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## **Evaluation of gellan gum fluid gels as modified release oral liquids**

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## **Abstract**

Oral liquids are often preferred for drug administration to patients for whom swallowing is difficult, however formulating modified release versions can be challenging. A potential route to achieve modified release in oral liquids is by using fluid (sheared) gels formed by introducing a shear field during gelation in gel-forming biopolymers. These fluid gels can act as pourable viscoelastic fluids but retain true gel micro/nano structure. Here, we have demonstrated that fluid gels have potential as paediatric oral liquids preventing release of ibuprofen in simulated gastric fluid. Subsequent release at pH 7.4 was affected by the duration of exposure and magnitude of acid pH with a linear relationship between onset of release and the preceding acidic exposure duration. Delayed release was a result of increasing gel stiffness, a consequence of the acidity of the initial release media and exposure time. A much faster release rate was measured when exposure time in acid was 10 min compared with 60 min. This study highlights the potential to design fluid gels that are tuned to have a specified stiffness at a particular pH and exposure time. This could enable the preparation oral liquids with modified release behaviour.

## 1 **1. Introduction**

2 There is an ever increasing demand for the development of age appropriate dosage forms, especially  
3 for paediatric patients and older adults who have difficulties in swallowing. This is most apparent in  
4 modified release formulations where the functional excipients responsible for controlling drug  
5 release can become ineffective due to manipulation prior to administration to children. Even over  
6 the counter antipyretic formulations have an increased risk of side effects in children. Worryingly,  
7 there are very few oral modified-release drug delivery platforms suitable for administration to  
8 paediatrics. Generally, for children and patients who find swallowing is difficult, syrup-based oral  
9 liquids are the preferred dosage form however formulating these dosage forms to have modified  
10 release properties can be challenging. Recently researchers have looked to develop such dosage  
11 forms using enteric coated micro-particles (Dalmoro et al., 2010) and ion exchange resins (Cuña et  
12 al., 2000) however these systems are often costly, suffer from poor mouth feel and are only suitable  
13 for use with specific drugs. There is therefore a real need for alternative formulations. A potential  
14 route to achieve modified release in oral liquids is by using polysaccharide solutions which undergo  
15 a sol gel transition on exposure to stomach acid. Indeed several authors have evaluated the oral  
16 sustained delivery of drugs such as theophylline, ambroxol, paracetamol and cimetidine in various  
17 in situ gelling polysaccharides which have included xyloglucan (Miyazaki et al., 2003; Itoh et al.,  
18 2008; Itoh et al., 2010), pectin (Itoh et al., 2008; Kubo et al., 2004; Miyazaki et al., 2005; Kubo et  
19 al., 2005), and sodium alginate (Itoh et al., 2010; Kubo et al., 2003). Although these systems have  
20 shown some promise as vehicles there are issues associated with their use such as leaching of water  
21 soluble drugs and lengthy gastric retention due to large bulk gel formation in situ (Kubo et al.,  
22 2003). These issues could potentially be overcome by using fluid gels.

23 Fluid gels (also referred to as sheared gels) can be defined as suspensions of gel particles prepared  
24 by introducing a shear field while gelation is occurring in biopolymer solutions. These fluid gels  
25 can be formulated so the bulk material acts as a pourable viscoelastic fluid whilst retaining a cross-

26 linked gel microstructure within the particles. The formation of these gelled particles has been  
27 previously described by a nucleation and growth mechanism, with the applied shear field limiting  
28 the molecular ordering to within individual gel particles by physically ensuring that the original  
29 formed gel nucleation sites remain separate from one another (Norton *et al.*, 1999). Along with the  
30 bulk viscosity, the size and strength of these micron-sized, gelled particles can also be controlled by  
31 varying the concentration of polymer and shearing rate used during production (Gabriele *et al.* 2009;  
32 Fernández Farrés *et al.*, 2014). This creates an attractive opportunity to incorporate drugs into an  
33 acid-resistant fluid gel which could potentially delay release in the stomach.

34 Gellan gum is a biopolymer particularly suited for producing fluid gels for such applications. It is a  
35 microbial exopolysaccharide produced by *Sphingomonas elodea* (Doner *et al.*, 1997; Dai *et al.*  
36 2008) and consists of repeating tetrasaccharide units of glucose, glucuronic acid, glucose and  
37 rhamnose residues (Chandrasekaran *et al.*, 1988). Gellan gum is an EU approved food additive  
38 (E418) that has been investigated by several groups for applications in pharmaceuticals (Deasy and  
39 Quigley, 1991; Carlfors *et al.*, 1998) and as a biomaterial (Smith *et al.*, 2007; Oliveira *et al.*, 2010;  
40 Jahromi *et al.*, 2011). At temperatures above 85 °C the gellan gum exists as a random coil, which  
41 forms helical structures upon cooling resulting in a “weak gel” formed by tenuous association of  
42 ridged ordered structures (Norton *et al.*, 1984) rather than by stronger associations of junction zones  
43 present in normal polysaccharide gels (Rees *et al.*, 1982). However, on addition of ions such as  
44 hydrogen, sodium, potassium and calcium true, self-supporting gels are formed. This occurs via a  
45 mechanism of aggregation of gellan double helices either by suppression of the negatively charged  
46 groups on the polymer with monovalent ions or by direct site binding of the helices with divalent  
47 cations (Grasdalen and Smidsrod, 1987; Sworn *et al.*, 1995; Morris *et al.*, 2012). The mechanical  
48 properties and gelation temperature can be controlled by salt concentration and species (Ogawa,  
49 1996). The ability of gellan gum to form acid-insoluble gels renders it a particularly attractive  
50 candidate for developing oral bioresponsive drug delivery systems. Indeed, these have been  
51 investigated in the form of gastro-retentive controlled release (Babu *et al.*, 2010), enteric release

52 (Smith et al., 2010) and as floating in situ gelling systems (Rajinikanth and Mishra, 2008).  
53 Furthermore oral sustained delivery using gellan solutions (which formed acid gels in the stomach)  
54 has also been explored and bioavailability from the gels formed in situ was similar to that of a  
55 commercially available suspension (Kubo et al., 2003). Unlike tablet or capsule formulations, there  
56 is no standard technique for measuring the dissolution properties of oral liquids. Biopharmaceutical  
57 measurements of such formulations are usually performed using modified USP dissolution  
58 apparatus which can lead to high variability. This is a particularly important issue when designing  
59 medicines for children as extrapolating adult biopharmaceutical measurements is difficult due to the  
60 difference in gastrointestinal physiology in paediatric patients (Batchelor et al., 2013). Moreover,  
61 large variations in physiology within paediatric populations are also evident from birth through to  
62 adolescence (Bowles et al., 2010) which further complicates the design suitable biopharmaceutical  
63 methodologies.

64 In the present study gellan gum fluid gels loaded with ibuprofen, (a BCS Class II drug that is  
65 currently available as modified release tablets) were investigated as a modified release oral liquid.  
66 Fluid gel formulations were investigated over a range of pH and acid exposure times to evaluate  
67 how variations in gastric physiology may impact the mechanical properties of these physiologically  
68 responsive fluid gels and the consequential release behaviour.

69

## 70 **2. Material and Methods**

### 71 *2.1. Materials*

72 Low acyl gellan gum (Kelcogel™) was kindly donated by CP Kelco (USA). Ibuprofen powder  
73 (Ibuprofen 38) was obtained from BASF. All other materials were obtained from Sigma–Aldrich,  
74 Poole, UK.

### 75 *2.2. Preparation of fluid gels*

76 Fluid gels were prepared by adding low acyl gellan gum at concentrations from 0.1 to 1% w/w to  
77 deionised water at 85 °C while stirring. Once fully dissolved, the solutions were allowed to cool to  
78 ~60 °C then a paediatric dose of ibuprofen (20 mg/ml) was added and the pH was adjusted to 7.4  
79 using 1 M NaOH. Solutions were then cooled further at 2 °C min<sup>-1</sup> whilst being sheared using  
80 Bohlin Gemini Nano HR rheometer at a shear rate of 500 s<sup>-1</sup>. To evaluate the potential to vary the  
81 particle size during formulation, fluid gels were prepared with changes to the processing conditions.  
82 To investigate the effect of cooling rate, 0.75% w/w gellan gum fluid gels were prepared as  
83 described above at a fixed shear rate of 500 s<sup>-1</sup> with cooling rates of 0.5 °C min<sup>-1</sup>, 2 °C min<sup>-1</sup> and 10  
84 °C min<sup>-1</sup>. Similarly, to investigate the effect of shear rate, 0.75% w/w gellan gum fluid gels were  
85 prepared at a fixed cooling rate of 2 °C min<sup>-1</sup> using shear rates of 100 s<sup>-1</sup>, 500 s<sup>-1</sup> and 1000 s<sup>-1</sup>.

