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1 **Chitosan Cross-linked Poly(acrylic acid) Hydrogels:**
2 **Drug Release Control and Mechanism**

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23 **Abstract:** Chitosan has been used to cross-link poly(acrylic acid) to give three
24 pH-sensitive hydrogels designed to control the release of the drugs amoxicillin and
25 meloxicam. The extent of cross-linking and solution pH was found to dominate the
26 swelling behavior of these hydrogels as shown by scanning electron microscopy and
27 swelling time dependencies. The rates of release of amoxicillin and meloxicam from
28 the loaded hydrogels increased with increase in pH consistent with the extent of
29 hydrogen bonding between hydrogel components and between the hydrogel and the
30 drugs being important determinants of release rate. Both the Korsmeyer-Peppas and
31 Weibull models fitted release data consistent with drug release occurred through a
32 combination of drug diffusion and hydrogel relaxation processes. These hydrogels
33 appear to provide an ideal basis for controlled drug delivery systems.

34

35 **Keywords:** Chitosan, pH sensitive hydrogel, Drug delivery, Release mechanism

36

37

38 **1. Introduction**

39 Hydrogels are generally composed of hydrophilic organic networks which
40 incorporate large amounts of water into their structures. This renders them both soft
41 and elastic properties which are compatible with human physiology. Many hydrogels
42 are also able to load a wide variety of drugs into their structures and substantially
43 protect them from physiological conditions, particularly those of the stomach where pH
44 is low and enzyme concentrations are high; conditions under which many drugs are

45 unstable. In addition to this protective characteristic, hydrogels may potentially be
46 designed to selectively release drugs under the physiological conditions at the disease
47 site in the body, and thereby achieve a targeted drug release. Consequently, hydrogels
48 have found wide application in drug delivery studies [1-4]. In addition to these
49 characteristics, the introduction of stimuli dependent phase changes into hydrogels
50 offers the possibility of developing sophisticated controlled drug release systems.
51 Examples of such stimuli are light [5], temperature [6] and pH change [7].

52 Apart from being physically compatible with human physiology, hydrogels must
53 also be biocompatible with body chemistry if they are to be viable as drug delivery
54 systems. Fortunately, there is range of biocompatible polymers which may be
55 converted to hydrogel networks through chemically cross-linking them. However, it
56 must be ensured that such cross-linking entities are not toxic [8-10]. While
57 cross-linking through physical interactions such as hydrogen bonding or hydrophobic
58 interactions has been proposed to avoid toxicity problems [11-13], such cross-linking
59 may be not be strong enough to produce a sufficiently stable hydrogel for effective
60 drug loading. Fortunately, polysaccharides may be used as chemical cross-linkers to
61 produce biocompatible hydrogels which present attractive applications in drug
62 delivery [14-17].

63 The naturally occurring polysaccharide chitosan (CS) has been shown to be
64 amenable to functionalization to produce a range of versatile materials with
65 substantial potential for biomedical applications [18-22]. In this work, a chitosan
66 derivative is used to cross-link poly(acrylic acid) (PAA) to give three pH sensitive

67 poly(acrylic acid)/chitosan hydrogels (PAACS-I, PAACS-II and PAACS-III) in which
68 the extent of chitosan cross-linking progressively increases, and which are designed to
69 control the release of the drugs amoxicillin and meloxicam (Scheme 1). These drug
70 releases are analyzed through the Korsmeyer-Peppas and Weibull drug release
71 models [23,24] to gain insight into the drug release mechanism and thereby improved
72 understanding for the design of more advanced and reliable hydrogel drug delivery
73 systems.

74

75 **Scheme 1.** Molecular structures of amoxicillin and meloxicam.

76

77 **2. Experimental**

78 **2.1 Materials:**

79 Chitosan (CS, degree of *N*-deacetylation = 95%, Mw = 200 kDa) was purchased
80 from Aoxing Biotechnology Co. Ltd., China. Maleic anhydride (MAH, 99%) was
81 purchased from Acros Co. Ltd. Ammonium persulfate (APS, 99%) and acrylic acid
82 (AA, 99%, distilled under vacuum pressure prior to use) were provided by Sigma
83 Aldrich. Amoxicillin and meloxicam were supplied by TCI, Japan. The water used in
84 all experiments was purified by reverse osmosis (Shanghai RO Micro Q). All other
85 reagents and solvents were used directly.

86

87 **2.2 Synthesis of chitosan-g-(maleic anhydride) (CSMAH)**

88 An aqueous solution of chitosan was prepared by dissolving 0.5 g of chitosan in

89 40 mL of 2.5 wt% acetic acid aqueous solution under vigorous stirring. Subsequently,
90 2.5 g maleic anhydride in 1 mL acetone were added slowly into the pre-prepared
91 chitosan solution under ice cooling within 10 min. The reaction mixture was allowed
92 to warm to room temperature and stand for 8 h. Finally, the viscous solution was
93 poured into 500 mL of acetone to precipitate the product. The solid product was
94 purified by extraction with acetone three times and subsequent drying under vacuum
95 at 50 °C for 48 h.

96

97 **2.3 Preparation of PAACS hydrogels**

98 The three hydrogels, PAACS-I, PAACS-II and PAACS-III, were prepared
99 through free radical polymerization, using APS as an initiator and the synthesized
100 CSMAH as a cross-linker. Briefly, to a solution of 1.4 g NaOH in 40 mL water at
101 room temperature, either 0.05, 0.10 or 0.15 g of CSMAH were added (for PAACS-I,
102 PAACS-II and PAACS-III, respectively) with stirring until a transparent solution was
103 obtained, whereupon 0.01 g APS was added (Table 1). These mixtures were each
104 transferred into a reaction vessel and a N₂ stream was passed through for 30 min to
105 eliminate dissolved oxygen. The copolymerizations were carried out at 70 °C for 2 h.
106 The gained hydrogels were placed in 500 mL of methanol/water (v/v = 7/3) for 24 h
107 to remove the residual reactants. Finally, the purified hydrogels were cut into thin
108 cylinders and dried to constant weight in an oven at 60 °C (hydrogel samples with 60
109 mg in weight, 2.5 mm in diameter, and 20 mm in length).

110

111

Table 1. Reactants amounts for the preparation of PAACS hydrogels.

