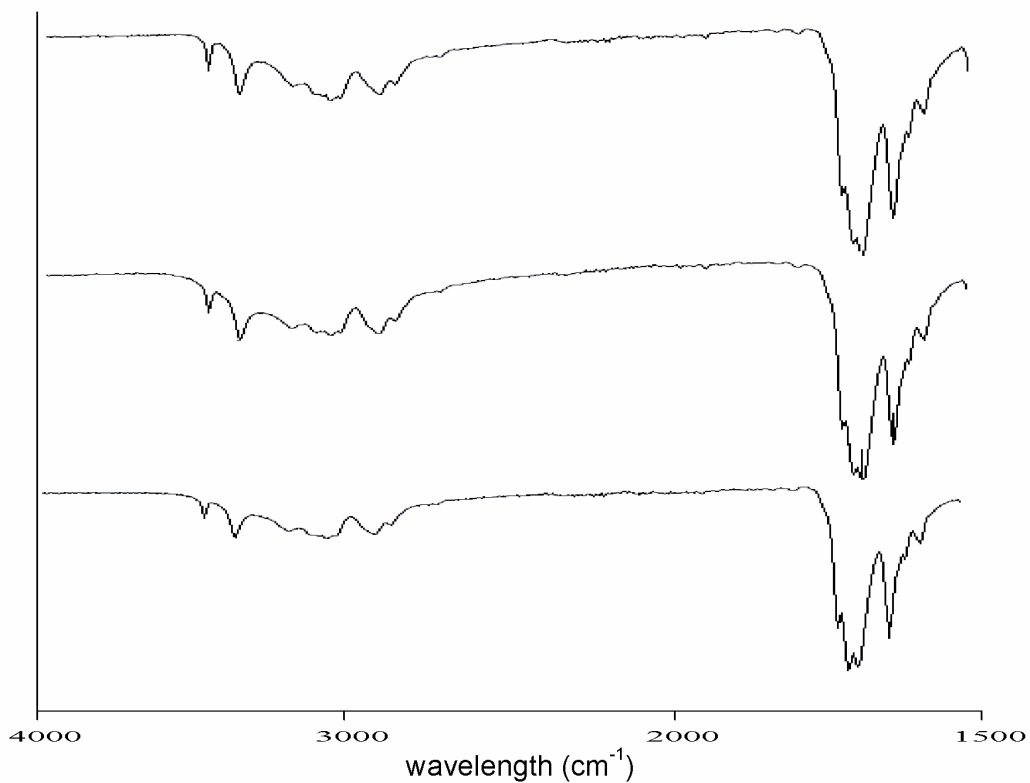
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617 **Figure 1.** A Fourier infrared spectroscopy trace of the fully hydrolysed poly(vinyl
 618 alcohol) (PVA) (top) the partially hydrolysed PVA (middle) and lorazepam with a coat
 619 composed of partially hydrolysed PVA blended with poly(vinyl pyrrolidone) (Lorz_{pva}).



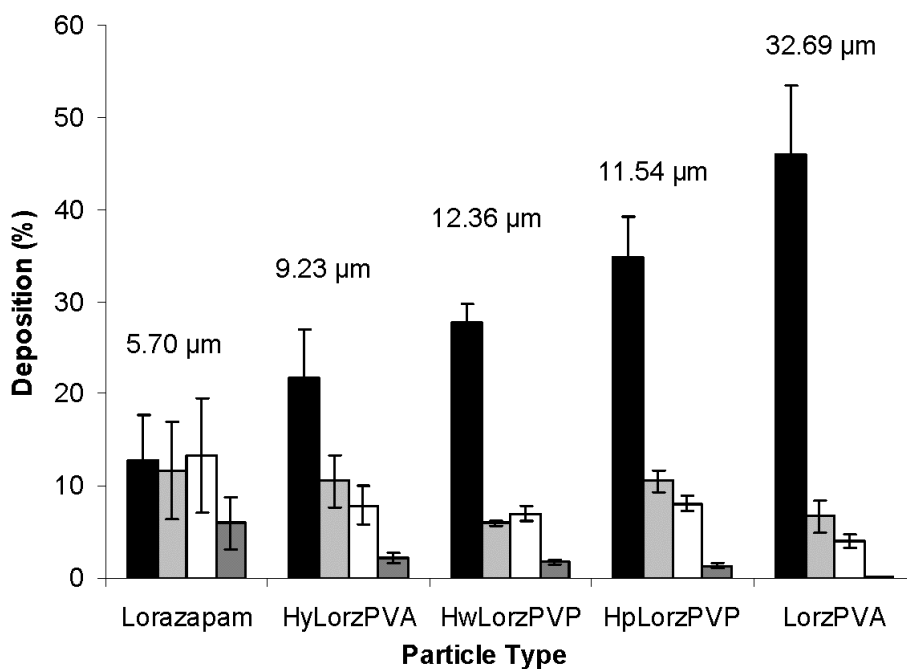
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623 **Figure 2.** A Fourier infrared spectroscopy trace of the lorazepam (top), lorazepam with
 624 a thin coat composed of partially hydrolysed PVA blended with poly(vinyl
 625 pyrrolidone) (middle HyLor_{pva}) and lorazepam with a thin coat composed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