### 86 *2.3. Preparation of control formulations*

#### 87 *2.3.1 Viscosity test controls*

88 To ensure the fluid gel formulations had a suitable viscosity profile a marketed paediatric ibuprofen  
89 suspension was used as a standard comparison and referred to as C1.

#### 90 *2.3.2 Dissolution test controls*

91 To ensure ibuprofen could be fully dissolved in the dissolution media (PBS pH 7.4) at the  
92 formulated dose following 20 min exposure to acid at pH 1.2 (and any delayed release was not an  
93 effect of the pKa of the ibuprofen), control solutions were prepared by adding drug (20 mg/ml) to  
94 deionized water at ~60 °C which were cooled to room temperature and the pH was adjusted to 7.4  
95 using 1 M NaOH (referred to as C2).

96 To ensure the same grade of ibuprofen was used in all dissolution experiments formulations based  
97 upon standard ibuprofen suspensions were prepared as a control by adding 0.3% w/w xanthan gum  
98 and 0.2% w/w sorbitol to deionized water / glycerol 50:50 at 85°C while stirring (to prevent any  
99 interference with UV analysis no preservatives, colouring agents or flavours were added). Once

100 fully dissolved, the solution was allowed to cool to ~60 °C then a paediatric dose of ibuprofen (20  
101 mg/ml) was added. The suspension was then cooled to room temperature and referred to as C3.

#### 102 *2.4. Viscosity measurements*

103 Viscosity of all samples was determined taken at 25 °C using the Bohlin Gemini Nano HR  
104 rheometer using the 55 mm parallel plate geometry across shear rates ranging from 1 s<sup>-1</sup> - 1000 s<sup>-1</sup>.

#### 105 *2.5. Microscopy*

106 Fluid gel samples were imaged using an optical microscope (Keyence VHX digital microscope RZ  
107 x 250- x2500 real zoom lens in high dynamic range). Samples were prepared for imaging by  
108 dispersing the fluid gel samples in 10 ml of 50 mM CaCl<sub>2</sub>. The suspension was then centrifuged at  
109 13000 rpm and the pellet was then examined under the microscope. CaCl<sub>2</sub> was used as the diluent  
110 during the processing of the sample prevent aggregation of the gel particles during the  
111 centrifugation step.

#### 112 *2.6. Dissolution studies*

113 A modified USP I apparatus (baskets at a stirring rate of 100 rpm) was used to study in vitro drug  
114 release. Each formulation (5 ml) was placed into dialysis tubing (12500 MWCO) then submerged  
115 (within the baskets) in small volume vessels containing 200 ml dissolution media at pH values of  
116 1.2, 2, 3, 4, 5, and 7.4 for 20 min. The media were subsequently changed to pH 7.4 phosphate  
117 buffered saline (sodium chloride 137 mM, potassium chloride 2.7 mM, disodium hydrogen  
118 phosphate 10 mM and potassium dihydrogen phosphate 2.0 mM). All buffers used were prepared at  
119 the same ionic strength and pH 7.4 was used to represent the highest pH the formulations may  
120 encounter during intestinal transit (terminal ileum). To understand how release in simulated  
121 intestinal conditions was affected by residence time in acidic media, samples were also exposed to  
122 pH 1.2 and pH 2 environments for time periods increasing from 5 min to 120 min before changing  
123 the media to pH 7.4 and recording the subsequent onset of release. Ibuprofen standards were



124 prepared at concentrations ranging from 10-1000  $\mu\text{g/ml}$  and measured using UV spectrophotometer  
125 at a wavelength of 254 nm to generate calibration curves which were plotted for all pH values. The  
126 concentration of ibuprofen released from the sample was determined from the corresponding  
127 calibration curves. All experiments were carried out in triplicate.

## 128 *2.7. Rheological Measurements*

129 The following rheological measurements were performed to investigate how gel stiffness changes  
130 during the *in vitro* dissolution tests and therefore enable correlation of stiffness ( $G'$ ) to drug release.

131 To understand how elastic modulus ( $G'$ ) was affected by residence time in acidic media, 5 ml of the  
132 formulation was placed into a dialysis tube (12500 MWCO) then submerged in 200 ml 0.1 M HCl  
133 at pH 1.2 for time periods increasing from 5 to 120 min before loading the sample on the rheometer.

134 To study the impact the change of dissolution media (to PBS pH 7.4) has on the stiffness of the gel  
135 following exposure to acid, another set of samples was also exposed to pH 1.2 for 10 and 60 min  
136 (batch A and B respectively). The medium was then changed to pH 7.4 for a period of time from 30  
137 to 600 min for batch A and 60 to 1200 min for batch B, prior to loading on the rheometer.

138 Rheological measurements were carried out using a Bohlin Gemini Nano HR rheometer. Oscillation  
139 mode was used to determine viscoelasticity of the gel. Mechanical spectra were obtained by taking  
140 measurements of the elastic (storage) modulus ( $G'$ ), viscous (loss) modulus ( $G''$ ) and complex  
141 dynamic viscosity ( $\eta^*$ ). The measurements were recorded at 10 rad/s angular frequency and 0.5%  
142 strain using a 55 mm parallel-plate geometry with a 0.5 mm gap. The strain amplitude chosen was  
143 within the linear viscoelastic region of the samples. All measurements were taken at 37 °C.

## 144 *2.8. Statistical analysis*

145 Statistical significance ( $P < 0.05$ ) between test groups was determined by one-way analysis of  
146 variance (ANOVA) and Tukey post-hoc test using Primer of Biostatistics version 4.

## 147 3. Results

### 148 3.1. Rheological Measurements

149 Using a rheometer, the formation of fluid gels can be characterised during manufacture alongside  
150 real-time measurements of the characteristic changes in viscosity that occur during formation.  
151 Figure 1 shows the relative viscosity vs temperature of a 0.1%, 0.375%, 0.5% 0.75% and 1% w/w  
152 gellan gum fluid gel during manufacture. As the temperature is decreased there is an increase in  
153 viscosity that occurs at the onset of gelation of the gellan, a maximal viscosity is then reached  
154 which is the temperature beyond which no further particles are formed ( $T_{\max}$ ), followed by a plateau  
155 in viscosity as the formed particles are smoothed. The results indicate that the viscosity of fluid gel  
156 is concentration dependant; onset of gelation increases from  $\sim 40$  °C for 0.1 % gellan gum to  $\sim 45$  °C  
157 for 1% gellan gum. Furthermore, the final viscosity (at  $500\text{ s}^{-1}$  and  $20$  °C) of the fluid gels increases  
158 with increasing concentration from  $\sim 0.01$  Pas for 0.1% w/w gellan gum up to  $\sim 0.1$  Pas for 1% w/w  
159 gellan gum. To evaluate the potential of gellan gum fluid gels as a modified oral liquid, samples  
160 were tested and compared with a proprietary ibuprofen suspension. The viscosity profiles of gellan  
161 gum fluid gel formulations (0.1-1 % w/w) are shown in figure 2A and have a shear thinning  
162 viscosity profile. The 0.75% fluid gel sample exhibited a viscosity profile that was most similar to  
163 that of a standard ibuprofen paediatric suspension. In addition the yield stress was sufficient to  
164 allow inversion of the fluid gel sample without any flow however following mild shaking of the  
165 sample it is easily poured on a dispensing spoon as illustrated in figure 3 (Supplementary Video 1) .  
166 This formulation was therefore used in further investigations. Dynamic small deformation  
167 oscillatory measurements of  $G'$  and  $G''$  (Fig 2B) highlight the viscoelasticity of the 0.75% w/w  
168 fluid gel with  $G'$  slightly greater than  $G''$  across a range of frequencies; this is typical 'weak gel'  
169 rheological behaviour. Figure 3 shows the effect of cooling rate on the viscosity during formation of  
170 a 0.75% w/w fluid gel at a fixed shear rate of  $500\text{ s}^{-1}$  (Fig 4A) and the effect of shear rate at a fixed

171 cooling rate of 2 °C/min (Fig 4B). The viscosity of the fluid gels during formation increased with  
172 increasing cooling rates and viscosity decreased when shear rate was increased.