Hydrogel	AA (g)	CSMAH (g)	APS (g)	NaOH (g)	Deionized Water (mL)
PAACS-I	2.8	0.05	0.01	1.4	40
PAACS-II	2.8	0.10	0.01	1.4	40
PAACS-III	2.8	0.15	0.01	1.4	40

112

113 **2.4 Determination of the hydrogel swelling ratios (*SR*)**

114 The dried hydrogel (0.5 g) was immersed in the 100 mL of aqueous phosphate
 115 buffer solutions at pH 1.2, 6.8, and 7.4. The hydrogels were taken out of solution and
 116 weighed after removing the residual solutions on the surface at a pre-determined time
 117 interval. The hydrogels were then returned to solution and the process was repeated
 118 until a constant *SR* was obtained as calculated through Equation (1), in which m_s and
 119 m_d are the weight of the hydrogel in the swollen and dry states, respectively.

$$120 \quad SR = \frac{m_s - m_d}{m_d} \quad (1)$$

121 **2.5 Rheological measurements**

122 The dynamic frequency sweep measurements were performed on a MCR501
 123 rheometer (Anton-Paar Physical Company). A parallel-plate made of stainless steel
 124 with a diameter of 25 mm was used. During all rheological measurements, the upper
 125 plate was set at a distance of 1 mm from the down plate. All the hydrogel samples
 126 were cut into a cylindrical shape with a thickness of 1 mm and a diameter of 25 mm
 127 for the measurement. The elastic modulus (G') and viscous modulus (G'') over a
 128 frequency range of 0.1 to 10 Hz were recorded at a constant strain of 1%, which was

129 in the linear range of the viscoelasticity. All measurements were performed at 37 °C.

130

131 **2.6 Drug loading**

132 Amoxicillin and meloxicam were loaded into the PAACS hydrogels by soaking
133 and swelling the dried hydrogels in solutions of drugs according to a reported method
134 [25]. This is exemplified by the loading of amoxicillin for which 60 mg of the dry
135 cylindrical hydrogels were immersed into 50 mL of 200 µg mL⁻¹ amoxicillin solutions
136 under moderate stirring for 24 h at 37 °C. Thereafter, the drug-loaded hydrogels were
137 taken out and rinsed with deionized water to remove any residual drugs from the
138 surface. It should be noticed that meloxicam is poorly water soluble and accordingly a
139 small amount of methanol was added to improve solubility; otherwise the procedure
140 was as for that of amoxicillin. The loaded drug amounts were determined by UV-vis
141 spectroscopy (SHIMADZU UV-2550 UV-vis) based on the decrease of the
142 concentration of drug loading solutions determined from UV-vis calibration curves for
143 amoxicillin and meloxicam at 228 nm and 361 nm, respectively. The encapsulation
144 efficiency (*EE*) and loading content (*LC*) of the drugs were calculated through
145 Equations (2) and (3) where m_e is the amount of encapsulated drug, m_o is the total
146 amount of added drug, and m_d is the amount of the dried hydrogel. The *EE* and *LC*
147 determined are listed in Table S1.

$$148 \quad EE(\%) = \frac{m_e}{m_o} \times 100 \quad (2)$$

$$149 \quad LC(\%) = \frac{m_e}{m_d} \times 100 \quad (3)$$

150 **2.7 drug release study**

151 The release of amoxicillin and meloxicam from PAACS hydrogels was carried
152 out in aqueous phosphate buffer solutions at pH 1.2, 6.8, and 7.4 at 37 °C. Basically,
153 either amoxicillin or meloxicam loaded hydrogel was placed into 60 mL of
154 moderately stirred aqueous buffer solution. At appropriate time intervals, 2.0 mL
155 samples of the aqueous buffer solutions were withdrawn and replaced by 2.0 mL fresh
156 aqueous buffer solutions. The amount of the released drugs in the withdrawn sample
157 was determined by UV-Vis absorbance at 228 nm for amoxicillin and 361 nm for
158 meloxicam according to the molar absorbance calibration curves of amoxicillin and
159 meloxicam. All release data were performed in in triplicate and averaged.

160

161 **2.8 Characterization**

162 All infrared spectra were obtained from dried samples in KBr pellets using a
163 Nicolet 6700 FTIR spectrophotometer. ¹H NMR spectra was taken by a 500 MHz
164 Bruker DRX500 spectrometer at 25 °C using D₂O as the solvent. The SEM was
165 performed using a Nova Nano SEM 50 field emission scanning electron microscope
166 (FE-SEM) at an acceleration voltage of 3 kV.

167

168 **3. Results and discussion**

169 As shown in scheme 2, CSMAH was synthesized by grafting MAH onto the main
170 chain of CS. Subsequently, CSMAH was employed to copolymerize with AA to create
171 the three hydrogels in which the extent of CS cross-linking increase in the sequence

172 PAACS-I < PAACS-II < PAACS-III as a consequence of the three-fold increase in
173 CSMAH concentration used in their respective preparations (Table 1).

174

175 **(Scheme 2 here)**

176

177 **Structure characterization**

178 Fig. 1A shows the ¹H NMR spectrum of CSMAH. The broad peaks at 3.2-4.2
179 ppm arise from the hydrogens of the pyranose units of CS (H3, H4, H5, and H6), the
180 peak at 3.05 ppm arises from H2, and the peak of methyl hydrogen of the *N*-acetyl
181 groups is located at 2.12 ppm. The two peaks at 5.85 and 6.32 ppm which are referred
182 to H7 and H8 of the grafted MAH. Thus, the ¹H NMR characterization indicates that
183 MAH modified CS was successfully synthesized. The averaging grafting degree (*GD*)
184 of MAH onto CS in CSMAH, defined as the number of grafted MAH per 100
185 pyranose units, was determined to be 27.3 ± 0.1 % based on the proton integration (Eq.
186 4), where $I_{6.32ppm}$ and $I_{3.2-4.2ppm}$ are the integrated peak area ratios of protons of the
187 MAH and CS components, respectively. It is anticipated that that *GD* varies over a
188 small range between individual chains.

$$189 \quad GD = \frac{5 \times I_{6.32ppm}}{I_{3.2-4.2ppm}} \times 100\% \quad (4)$$

190 FTIR spectra of PAA, CS, CSMAH, and PAACS hydrogels are displayed in Fig.
191 1B. For PAA, a broad absorption band from 3000 to 3600 cm⁻¹ is stemmed from the
192 O-H stretching vibration. The peaks appeared at 1637 and 1151 cm⁻¹ are contributed
193 by the stretching vibration of C=O and C-O of the carboxylic group. Another two