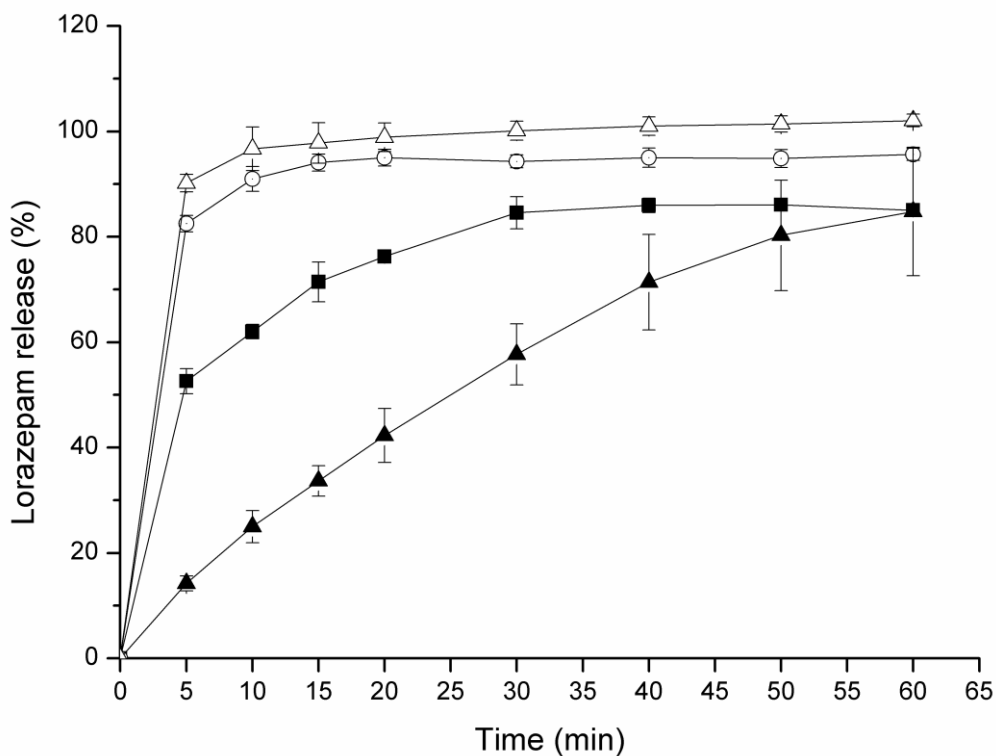
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631 **Figure 3.** Aerosolisation of lorazepam and lorazepam coated microparticles into the
 632 Multi-stage Liquid Impinger (MSLI) at the airflow rate of 30 (L.min⁻¹). Black bar is
 633 stage 1 (cut off diameter 18.39 µm); light grey is stage 2 (cut-off 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).



637
 638
 639

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, HyLor_{pva} (▲) and HpLor_{pvp} (Δ) and
 643 Lor_{pva} (○), particle composition details can be found in Table 2 (data expressed as
 644 mean ± standard deviation, n = 4-6).