173

### 174 *3.2 Effect of gellan gum concentration*

175 Microscopy images in figure 5 reveal particle sizes of fluid gel are highly dependent on  
176 concentration. At 0.1% gellan the particles were in the region of 1-5 µm and were generally  
177 spherical in shape (Fig. 5A). As the concentrations increased to 0.5% the particles had a larger,  
178 binomial size distribution with a population of micron sized particles (similar to 0.1% w/w) and a  
179 population and a population in the region of 10-20 µm (Fig. 5B). At 0.75% w/w the particles appear  
180 less polydisperse than at 0.5% and more spherical with the majority of the population in the region  
181 of 20 µm (Fig. 5C). When the concentration is increased further to 1% the particles were much  
182 larger and irregular in shape (Fig. 5D).

183

### 184 *3.3 Effect of cooling rate and shear rate*

185 Figure 6 shows the effect of increasing cooling rates on the particle size of 0.75% w/w at fixed  
186 shear rate of 500 s<sup>-1</sup> (Fig 6A-C) and the effect of increasing shear rates on the particle size of same  
187 concentration of gellan at fixed cooling rate of 2 °C/min (Fig 6D-F). These micrographs indicate  
188 that a smaller particle size can be obtained by decreasing cooling rate and increasing the shear rate  
189 when forming the fluid gels.

190

### 191 *3.3 Dissolution behaviour*

192 To investigate the effects of exposure to low pH for the fluid gels a 5 ml sample of each was  
193 dispensed into 0.1M HCl at pH 1.2. A proprietary ibuprofen suspension (C1) was also used for  
194 comparison. The proprietary formulation formed a cloudy dispersion in the acid which is attributed  
195 to the poor solubility of ibuprofen at low pH. The gellan fluid gel on the other hand formed an acid  
196 gel with the ibuprofen remaining associated with the gellan. This remained as large aggregated gel

197 pieces for over 6 hours. This was supported by dissolution experiments which showed no ibuprofen  
198 was released at pH 1.2 (results not shown) and in Figure 7 where ibuprofen crystals can be seen to  
199 remain entrapped within the fluid gel particles.

200 Figure 8 illustrates the *in vitro* release of ibuprofen from different gellan gum fluid gel  
201 concentrations ranging from (0.0 % (ibuprofen alone) to 0.75% w/w) determined at pH 1.2 then the  
202 release media was changed after 20 minutes to PBS pH 7.4 . The results show that there was a small  
203 quantity of ibuprofen released in acidic media for the gels containing lower concentrations of gellan  
204 and control formulations (C2 and C3). At 0.75% w/w however, there was no release in acid medium  
205 and subsequent release was retarded in PBS for 30 min.

206 To account for the wide variation in stomach pH found in paediatric patients, release characteristics  
207 were determined *in vitro* at different pH values (1.2, 2, 3, 4, 5 and 7.4) then the release medium was  
208 changed after 20 minutes to PBS pH 7.4. Figure 9 highlights that the release of ibuprofen from the  
209 gellan gum fluid gel was strongly affected by pH of the dissolution media. There was no significant  
210 difference ( $p > 0.05$ ) in release between samples initially immersed in pH 7.4, pH 5 and pH 4. At  
211 pH 3 however, subsequent release of ibuprofen was retarded. The retardation of release became  
212 progressively more pronounced as the pH was dropped further, to the point where exposure to pH  
213 1.2 for just 20 min delayed the onset of drug release for a further 60 min when transferred to pH  
214 7.4. The duration of exposure to acidic pH was shown to dramatically affect the lag time to onset of  
215 release following transfer to pH 7.4. Figure 10 illustrates the linear relationship between onset of  
216 release in pH 7.4 and the preceding exposure time at pH 1.2 and pH 2. The onset of release in pH  
217 7.4 was shown to be dramatically affected by the acidity of the initial dissolution medium taking  
218 almost 3 hours after exposure to pH 1.2 (for 2 hours) compared with 30 minutes to onset of release  
219 following exposure to pH 2 (for 2 hours). This lag time was shown to be dependent on gel stiffness.  
220 Figure 11 shows onset of release time rises exponentially with increase in  $G'$ , which in turn is  
221 dependent on exposure time to pH 1.2 as highlighted in figure 12. Interestingly, when the fluid gel  
222 was transferred to PBS pH 7.4 following 10 minutes in pH 1.2 medium, the gel stiffness continued

223 to increase, albeit at a slower rate, until a plateau was reached (following 90 minutes in pH 7.4)  
224 where  $G'$  is approximately 1200 Pa. When the gel was exposed to pH 1.2 for 60 minutes the  
225 stiffness was almost an order of magnitude greater than after 10 minutes exposure. However  
226 following transfer to PBS pH 7.4 the stiffness gradually decreased over a period of 180 minutes to  
227 the plateau where  $G'$  is approximately 1200 Pa. The relationship between gel stiffness and release  
228 in pH 7.4 is highlighted in figure 13. Following 60 minutes exposure to 0.1 M HCl the gel stiffness  
229 was 8000 Pa which gradually decreased on transfer to PBS. No released drug was detected until the  
230 stiffness of the gel had reduced to ~2000 Pa (which took 2 hours), following which zero order  
231 release 0.15 mg/min was apparent (Fig. 13A). When the sample was exposed to pH 1.2 for 10  
232 minutes, the gel stiffness was only ~600 Pa and gradually increased to ~1300 Pa on transfer to PBS  
233 pH 7.4. In this system the zero order drug release occurred within 40 minutes and at an increased  
234 rate of 0.44 mg/min (Fig. 13B). After this time, the gel disintegrated and was no longer included in  
235 this study. These results highlight that increased gel stiffness can reduce the release rate.

236

#### 237 **4. Discussion**

238 The use of fluid gels as a platform technology for pharmaceutical formulations has great potential  
239 due to the tuneable mechanical properties and their ease of manufacture. It has been previously  
240 shown that fluid gels can be prepared with many different biopolymers including gelatin (de  
241 Carvalho and Djabourov, 1997), agarose (Norton et al., 1998),  $\kappa$ -carrageenan (Garrec and Norton,  
242 2012; Gabriele et al., 2009) and gellan gum (Sworn et al., 1995). Most of these investigations have  
243 been focused towards applications in foods to improve stability and improve texture. Here we have  
244 investigated the potential of gellan gum fluid gels as a modified release oral drug delivery system.  
245 The preparation of fluid gels is a simple process, producing gelled particles that are dispersed in an  
246 un-gelled medium. Production using a rheometer allows the cooling rate and the shear rate to be  
247 accurately controlled and the characteristic change in viscosity monitored (the process however, is  
248 easily carried out on a larger scale using application of shear). When the gellan gum fluid gels were

249 formed containing ibuprofen, the onset of ordering increased with increasing gellan concentration  
250 (Fig 1) which can be explained by the consequential increase in concentration of the counterions to  
251 the charged group of the polymer promoting aggregation (Morris et al 2012). Interestingly, this  
252 onset of ordering occurs at a slightly lower temperature that has been previously reported for gellan  
253 gum fluid gels without a drug load (Sworn et al., 1995). This is thought to be due to the competitive  
254 inhibition by the negatively charged ibuprofen binding some of the Na<sup>+</sup> ions (introduced during pH  
255 adjustment with NaOH) reducing the overall ionic strength of the bulk, consequently reducing the  
256 viscosity and gelation temperature. Once manufactured, the bulk fluid gels containing ibuprofen  
257 showed shear thinning behaviour similar to that of a proprietary paediatric oral ibuprofen  
258 suspension with the 0.75% w/w fluid gel having the closest match (Fig 2). However, at very low  
259 shear rates the viscosity was sufficient for the preparation to be inverted without any steady state  
260 flow as illustrated in figure 3 (Supplementary Video 1). This is due to the weak gel properties of the  
261 ibuprofen gellan fluid gel (Fig 2B) which are thought to be a result of particle-particle interactions  
262 (Garrec et al., 2013).