194 peaks appeared at 1454 and 1409 cm^{-1} are caused by the O-H bending vibration of
195 PAA. The characteristic peaks of CS located at 3346 cm^{-1} (O-H and N-H stretching),
196 2921 and 2854 cm^{-1} (C-H stretching), and 1654 cm^{-1} (NH-CO (I) stretching) can be
197 observed clearly in the FT-IR spectrum. In the CSMAH spectrum, the new peaks
198 appeared at 1658 and 1564 cm^{-1} are attributed to C-O groups of the opened MAH, it
199 further approves the successful modification of CS. The peak at 1700 cm^{-1} is caused
200 by the carboxyl stretching vibration of carboxylic acid. With regard to the spectrum of
201 PAACS hydrogel, some absorption peaks are changed by comparing with CSMAH
202 and PAA. A broad peak at the range of 3000-3500 cm^{-1} arises from the overlapping of
203 the O-H stretching vibrations of PAA and N-H stretching vibrations of CSMAH. The
204 characteristic stretching absorption band of C=O in PAA presents at 1637 cm^{-1} . In
205 particular, the characteristic absorption bands of CS at 2921 and 2854 cm^{-1} consistent
206 with the participation of CSMAH in the polymerization to for PAACS hydrogels.

207

208 **(Fig. 1 here)**

209

210 **X-Ray powder diffraction (XRD)**

211 XRD was employed to reveal the crystallinity of CS, CSMAH, PAA, PAACS-I,
212 PAACS-II and PAACS-III. As shown in Fig. 1C, the XRD pattern of CS shows two
213 major peaks at 10° and 19° which transforms into a single broad peak at 20° in the
214 XRD pattern of CSMAH caused by the grafting of MAH onto CS. Upon
215 polymerization with AA, a substantial decrease in intensity occurs in the region

216 centered at 10° where both CS and CSMAH absorb, and the broad peaks of PAA
217 appear in the range 15°-40°. This is consistent with the copolymerization of CSMAH
218 and AA progressing in a random way and a consequent decrease in crystallinity by
219 comparison with that of CS, and also a decrease in inter- and intra-molecular
220 hydrogen bonding.

221

222 **Rheology**

223 The rheological properties are important indicators of soft materials performances
224 [26]. As shown in Fig. 1D, for each of the three hydrogels, PAACS-I, PAACS-II and
225 PAACS-III, the elastic modulus, G' , was higher than their viscous modulus, G'' , over
226 the measured frequency range. This is consistent with the hydrogels being present as
227 solids under the measuring conditions; thereby constituting a stable structure for drug
228 loading. It is also observed that G' increases in the sequence PAACS-I < PAACS-II <
229 PAACS-III coincident with the increasing CS cross-linker content. Additionally, the
230 reacted ratio of MAH groups in CSMAH was estimated by Eq. 5, where ρ is the
231 density of PAA, R is the ideal gas constant, T is temperature, and \bar{M}_c is the average
232 molecular weight of PAA between two adjacent cross-linking points [27], here we
233 hypothesize a complete copolymerization is achieved.

$$234 \quad G = \frac{\rho RT}{\bar{M}_c} \quad (5)$$

235 The calculation results demonstrated that the cross-linking efficiency is not very
236 high which might stem from the big molecular volume of chitosan, for instance, only
237 ~0.5% MAH groups in CSMAH was presented in cross-linking PAA chains (Fig. 1D).

238 This is also responsible for the low elastic modulus of these hydrogels.

239

240 **Morphology of PAACS hydrogels**

241 The micro-morphologies of the freeze-dried PAACS hydrogels were shown to
242 possess well-defined network structures by SEM (Fig. 2). A statistical analyses of the
243 pore size of these hydrogels indicated that increase in the extent of CS cross-linking
244 significantly decreased pore size. The average pore size of PAACS-I is around ~126
245 μm , while those of PAACS-II and PAACS-III are smaller, ~86 and ~51 μm ,
246 respectively. While it has been proposed that the pore size of the hydrogel depends on
247 the size of the ice crystals which are formed during the freeze-drying treatment of the
248 samples [28], the greater the extent of CS cross-linking the greater will be the restraint
249 on the capacity of the hydrogel to swell with water absorption. As a result, the size of
250 the ice crystals and hydrogel pores will decrease with increase in CS cross-linking [29,
251 30].

252

(Fig. 2 here)

254

255 **Swelling behavior**

256 The swelling properties of PAACS hydrogels were investigated by soaking the
257 freeze-dried hydrogels in aqueous buffer solutions at pH 1.2, 6.8 and 7.7 and
258 recording the weight changes with time at 37 °C. It is seen from Fig. 3 that PAACS-I,
259 PAACS-II and PAACS-III each exhibits an increase in swelling ratio (*SR*) as pH

260 increases. It is also seen that at a given pH *SR* decreases in the sequence PAACS-I >
261 PAACS-II > PAACS-III as the extent of CS cross-linking increases. At pH 1.2, the
262 carboxylic acid groups in PAA chains are almost protonated and substantial
263 hydrogen-bonding occurs between them and the repulsion force between polymer
264 chains in the networks is reduced so that the water diffusion into the hydrogel is
265 impeded and swelling is reduced [31-34]. However, at pH 7.4, the carboxylic groups
266 were deprotonated and hydrogen-bonding between them is absent while their negative
267 charges cause electrostatic repulsion between the PAA chains [35]. The overall effect
268 is that the hydrogel network has a looser structure at pH 7.4 than that at pH 1.2 which
269 permits an increased diffusion of water into the hydrogel and an increased swelling.

270 The effect of pH change on hydrogel swelling superimposes on the increase in the
271 extent CS of cross-linking in the sequence: PAACS-I < PAACS-II < PAACS-III and
272 the corresponding decrease in *SR* in the sequence: PAACS-I > PAACS-II >
273 PAACS-III at the three pH conditions studied. Thus, an increase in CS cross-linking
274 tightens the hydrogel network thereby impeding diffusion of water into it and
275 decreasing the *SR*.