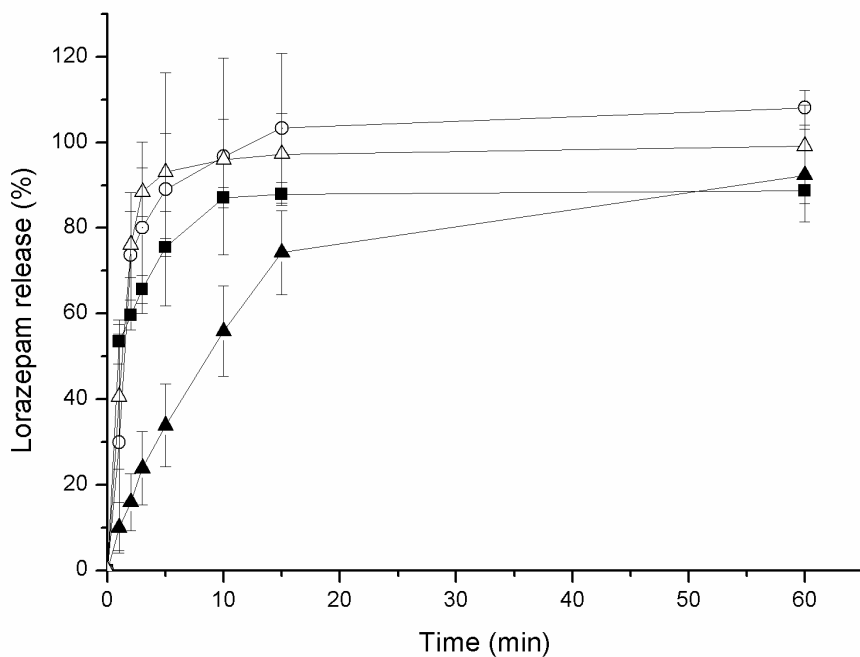


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648 **Figure 5.** Dissolution profiles of lorazepam from various formulations using 0.5%
 649 (w/v) sodium dodecyl sulfate (SDS) aqueous solution as the dissolution media.
 650 Lorazepam alone (■) was used as control, HyLor_z_{pva} (▲) and HpLor_z_{pvp}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

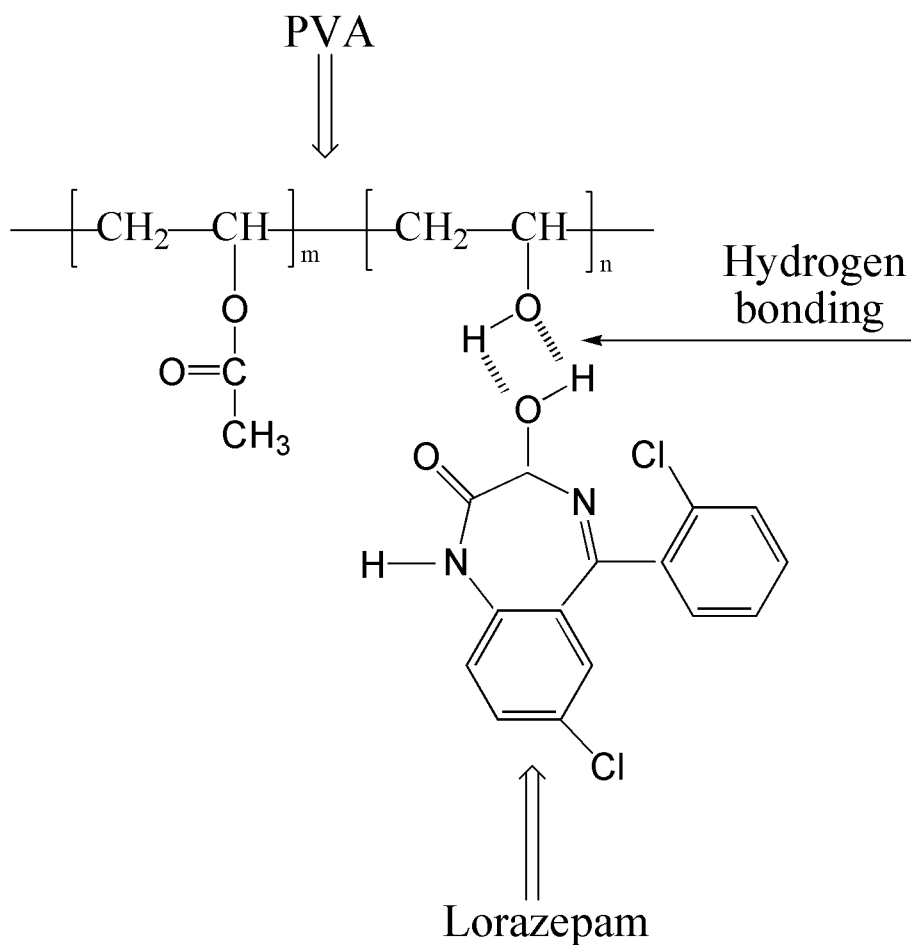


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656 **Figure 6.** The mode of hydrogen bonding between lorazepam and partially
 657 hydrolysed poly(vinyl alcohol) (PVA).



658