263 Oral liquid formulations with relatively high values of zero shear viscosity that rapidly shear thin to  
264 enable dispensing would be greatly beneficial by suspending the drug more efficiently during  
265 product storage while not impacting on the ease of administration. Furthermore, producing oral  
266 liquid formulations with modified release properties would provide an alternative dosage form for  
267 paediatric patients in particular. The physical properties of gellan gum fluid gels can be tuned by  
268 simply changing the concentration of the polymer or by the rate of cooling and/or shear rate during  
269 fluid gel formation (Fig 4). This has previously been demonstrated in food based applications with  
270 agarose and carrageenan fluid gels (Norton et al 1998; Gabriele et al 2009). This allows the particle  
271 size to be controlled as shown in figures 5 and 6. Gellan gum has previously shown promise as a  
272 sustained release oral liquid which gels in situ (Miyazaki et al., 1999). In this study we present a  
273 gellan gum oral liquid which was formulated to have a physically cross-linked microstructure prior  
274 to exposure to an acidic gastric environment. The weak acid gel contains pre-gelled particles and

275 ungelled gellan effectively immobilising the pre-gelled particles. This system was shown to prevent  
276 the dispersion of ibuprofen in the gastric fluid as occurred with a proprietary oral liquid and the  
277 drug remained associated with the gellan gum for over 6 hours at pH 1.2. A problem often  
278 associated with hydrogel drug delivery systems is drug leaching through the pores of the gel. In this  
279 system however, the poor solubility of ibuprofen resulted in precipitation within the gel when  
280 exposed to 0.1M HCl pH 1.2, illustrated by the opaque nature of the gel with the precipitated drug  
281 particles remaining entrapped within gel particles as illustrated in figure 7.

282 To develop a modified release oral liquid designed particularly for children it is vitally important to  
283 take into account paediatric gastrointestinal physiology when designing *in vitro* biopharmaceutical  
284 tests. Variables such as stomach acid volume, gastric pH and small intestinal transit time, which are  
285 important for drug release, are well documented (Bowles et al., 2010). For example, in paediatric  
286 patients the age at which gastric acid secretion reaches adult values is often quoted as 6 months,  
287 however in reality the pH remains variable and the time that intragastric pH is maintained below pH  
288 2 increases as a function of age. Nagita et al. (1996) reported that gastric acidity rapidly increased  
289 from infancy to 3 years of age and then slowly increased and attained adult levels (< pH 2 for 65%  
290 of a 24 h period) by adolescence (age 14). In vitro release data shown in figure 8 reveal that even at  
291 concentrations as low as 0.1%, gellan fluid gels have the ability to retard the release of ibuprofen  
292 following 20 minutes exposure to 0.1 M HCl pH 1.2 compared with the control formulations (C2  
293 and C3). There is however, still some ibuprofen (approximately 5%) released while exposed to pH  
294 1.2. Increasing gellan concentration further slows release and at 0.75%, no ibuprofen was measured  
295 during acid exposure. Moreover, when the medium was changed to pH 7.4, there was a lag time of a  
296 further 30 minutes before onset of release.

297 The effects of varying acidic pH on the subsequent release of ibuprofen from the 0.75% gellan gum  
298 fluid gel following transfer to pH 7.4 was also evaluated. It was found that the release of ibuprofen  
299 from a 0.75% gellan gum fluid gel was strongly affected by the pH of the dissolution media (Fig.

300 9). There was no significant difference in release between samples that were initially exposed to pH  
301 4, pH 5, and pH 7.4. However, as the pH was decreased below the  $pK_a$  of the carboxyl group of the  
302 gellan gum ( $\sim 3.4$ ), an acid gel was formed, preventing the dissolution of the gel, thus retarding  
303 ibuprofen release. This lag time became progressively more pronounced as the pH was dropped  
304 further and the acid gel strengthened; exposure to pH 1.2 for just 20 min prevented the onset of  
305 release for a further 60 min following transfer to pH 7.4. Moreover, there was a linear relationship  
306 between onset of release in pH 7.4 and the preceding exposure time at pH 1.2 for up to 120 min  
307 (Fig. 10). A linear relationship was also found following exposure to pH 2 although the effect was  
308 substantially less pronounced. This was thought to be due to fewer  $H^+$  ions present at pH 2  
309 compared with pH 1.2. This will result in formation of a weaker acid gel with an associated increase  
310 in hydration and dissolution of the ibuprofen when transferred to pH 7.4. Indeed, the stiffness of the  
311 gel had an exponential relationship with onset of release in pH 7.4 media (Fig. 11). Furthermore the  
312 stiffness of the gellan was dependent on the duration of exposure to acidic pH which has also  
313 recently been reported by Bradbeer et al. (2014). Interestingly, regardless of the duration of acid  
314 exposure, the stiffness of gellan fluid gels eventually plateaued at approximately 1200 Pa when  
315 transferred to pH 7.4 (Fig. 12). Subsequently, the gel stiffness as a function of exposure time  
316 relates to *in vitro* release. When the gel was exposed to pH 1.2 for 60 min,  $G'$  was approximately  
317 8000 Pa and release was retarded, probably due to the time required for ion exchange to occur  
318 between the  $H^+$  cross-linked gel and the phosphate buffer. This exchange gradually reduces the gel  
319 strength until drug release is enabled. Moreover, the diffusion of the phosphate buffer into the gel  
320 also increases the solubility of the ibuprofen by increasing the pH within the gel. This is thought to  
321 have facilitated drug diffusion into the surrounding release medium increasing release rate as  
322 highlighted, with a much faster release rate of 0.44 mg/min when the exposure time in acid was  
323 only 10 min compared with 0.15 mg/min following 60 min exposure (Fig. 13). This dependence on  
324 acidic residence time and the strength of acidic pH may be problematic in determining reproducible  
325 pharmacokinetics between patients where gastro intestinal physiology can vary. Therefore strategies



326 to overcome this issue would need to be addressed if such a carrier was to be used in clinical  
327 practice. However, by understanding the three way relationship between acid exposure time, gel  
328 stiffness and onset of release, there is potential for controlling release behaviour by tuning the fluid  
329 gels to have a specified stiffness at a particular pH and duration of exposure. Furthermore,  
330 producing formulations using this relatively simple method is particularly attractive and by careful  
331 design of processing parameters, the microgel particles' size, shape, viscoelasticity and behaviour in  
332 physiological fluids can be manipulated to suit the application. This could open the door to multiple  
333 applications of fluid gel systems in pharmaceutical technology in addition to use as modified release  
334 oral liquids.

335

## 336 **5. Conclusion**

337 In this study we have demonstrated that fluid gels have the potential to be formulated with a similar  
338 viscosity profile to that of a marketed paediatric oral liquid with a yield stress sufficient that the  
339 sample can be inverted without any immediate flow but shear thins sufficiently by shaking, to be  
340 poured onto a dispensing spoon. Furthermore, we have shown that it is possible to modify the  
341 release of ibuprofen from gellan gum fluid gels, providing a simple and effective technology in  
342 formulating modified release oral liquids. The release behaviour of ibuprofen from gellan gum fluid  
343 gels in a simulated intestinal pH environment was dependent on the stiffness of the gel following  
344 exposure to simulated gastric pH media. The stiffness, and hence drug release, could be controlled  
345 with exposure time and acidity of the simulated gastric pH environment. This work highlights the  
346 potential application of gellan gum fluid gels as modified release oral liquids while at the same  
347 time, illustrates the importance of understanding how subtle differences in patient physiology could  
348 impact on drug release from such formulations. A realization of this is very important especially  
349 when designing medicines for paediatrics.

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352 Mohammed Mahdi and Professor Ian Norton and Dr Fotis Spyropoulos of the University of  
353 Birmingham for their helpful discussions.