276

277 **(Fig. 3 here)**

278

279 **Study of pH triggered drug release**

280 The release curves for amoxicillin and meloxicam are displayed in Fig. 4. It
281 demonstrated drug release rate decreases in the hydrogel sequence PAACS-I >

282 PAACS-II > PAACS-III and that for each hydrogel the release rate increases with
283 increase in pH. This pattern bears a striking similarity to that for the hydrogel *SR*
284 shown in Fig. 3 and suggests that the increase in drug mobility is directly related the
285 increase in hydrogel pore size as pH increases [36].

286 For PAACS-I, ~30%, ~60% and ~80% of amoxicillin is released after 800 min at
287 pH 1.2, 6.8 and 7.4, respectively (Fig. 4). The analogous values for meloxicam are
288 ~20%, ~70% and ~90% at pH 1.2, 6.8 and 7.4, respectively. Both drugs are released
289 more slowly from PAACS-II and PAACS-III, and release from both hydrogels shows
290 an increase in rate with increase in pH. It has been suggested that many drugs are
291 released from hydrogels through a diffusion process which is dominated by the
292 swelling behavior of the hydrogel [36]. Thus, the lower release rate of amoxicillin and
293 meloxicam at pH 1.2 is probably largely contributed by the pore size decrease (Fig.
294 S1) due to greater hydrogen bonding between the PAA and CS chains in hydrogel
295 networks (Scheme 1) and a consequent decrease in hydrogel flexibility and an
296 inhibition of both drug and water diffusion. The hydrogel flexibility is further
297 decreased as cross-linking increases with the consequence that drug release is further
298 slowed as seen from Fig. 4.

299 It has been revealed that the chemical structure of both the drug and the hydrogel
300 determine the nature and extent of interactions between them and that this impinges
301 on the magnitude of drug release rates [37]. From the release curves for amoxicillin
302 and meloxicam (Fig. 4), we can see obviously that the release rate of amoxicillin is
303 higher than that of meloxicam at pH 1.2 whereas the reverse is the case at pH 6.8 and

304 7.4. This reflects the variation of the effects of hydrogen bonding between the
305 hydrogel PAA and CS chains and probably between them and the two drugs.
306 Amoxicillin is more hydrophilic than is meloxicam as assessed on the basis of the
307 higher water solubility of amoxicillin. This is likely to differentiate the behaviour of the
308 two drugs within the hydrogel but a more detailed analysis is not possible on the basis
309 of the currently available data.

310

311 **(Fig. 4 here)**

312

313 **Mechanism of drug release from hydrogels**

314 The mechanism of drug released from hydrogels may be envisaged as occurring
315 in three main steps as shown in Fig. 5. In the initial step, a), the drug-loaded hydrogel
316 contains a minimum amount of water, the hydrogel exhibits its minimum flexibility,
317 pore size is small and drug mobility is limited. In the second step, b), water diffuses
318 into the hydrogel which undergoes relaxation to become more flexible, pore size
319 grows and drug mobility increases with increased hydration. In the final stage, c), the
320 hydrogel is fully relaxed and hydrated and pore size is at a maximum, as is the rate of
321 drug diffusion from the hydrogel [38, 39].

322

323 **(Fig. 5 here)**

324

325 The mathematical modeling of drug release from hydrogel is a facile and an

326 important approach to understand the elusive release mechanism [24, 39-44].
327 Accordingly, We have employed both Korsmeyer-Peppas [39-42] and Weibull [24]
328 models to elucidate the release mechanism of amoxicillin and meloxicam. The widely
329 used Korsmeyer-Peppas model expresses the rate of drug release up to the stage
330 where 60% of the drug is released through Eq. 5 where M_t and M_∞ are the amounts of
331 drug released at time t and when equilibrium is reached, respectively; k is a kinetic
332 constant, and n is an exponent typifying the release mechanism.

$$333 \quad \frac{M_t}{M_\infty} = kt^n \quad (5)$$

334 The release data for both amoxicillin and meloxicam is well-fitted by Eq. 5 for up
335 to 60 % of drug release as shown in Fig. S1a and c). These fittings correspond to n
336 values in the range between 0.51 and 0.85 for amoxicillin and between 0.63 and 0.87
337 for meloxicam (Table S2) consistent with the drugs being released through so-called
338 anomalous diffusion, in which the effects of drug diffusion and hydrogel relaxation
339 are comparable. [36, 39-42]. It can also be seen clearly that at a given pH value, the n
340 values more closely approach 0.89 at which only the relaxation of hydrogel governs
341 the drug release as the extent of cross-linking increases in the sequence PAACS-I <
342 PAACS-II < PAACS-III in the hydrogels [39-42]. That is because increases in
343 cross-linking decrease the hydrogel flexibility such that the hydrogel relaxation
344 process becomes the controlling factor for drug release. The n values characterizing
345 amoxicillin release are smaller than those for meloxicam release which may indicate
346 that amoxicillin interacts more strongly with the hydrogels and is therefore less
347 dependent upon hydrogel relaxation for release. This can also be seen from the

348 diffusion coefficients of amoxicillin (D_1) and meloxicam (D_2) in the hydrogels (Fig.
349 S3 and Table S3). At higher pH (pH 6.8 and 7.4), we found that the hydrogels relaxed
350 completely within ~ 300 min, after which the drugs were released in a stable diffusion
351 process. By estimating the diffusion coefficient, we found that D_1 was smaller than D_2
352 demonstrating the higher interaction between amoxicillin and hydrogel. Consequently,
353 the n values for amoxicillin release more closely approach 0.45 (at which only
354 diffusion controls drug release) than is the case for meloxicam. However, the overall
355 conclusion is that both amoxicillin and meloxicam are released from the hydrogels
356 through a combination of diffusion and hydrogel relaxation under the conditions of
357 this study.

358 As we mentioned previously, Korsmeyer-Peppas equation is only valid for the
359 first 60% of the release curve. In order to give a more reliable mechanism revealing,
360 another model, Weibull model, which covers the entire drug release process, is
361 described through Eq. 6, where a is a constant, and b is an exponent which reflects the
362 underlying release mechanism. A value of b in the range of 0.35 \sim 0.75 signifies a
363 diffusion dominated drug release process and a b value in the range 0.75 \sim 1.0
364 indicates a combined diffusion and hydrogel relaxation mechanism [24].