354

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#### 443 **Figure Captions**

444 **Figure 1 Viscosity of gellan gum during fluid gel formation (cooling at 2°C /min at a shear**  
445 **rate of 500 s<sup>-1</sup>) for 0.1% (filled diamonds) 0.375 % (open squares) 0.5% (open circles) 0.75%**  
446 **(filled triangles) and 1% (black crosses) w/v gellan gum loaded with 20 mg/ml ibuprofen.**

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448 **Figure 2 A) Viscosity vs. shear rate at 25°C for 0.1% (filled diamonds) 0.375 % (open squares)**  
449 **0.5% (open circles) 0.75% (filled triangles) and 1% (black crosses) w/v gellan gum loaded**  
450 **with 20 mg/ml ibuprofen. Black line indicates a proprietary ibuprofen paediatric suspension.**

451 **B) Mechanical spectrum (0.5% strain; 37 °C) of a 0.75% Gellan Gum fluid gel loaded with 20**  
452 **mg/ml ibuprofen showing variation of G' (filled squares), G'' (open squares) and  $\eta^*$  (filled**  
453 **triangles) with angular frequency.**

454 **Figure 3 Images illustrating the shear thinning behaviour of an ibuprofen loaded fluid gel**  
455 **sample with the ability to invert without any flow.**

456

457 **Figure 4 Viscosity of 0.75% w/v gellan gum loaded with 20 mg/ml ibuprofen during fluid gel**  
458 **formation using A) different cooling rates; 10 °C/min (open circles), 2 °C/min (filled**  
459 **diamonds), 0.5 °C/min (open triangles) at a shear rate of 500 s<sup>-1</sup> and B) different shear rates**  
460 **cooling at 2 °C/min; 1000 s<sup>-1</sup> (open diamonds), 500 s<sup>-1</sup> (filled diamonds), 100 s<sup>-1</sup> (black**  
461 **crosses).**

462

463 **Figure 5 Light microscopy images of gellan gum fluid gels prepared at different**  
464 **concentrations loaded with 20mg/ml ibuprofen A) 0.1% w/v B) 0.5 % w/v C) 0.75 % w/v D) 1**  
465 **% w/v.**

466

467 **Figure 6 Light microscopy images of 0.75% w/v gellan gum loaded with 20 mg/ml ibuprofen**  
468 **prepared at a shear rate of 500 s<sup>-1</sup> using different cooling rates (A-C) A) 0.5 °C/min B)**  
469 **2°C/min C) 10 °C/min and different shear rates cooling at 2 °C/min (D-F); D)100 s<sup>-1</sup> E) 500 s<sup>-1</sup>**  
470 **F) 1000 s<sup>-1</sup>**

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472 **Figure 7 Light microscopy images of gellan gum fluid showing crystallised ibuprofen**  
473 **entrapped within gel particles**

474

475 **Figure 8 Cumulative % release of ibuprofen from fluid gels prepared at different**  
476 **concentrations of gellan gum compared with a standard ibuprofen suspension. Dotted line**  
477 **indicates the point the media was changed from 0.1 M HCl at pH 1.2 to PBS at pH 7.4. Values**  
478 **are represented as mean  $\pm$  SD (n=3)**

479

480 **Figure 9 Cumulative % release of ibuprofen from 0.75% w/v gellan gum fluid gel loaded with**  
481 **20 mg/ml ibuprofen exposed to different acidic pH values for a period of 20 minutes. Dotted**  
482 **line indicates the point the media was changed to PBS at pH 7.4. Values are represented as**  
483 **mean  $\pm$  SD (n=3)**

484

485 **Figure 10 Relationship between onset of release at pH 7.4 and preceding exposure time in**  
486 **simulated gastric fluid at pH 1.2 (filled diamonds) and pH 2 (open diamonds).**

487

488 **Figure 11 Exponential relationship between the onset of release in SIF pH 7.4 as a function of**  
489 **gel stiffness (G').**

490

491 **Figure 12 Effect of time exposed to pH 1.2 on gel stiffness (G') and subsequent stiffness on**  
492 **transfer to pH 7.4. The red line (filled diamonds) indicates the stiffness of the gel when**  
493 **exposed to pH 1.2 (0.5% strain; 37 °C at 10rad s<sup>-1</sup>). The green dashed line (open triangles)**  
494 **represents the stiffness of the gel in PBS at pH 7.4 following 10 min exposure to pH 1.2. The**  
495 **blue dashed line (filled squares) represents the stiffness of the gel in PBS at pH 7.4 following**  
496 **60 min exposure to pH 1.2.**

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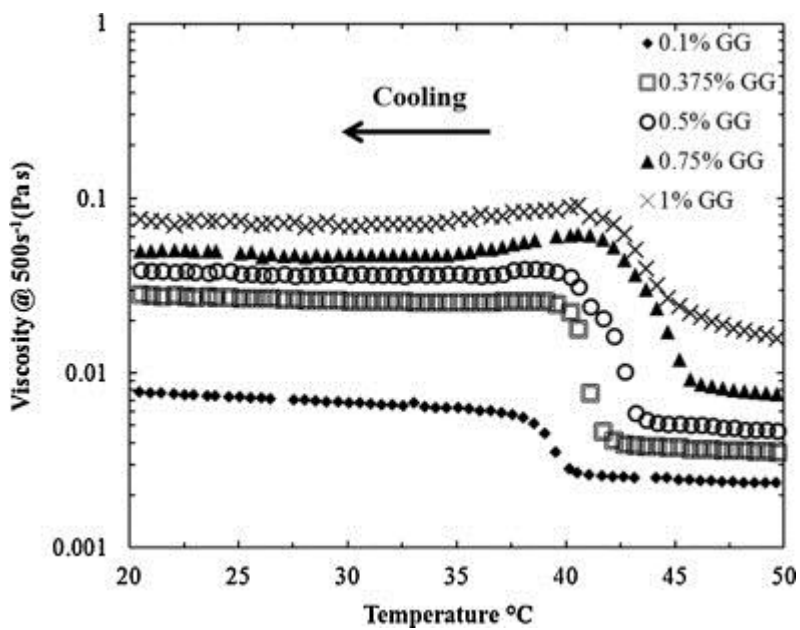


498 **Figure 13 Cumulative % release (primary vertical axis) and gel stiffness (G') (secondary**  
499 **vertical axis) versus time following A) 60 min exposure to pH 1.2 and B) 10 min exposure to**  
500 **pH 1.2.**

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Fig. 1.

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Viscosity of gellan gum during fluid gel formation (cooling at 2 °C/min at a shear rate of 500 s<sup>-1</sup>) for 0.1% (filled diamonds),  
507 0.375% (open squares), 0.5% (open circles), 0.75% (filled triangles) and 1% (black crosses) w/v gellan gum loaded with  
508 20 mg/ml ibuprofen.

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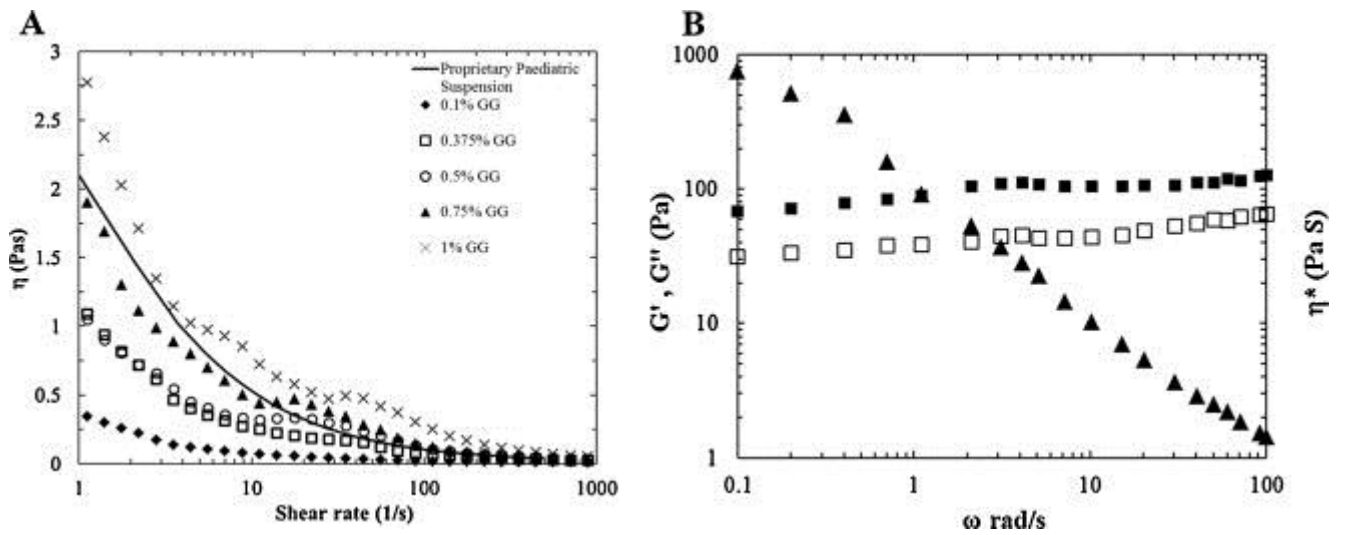


Fig. 2.