365
$$\frac{M_t}{M_\infty} = 1 - \exp(-at^b) \quad (6)$$

366 It can be seen from Fig. S2b and d that Eq. 6 can fit the drug release data very
367 well. From the fitting results (Table S2), we can see that most of the b values fall in
368 the range of 0.75 \sim 1.0, indicating a combination release process of diffusion and
369 hydrogel relaxation which is in good consistent with the results derived from

370 Korsemeier-Peppas model. Thus, it is concluded that both amoxicillin and meloxicam
371 are released from the hydrogels through a combination of diffusion and hydrogel
372 relaxation as was also deduced from the Korsemeier-Peppas model.

373

374 **Conclusions**

375 A series of chitosan cross-linked PAACS hydrogels with different degrees of
376 cross-linking were prepared and found an increase in swelling and pore size as pH
377 was increased and as the extent of cross-linking decreased. The drugs amoxicillin and
378 meloxicam were readily loaded into the hydrogels, and their release rates were found
379 to increase with increase in pH and to decrease with increase in cross-linking. Fitting
380 of two models for drug release to the experimental release data indicated that the rates
381 of drug release are controlled to varying extents by a combination of diffusion and
382 hydrogel relaxation.

383

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391

392

393 **References**

- 394 [1] A. Vashist, A. Vashist, Y.K. Gupta and S. Ahmad, Recent advances in hydrogel
395 based drug delivery systems for the human body, *J Mater Chem B*, 2 (2014)
396 147-166.
- 397 [2] P. Matricardi, C. Di Meo, T. Coviello, W.E. Hennink and F. Alhaique,
398 Interpenetrating Polymer Networks polysaccharide hydrogels for drug delivery
399 and tissue engineering, *Advanced Drug Delivery Reviews*, 65 (2013) 1172-1187.
- 400 [3] D. Costa, A.J.M. Valente, M.G. Miguel and J. Queiroz, Gel Network
401 Photodisruption: A New Strategy for the Codelivery of Plasmid DNA and Drugs,
402 *Langmuir*, 27 (2011) 13780-13789.
- 403 [4] Y. Qiu and K. Park, Environment-sensitive hydrogels for drug delivery,
404 *Advanced Drug Delivery Reviews*, 64 (2012) 49-60.
- 405 [5] M.B. Charati, I. Lee, K.C. Hribar and J.A. Burdick, Light-Sensitive Polypeptide
406 Hydrogel and Nanorod Composites, *Small*, 6 (2010) 1608-1611.
- 407 [6] Z.Q. Lin, W. Gao, H.X. Hu, K. Ma, B. He, W.B. Dai, X.Q. Wang, J.C. Wang, X.
408 Zhang and Q. Zhang, Novel thermo-sensitive hydrogel system with paclitaxel
409 nanocrystals: High drug-loading, sustained drug release and extended local
410 retention guaranteeing better efficacy and lower toxicity, *Journal Of Controlled*
411 *Release*, 174 (2014) 161-170.
- 412 [7] J.H. Jeong, J.J. Schmidt, C. Cha and H. Kong, Tuning responsiveness and
413 structural integrity of a pH responsive hydrogel using a poly(ethylene glycol)

- 414 cross-linker, *Soft Matter*, 6 (2010) 3930-3938.
- 415 [8] W.E. Hennink and C.F. van Nostrum, Novel crosslinking methods to design
416 hydrogels, *Advanced Drug Delivery Reviews*, 64 (2012) 223-236.
- 417 [9] M.T. Popescu, S. Mourtas, G. Pampalakis, S.G. Antimisiaris and C. Tsitsilianis,
418 pH-Responsive Hydrogel/Liposome Soft Nanocomposites For Tuning Drug
419 Release, *Biomacromolecules*, 12 (2011) 3023-3030.
- 420 [10] C.X. Ding, L.L. Zhao, F.Y. Liu, J. Cheng, J.X. Gu, Shan-Dan, C.Y. Liu, X.Z. Qu
421 and Z.Z. Yang, Dually Responsive Injectable Hydrogel Prepared by In Situ
422 Cross-Linking of Glycol Chitosan and Benzaldehyde-Capped PEO-PPO-PEO,
423 *Biomacromolecules*, 11 (2010) 1043-1051.
- 424 [11] E.A. Appel, R.A. Forster, M.J. Rowland and O.A. Scherman, The control of
425 cargo release from physically crosslinked hydrogels by crosslink dynamics,
426 *Biomaterials*, 35 (2014) 9897-9903.
- 427 [12] P. Ilg, Stimuli-responsive hydrogels cross-linked by magnetic nanoparticles, *Soft*
428 *Matter*, 9 (2013) 3465-3468.
- 429 [13] H.B. Zhang, F. Zhang and J. Wu, Physically crosslinked hydrogels from
430 polysaccharides prepared by freeze-thaw technique, *React Funct Polym*, 73 (2013)
431 923-928.
- 432 [14] F. Corrente, H.M. Abu Amara, S. Pacelli, P. Paolicelli and M.A. Casadei, Novel
433 injectable and in situ cross-linkable hydrogels of dextran methacrylate and
434 scleroglucan derivatives: Preparation and characterization, *Carbohydrate*
435 *Polymers*, 92 (2013) 1033-1039.

- 436 [15] D. Das, P. Ghosh, S. Dhara, A.B. Panda and S. Pal, Dextrin and Poly(acrylic
437 acid)-Based Biodegradable, Non-Cytotoxic, Chemically Cross-Linked Hydrogel
438 for Sustained Release of Ornidazole and Ciprofloxacin, *Acs Appl Mater Inter*, 7
439 (2015) 4791-4803.
- 440 [16] L. Yang, L. Shi, J. Chen, Y. Pei, F. Zhu and Y. Xia, Preparation and
441 Characterization of pH - sensitive Hydrogel Film of Chitosan/Poly (acrylic acid)
442 Copolymer, in: *Macromolecular Symposia*, Vol 225, Wiley Online Library,
443 2005, pp. 95-102.
- 444 [17] L. Shi, L. Yang, J. Chen, Y. Pei, M. Chen, B. Hui and J. Li, Preparation and
445 characterization of pH-sensitive hydrogel of chitosan/poly (acrylic acid)
446 co-polymer, *Journal of Biomaterials Science, Polymer Edition*, 15 (2004)
447 465-474.
- 448 [18] Y.M. Wang, J. Wang, T.S. Wang, Y.S. Xu, L. Shi, Y.T. Wu, L. Li and X.H. Guo,
449 Pod-Like Supramicelles with Multicompartment Hydrophobic Cores Prepared by
450 Self-Assembly of Modified Chitosan, *Nano-Micro Letters*, 8 (2016) 151-156.
- 451 [19] A.S. Carreira, F.A.M.M. Goncalves, P.V. Mendonca, M.H. Gil and J.F.J. Coelho,
452 Temperature and pH responsive polymers based on chitosan: Applications and
453 new graft copolymerization strategies based on living radical polymerization,
454 *Carbohydrate Polymers*, 80 (2010) 618-630.
- 455 [20] Y.M. Wang, J. Wang, H.Y. Han, J.J. Liu, H.Q. Zhao, M.X. Shen, Y.S. Xu, J. Xu,
456 L. Li and X.H. Guo, Self-assembled micelles of N-phthaloylchitosan-g-poly
457 (N-vinylcaprolactam) for temperature-triggered non-steroidal anti-inflammatory

458 drug delivery, *J Mater Sci*, 51 (2016) 1591-1599.

459 [21] L.M. Hu, Y. Sun and Y. Wu, Advances in chitosan-based drug delivery vehicles,
460 *Nanoscale*, 5 (2013) 3103-3111.

461 [22] A. Bernkop-Schnurch and S. Dunnhaupt, Chitosan-based drug delivery systems,
462 *Eur J Pharm Biopharm*, 81 (2012) 463-469.

463 [23] R.W. Kormsmeier, R. Gurny, E. Doelker, P. Buri and N.A. Peppas, Mechanisms
464 Of Solute Release From Porous Hydrophilic Polymers, *Int J Pharmaceut*, 15
465 (1983) 25-35.

466 [24] V. Papadopoulou, K. Kosmidis, M. Vlachou and P. Macheras, On the use of the
467 Weibull function for the discernment of drug release mechanisms, *Int J*
468 *Pharmaceut*, 309 (2006) 44-50.

469 [25] P. Gupta, K. Vermani and S. Garg, Hydrogels: from controlled release to
470 pH-responsive drug delivery, *Drug Discov Today*, 7 (2002) 569-579.

471 [26] K.L. Liu, Z.X. Zhang and J. Li, Supramolecular hydrogels based on
472 cyclodextrin-polymer polypseudorotaxanes: materials design and hydrogel
473 properties, *Soft Matter*, 7 (2011) 11290-11297.

474 [27] M. Oyen, Mechanical characterisation of hydrogel materials, *International*
475 *Materials Reviews*, 59 (2014) 44-59.

476 [28] X. Hu, W. Wei, X. Qi, H. Yu, L. Feng, J. Li, S. Wang, J. Zhang and W. Dong,
477 Preparation and characterization of a novel pH-sensitive Salectan-g-poly(acrylic
478 acid) hydrogel for controlled release of doxorubicin, *J. Mater. Chem. B*, 3 (2015)
479 2685-2697.

- 480 [29] M. Jaiswal, A.K. Dinda, A. Gupta and V. Koul, Polycaprolactone diacrylate
481 crosslinked biodegradable semi-interpenetrating networks of polyacrylamide and
482 gelatin for controlled drug delivery, *Biomedical Materials*, 5 (2010) 065014.
- 483 [30] M.-K. Yoo, H.Y. Kweon, K.-G. Lee, H.-C. Lee and C.-S. Cho, Preparation of
484 semi-interpenetrating polymer networks composed of silk fibroin and poloxamer
485 macromer, *International journal of biological macromolecules*, 34 (2004)
486 263-270.
- 487 [31] X. Gao, C. He, C. Xiao, X. Zhuang and X. Chen, Biodegradable pH-responsive
488 polyacrylic acid derivative hydrogels with tunable swelling behavior for oral
489 delivery of insulin, *Polymer*, 54 (2013) 1786-1793.
- 490 [32] S. Hua, H. Ma, X. Li, H. Yang and A. Wang, pH-sensitive sodium alginate/poly
491 (vinyl alcohol) hydrogel beads prepared by combined Ca²⁺ crosslinking and
492 freeze-thawing cycles for controlled release of diclofenac sodium, *International
493 journal of biological macromolecules*, 46 (2010) 517-523.
- 494 [33] A. Shefer, A.J. Grodzinsky, K.L. Prime and J.P. Busnel, Novel model networks
495 of poly (acrylic acid): synthesis and characterization, *Macromolecules*, 26 (1993)
496 5009-5014.
- 497 [34] F. Oosawa, *Polyelectrolytes*, in: *Polyelectrolytes*, Marcel Dekker, 1971.
498
- 499 [35] R. Jin, L.M. Teixeira, P.J. Dijkstra, M. Karperien, C. Van Blitterswijk, Z. Zhong
500 and J. Feijen, Injectable chitosan-based hydrogels for cartilage tissue
501 engineering, *Biomaterials*, 30 (2009) 2544-2551.

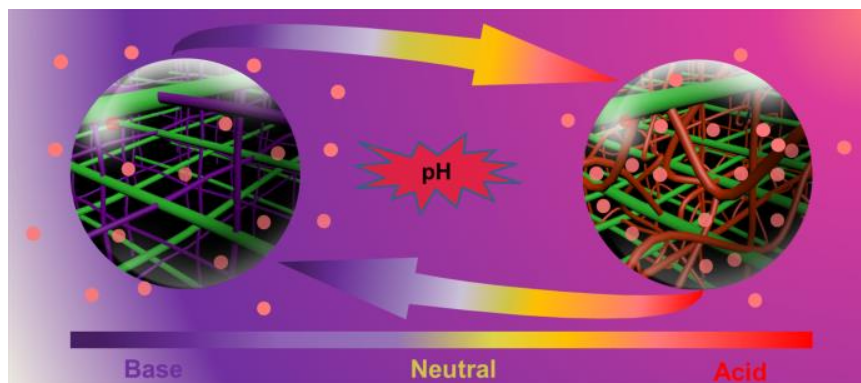
- 502 [36] D. Das, R. Das, P. Ghosh, S. Dhara, A.B. Panda and S. Pal, Dextrin cross
503 linked with poly(HEMA): a novel hydrogel for colon specific delivery of
504 ornidazole, *Rsc Adv*, 3 (2013) 25340-25350.
- 505 [37] G.R. Bardajee, A. Pourjavadi and R. Soleyman, Novel nano-porous hydrogel as
506 a carrier matrix for oral delivery of tetracycline hydrochloride, *Colloid Surface A*,
507 392 (2011) 16-24.
- 508 [38] S. Kiortsis, K. Kachrimanis, T. Broussali and S. Malamataris, Drug release
509 from tableted wet granulations comprising cellulosic (HPMC or HPC) and
510 hydrophobic component, *Eur J Pharm Biopharm*, 59 (2005) 73-83.
- 511 [39] N. A. Peppas, Analysis of Fickian and non-Fickian drug release from polymers,
512 *Pharm. Acta. Helv.*, 60 (1985) 110-111.
- 513 [40] P. L. Ritger and N. A. Peppas, A simple equation for description of solute release
514 II. Fickian and anomalous release from swellable devices, *J. Controll. Release*, 5
515 (1987) 37-42.
- 516 [41] N. A. Peppas and J. J. Sahlin, A simple equation for the description of solute
517 release. III. Coupling of diffusion and relaxation. *Int. J. Pharm.*, 57 (1989)
518 169-172.
- 519 [42] J. Siepmann and F. Siepmann, Mathematical modeling of drug delivery, *Int J*
520 *Pharmaceut*, 364 (2008) 328-343.
- 521 [43] J. Siepmann and N.A. Peppas, Modeling of drug release from delivery systems
522 based on hydroxypropyl methylcellulose (HPMC), *Advanced Drug Delivery*
523 *Reviews*, 48 (2001) 139-157.