(A) Viscosity vs. shear rate at 25 °C for 0.1% (filled diamonds), 0.375% (open squares), 0.5% (open circles), 0.75% (filled triangles) and 1% (black crosses) w/v gellan gum loaded with 20 mg/ml ibuprofen. Black line indicates a proprietary ibuprofen paediatric suspension. (B) Mechanical spectrum (0.5% strain; 37 °C) of a 0.75% gellan gum fluid gel loaded with 20 mg/ml ibuprofen showing variation of  $G'$  (filled squares),  $G''$  (open squares) and  $\eta^*$  (filled triangles) with angular frequency.



Fig. 3.

Images illustrating the shear thinning behaviour of an ibuprofen loaded fluid gel sample with the ability to invert without any flow.

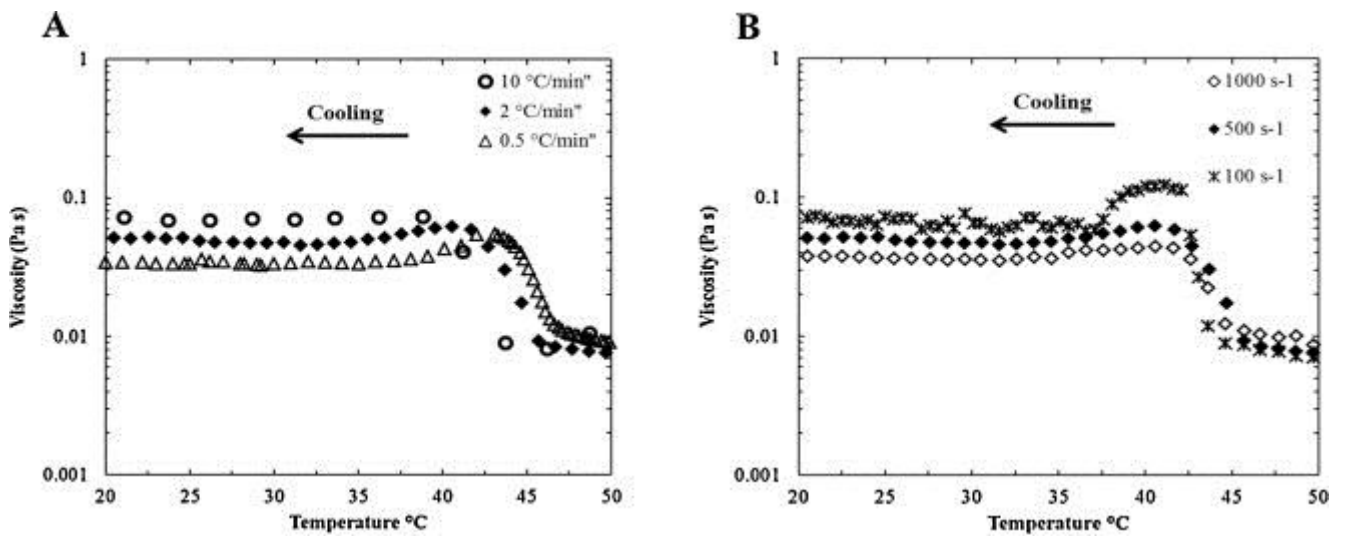


Fig. 4.

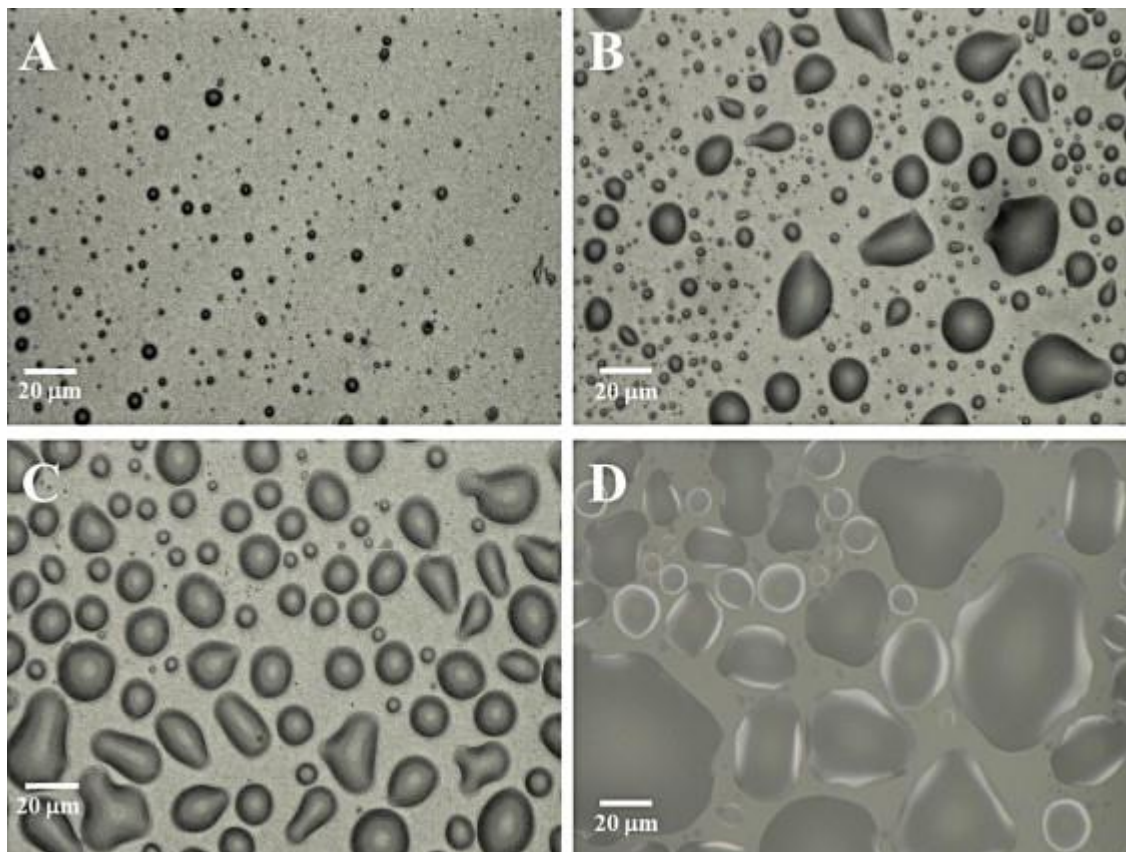
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Viscosity of 0.75% w/v gellan gum loaded with 20 mg/ml ibuprofen during fluid gel formation using (A) different cooling rates; 10 °C/min (open circles), 2 °C/min (filled diamonds), 0.5 °C/min (open triangles) at a shear rate of 500 s<sup>-1</sup> and (B) different shear rates cooling at 2 °C/min; 1000 s<sup>-1</sup> (open diamonds), 500 s<sup>-1</sup> (filled diamonds), 100 s<sup>-1</sup> (black crosses).



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Fig. 5. Light microscopy images of gellan gum fluid gels prepared at different concentrations loaded with 20 mg/ml ibuprofen (A) 0.1% w/v, (B) 0.5% w/v, (C) 0.75% w/v and (D) 1% w/v.

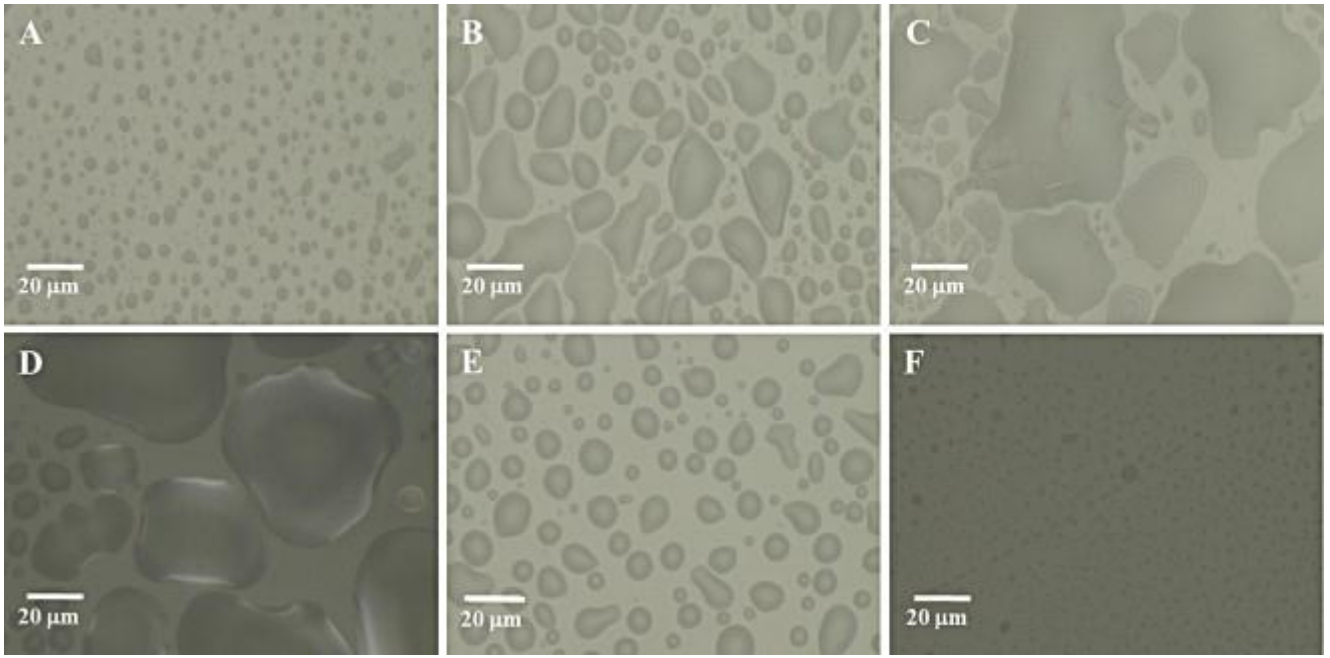


Fig. 6.

Light microscopy images of 0.75% w/v gellan gum loaded with 20 mg/ml ibuprofen prepared at a shear rate of  $500 \text{ s}^{-1}$  using different cooling rates (A–C). (A)  $0.5 \text{ }^{\circ}\text{C}/\text{min}$ , (B)  $2 \text{ }^{\circ}\text{C}/\text{min}$ , (C)  $10 \text{ }^{\circ}\text{C}/\text{min}$  and different shear rates cooling at  $2 \text{ }^{\circ}\text{C}/\text{min}$  (D–F), (D)  $100 \text{ s}^{-1}$ , (E)  $500 \text{ s}^{-1}$  and (F)  $1000 \text{ s}^{-1}$ .

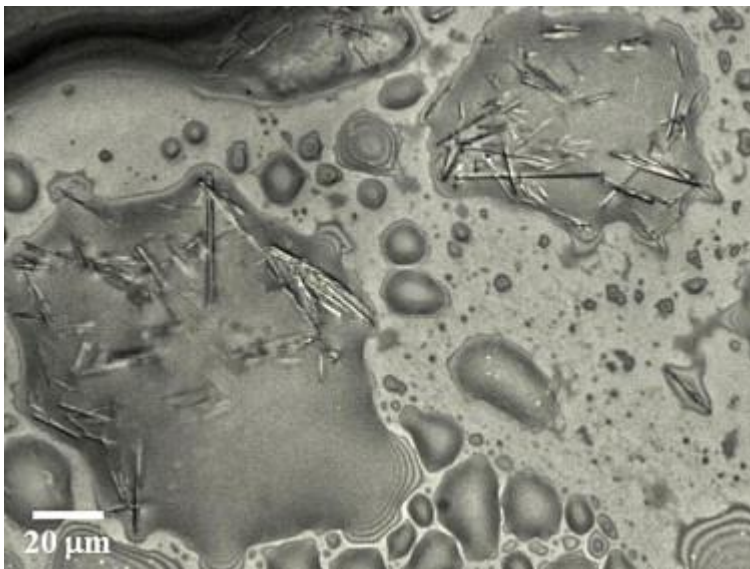


Fig. 7.

Light microscopy images of gellan gum fluid showing crystallised ibuprofen entrapped within gel particles.

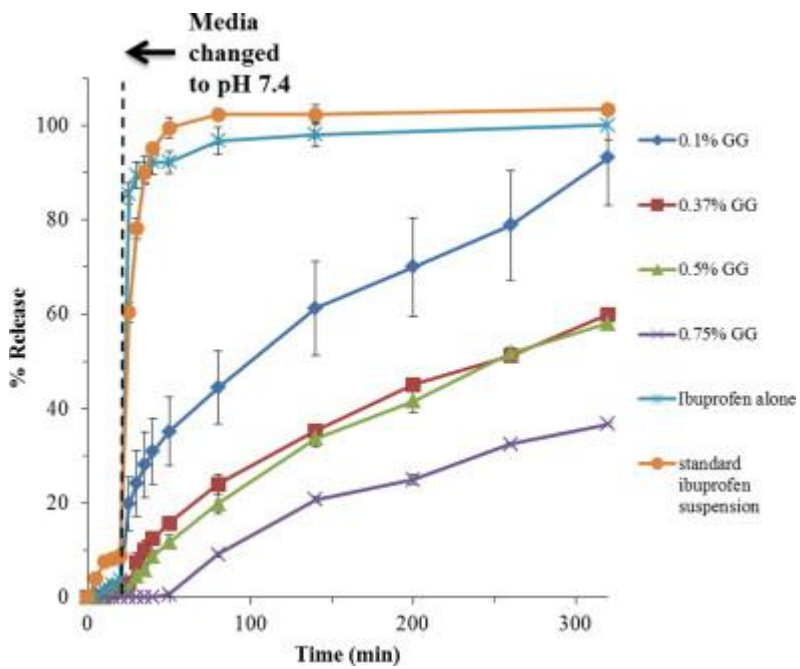


Fig. 8. Cumulative % release of ibuprofen from fluid gels prepared at different concentrations of gellan gum compared with a standard ibuprofen suspension. Dotted line indicates the point the media was changed from 0.1 M HCl at pH 1.2 to PBS at pH 7.4. Values are represented as mean  $\pm$  SD ( $n = 3$ ).

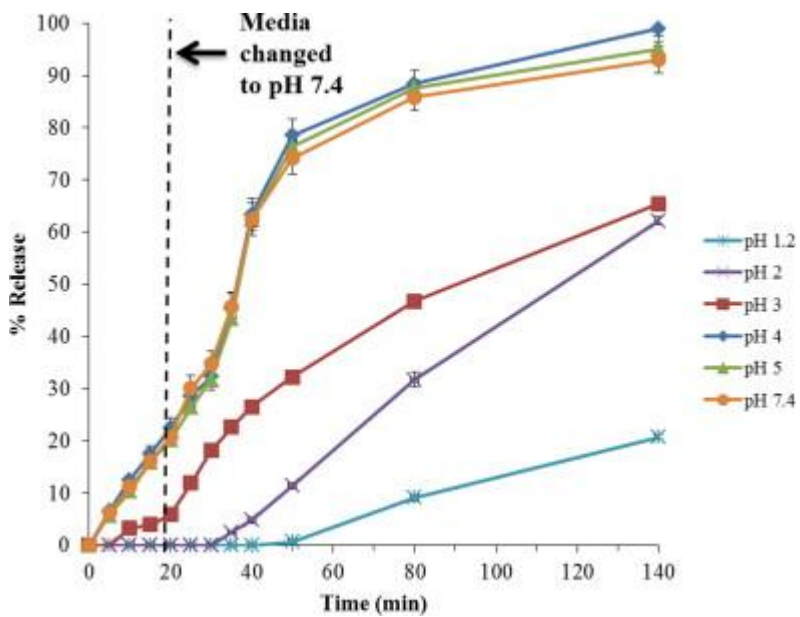


Fig. 9. Cumulative % release of ibuprofen from 0.75% w/v gellan gum fluid gel loaded with 20 mg/ml ibuprofen exposed to different acidic pH values for a period of 20 min. Dotted line indicates the point the media was changed to PBS at pH 7.4. Values are represented as mean  $\pm$  SD ( $n = 3$ ).