524 [44] K. Kosmidis, E. Rinaki, P. Argyrakis and P. Macheras, Analysis of Case II drug
525 transport with radial and axial release from cylinders, Int J Pharmaceut, 254
526 (2003) 183-188.
527

528 **Graphical abstract:**

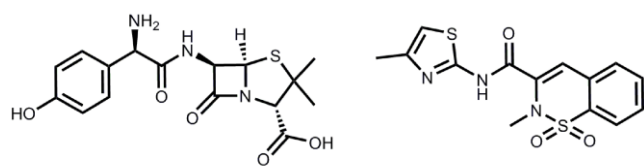
529 Drug loaded chitosan cross-linked poly(acrylate) hydrogels exhibit pH-dependent
530 drug release through a mechanism involving drug diffusion and hydrogel relaxation.

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Figure Captions



Amoxicillin

Meloxicam

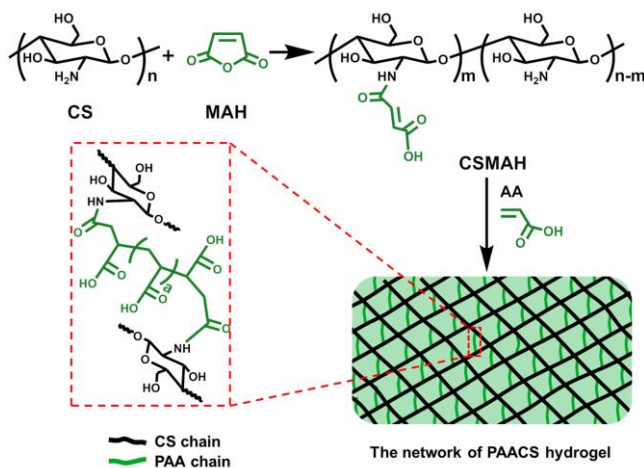
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539 **Scheme 1.** Molecular structures of amoxicillin and meloxicam.

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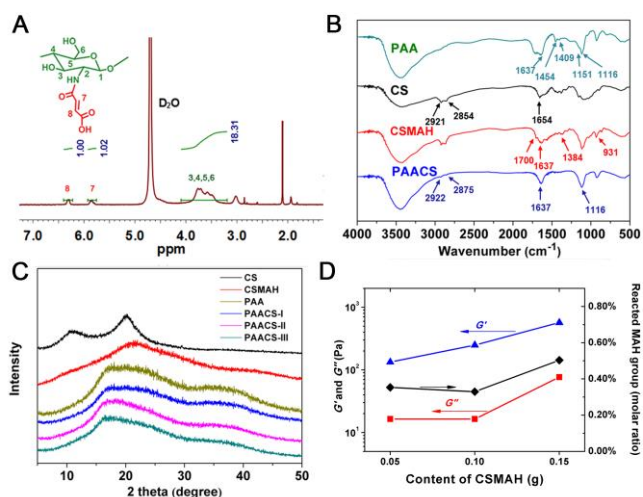
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544 **Scheme 2.** Preparation of PAACS hydrogels.

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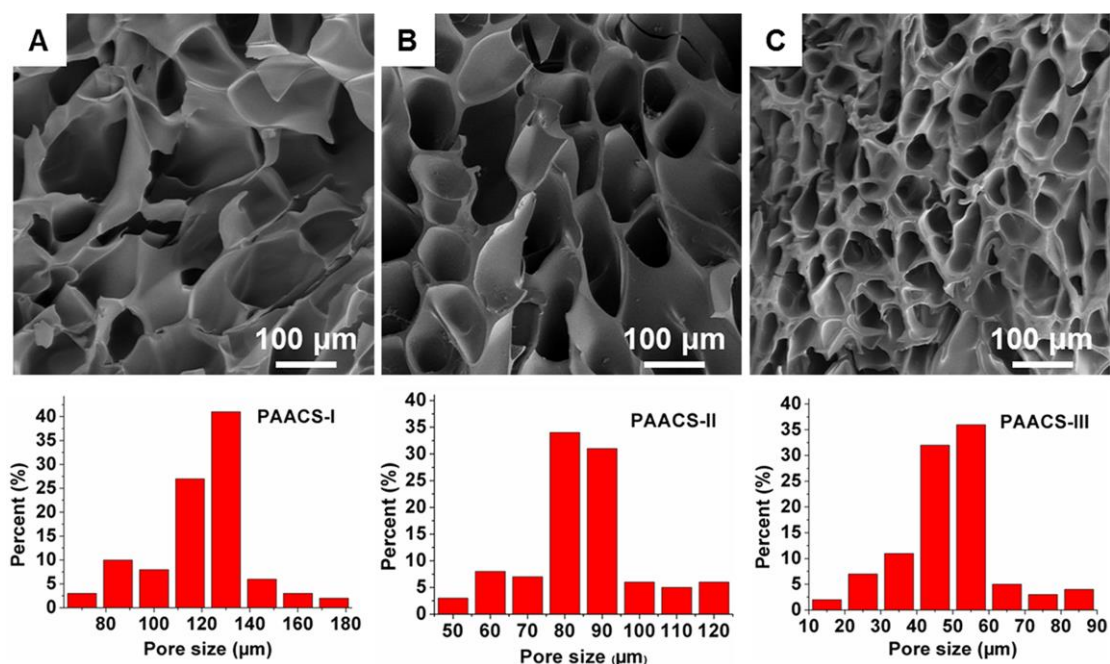
549 **Fig. 1.** ¹H NMR spectrum of CSMAH (A); FTIR spectra (B) and XRD patterns (C) of

550 CS, CSMAH, PAA and PAACS hydrogels; Elastic modulus G' and viscous modulus

551 G'' of PAACS hydrogels as a function of frequency (D).

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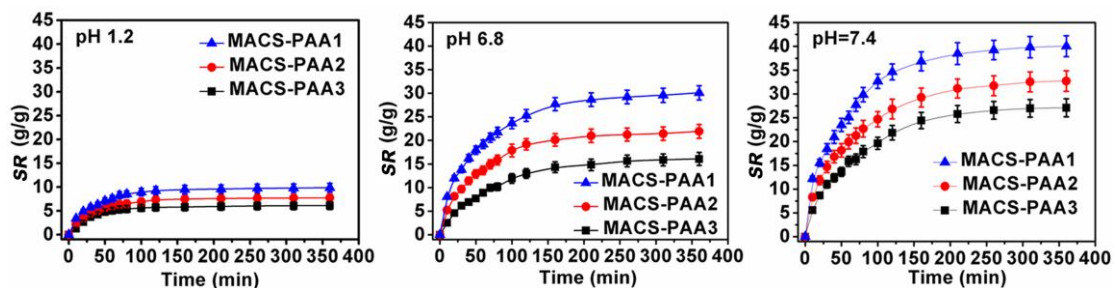
555 **Fig. 2.** The network structures and the pore size distributions of the hydrogels: A)

556 PAACS-I; B) PAACS-II; C) PAACS-III (each statistical result was obtained by

557 counting 100 pores from the SEM image).

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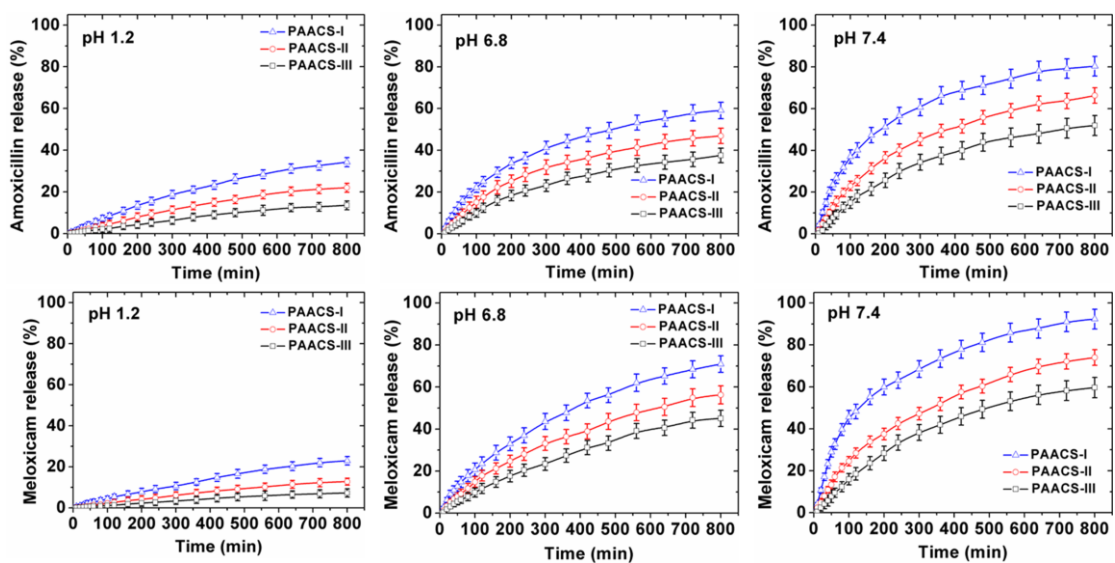
560

561 **Fig. 3.** Swelling kinetics of PAACS hydrogels at different pH, error bars are the

562 standard error of the mean taken from three samples.

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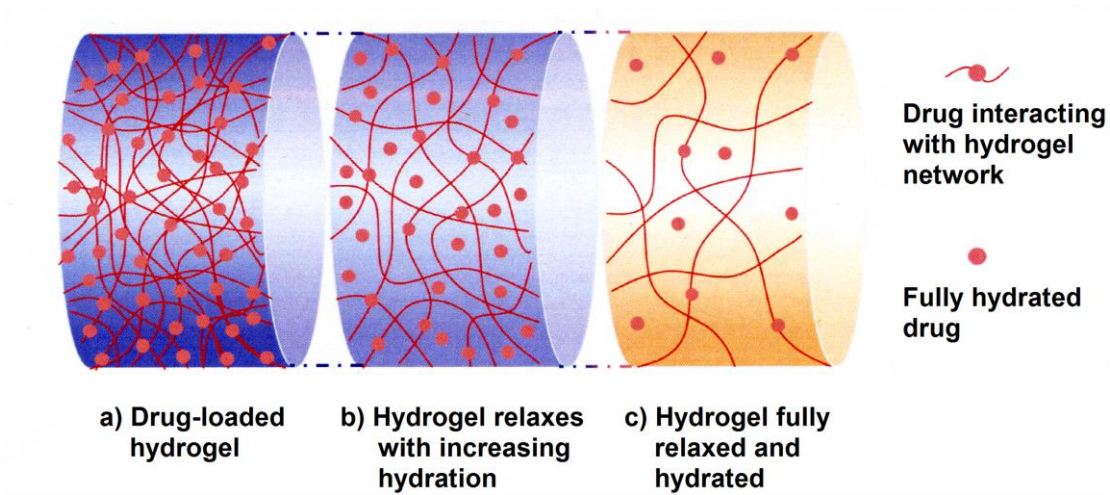
565

566 **Fig. 4.** The release curves of amoxicillin and meloxicam at different pH, error bars are

567 the standard error of the mean taken from three samples.

568

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570

571 **Fig. 5.** Schematic illustration of the process of drug release from hydrogel.