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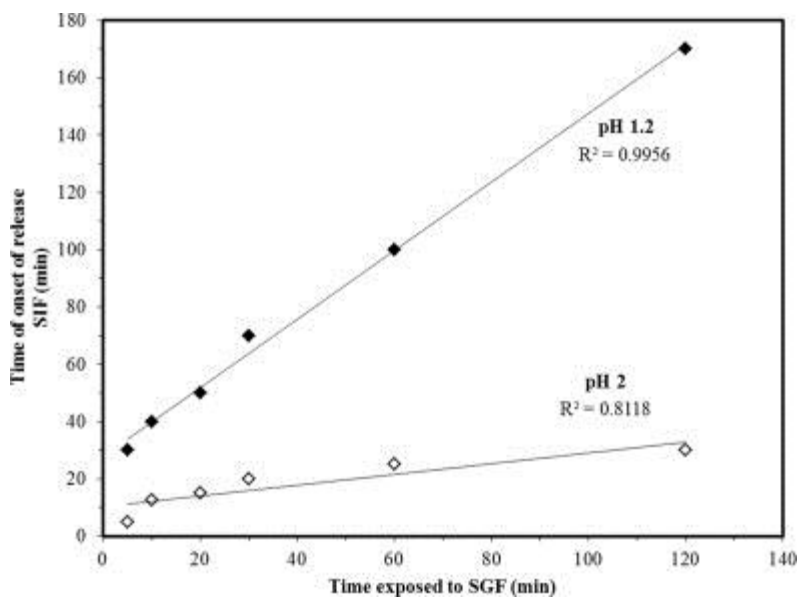


Fig. 10.

Relationship between onset of release at pH 7.4 and preceding exposure time in simulated gastric fluid at pH 1.2 (filled diamonds) and pH 2 (open diamonds).

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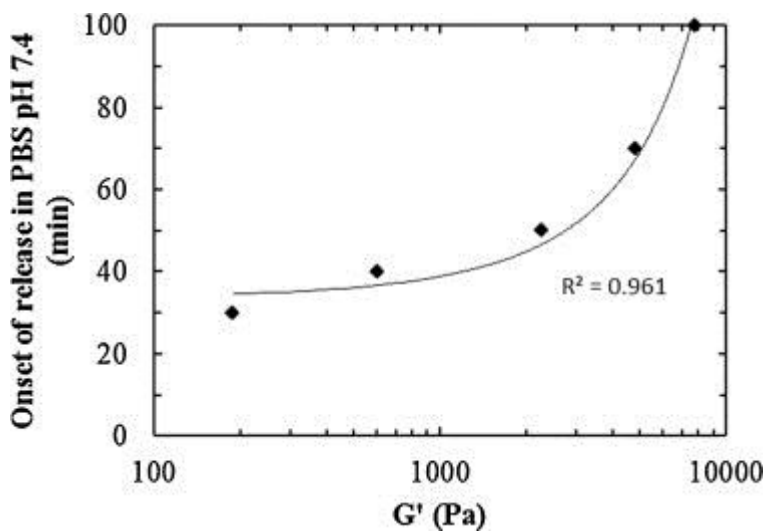
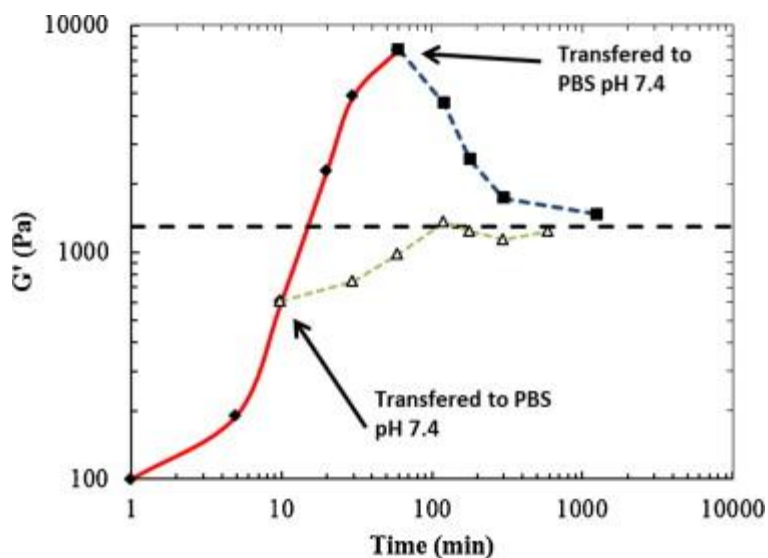


Fig. 11.

Exponential relationship between the onset of release in SIF pH 7.4 as a function of gel stiffness ( $G'$ ).

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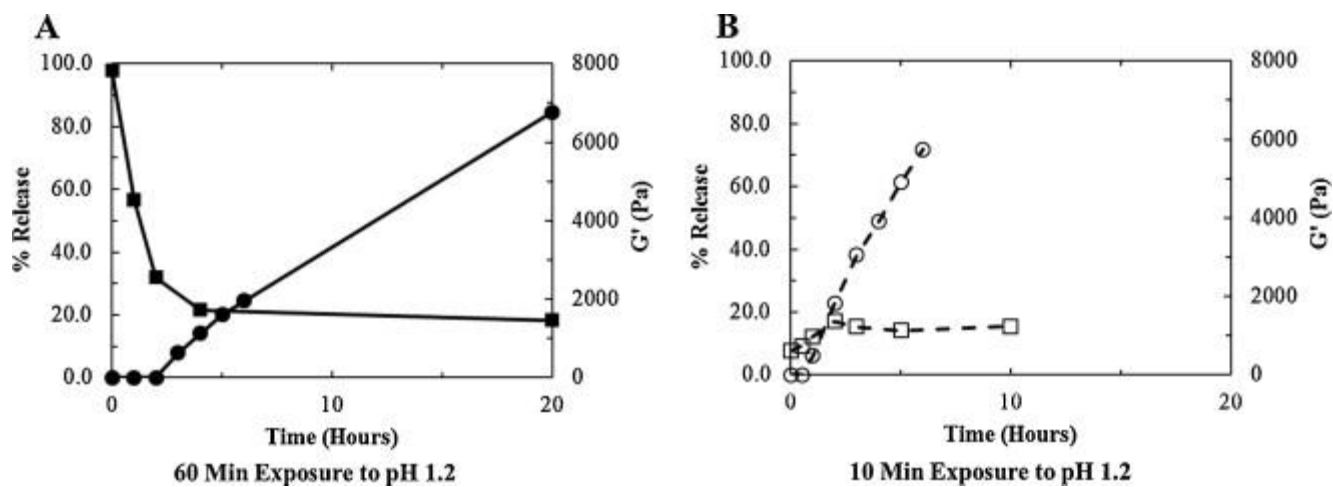




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Fig. 12.

Effect of time exposed to pH 1.2 on gel stiffness ( $G'$ ) and subsequent stiffness on transfer to pH 7.4. The red line (filled diamonds) indicates the stiffness of the gel when exposed to pH 1.2 (0.5% strain; 37 °C at 10 rad s<sup>-1</sup>). The green dashed line (open triangles) represents the stiffness of the gel in PBS at pH 7.4 following 10 min exposure to pH 1.2. The blue dashed line (filled squares) represents the stiffness of the gel in PBS at pH 7.4 following 60 min exposure to pH 1.2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



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Fig. 13.

Cumulative % release (primary vertical axis) and gel stiffness ( $G'$ ) (secondary vertical axis) vs. time following (A) 60 min exposure to pH 1.2 and (B) 10 min exposure to pH 1.2.