Kinetic release studies of nitrogen-containing bisphosphonate from gum acacia crosslinked hydrogels

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Natural polymer hydrogels are useful for controlling release of drugs. In this study, hydrogels containing gum acacia were synthesized by free-radical polymerization of acrylamide with gum acacia. The effect of gum acacia in the hydrogels on the release mechanism of nitrogen-containing bisphosphonate (BP) was studied at pH 1.2 and 7.4. The hydrogels exhibited high swelling ratios at pH 7.4 and low swelling ratios at pH 1.2. The release study was performed using UV–Visible spectroscopy via complex formation with Fe(III) ions. At pH 1.2, the release profile was found to be anomalous while at pH 7.4, the release kinetic of BP was a perfect zero-order release mechanism. The hydrogels were found to be pH-sensitive and the release profiles of the BP were found to be influenced by the degree of crosslinking of the hydrogel network with gum acacia. The preliminary results suggest that these hydrogels are promising devices for controlled delivery of bisphosphonate to the gastrointestinal region.

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1. Introduction

Hydrogels are three-dimensional, hydrophilic and polymeric networks which are capable of absorbing a large amount of water or biological fluid [1]. Hydrogels exhibit unique properties such as: (1) porous structure that permits loading and consequent release of drugs, (2) biocompatibility due to its hydrophilic nature, (3) biodegradability and (4) deformability [2]. These unique properties have attracted their application in drug delivery. Hydrogels can be prepared from natural polymers, synthetic polymers and from a combination of natural and synthetic polymers. Natural polymers are biocompatible with good cell affinity, whereas synthetic polymers exhibit excellent mechanical strength, water content and degradation rate [3]. Hydrogels prepared from a combination of natural and synthetic polymers capitalizes on the advantages of both synthetic and natural polymers. Hydrogels can be classified based on the method of preparation such as: (1) homopolymer hydrogel is prepared from one type of hydrophilic monomer, (2) copolymer hydrogel is obtained from crosslinking two monomers, (3) multi-polymer hydrogel is prepared from crosslinking of three or more comonomers and (4) interpenetrating polymer hydrogel, the first polymer matrix formed is swollen in a monomer to form a second intermesh network structure [4]. Hydrogels are used in tissue engineering to replace or regenerate tissues and organs for transplant [5], in tissue-engineering scaffolds to deliver signals to the cells [6], for wound dressing [7], for control and sustained drug-delivery systems. They are used for drug delivery to the oral cavity [8], gastrointestinal tract [9], rectum [10], eyes [11] and skin [12].

Gum acacia (GA) is a natural occurring polymer and it is built from (1→3) and (1→6)-linked β-D-galactopyranosyl units along with (1→6)-linked β-D-glucopyranosyluronic acid units. The side branch contains α-L-rhamnopyranose, β-D-glucuronic acid, β-D-galactopyranose and α-L-arabinofuranosyl units with (1→3), (1→4) and (1→6) glycosidic linkages [13]. Gum acacia is biodegradable, readily available, non-toxic and is an environmentally friendly polymer [14]. Gum acacia is used in pharmaceuticals as a demulcent and for healing wounds [15]. It has been found to inhibit the growth of periodontal bacteria [16]. It exhibits anti-inflammatory activity [17] and it is used to reduce the frequency of dialyses [18,19]. Recently, Raghavendra et al. [20] reported the excellent
antibacterial activity of nanocomposites containing gum acacia against *E. coli*. However, there are several research reports on the application of gum acacia containing biomaterials amongst which are: as surfactant to improve the porosity of hydrogel for cell penetration and proliferation enhancement [21], for sustained release profile of ampicillin [22], crosslinking with poly(2-hydroxyethyl methacrylate-co-acrylic acid) for antibacterial application [23] and for saline water treatment [24].

In this study, sodium hydrogen (5-amino-1-hydroxy-1-phosphonopentyl) phosphate (BP) was chosen as a drug of choice. It is a nitrogen-containing bisphosphonate and it is similar in structure to alendronate. Nitrogen-containing bisphosphonates have been found to have increased antiresorptive potency and are used: to treat sleeping sickness [25], for dental transplant [26], for the prevention and treatment of osteoporosis [27] and they exhibit: antibacterial [28], antimicrobial [29], anti-malarial [30] and anticancer activities [31]. They, however, suffer from severe pharmacological draw backs, such as: gastrointestinal irritation [32], esophagitis resulting from direct contact of the whole tablet with the esophageal mucosa [33], low intestinal adsorption which is believed to be as the consequence of its bulky phosphonates moieties, toxicity and poor bioavailability [34]. These findings have prompted several researchers to develop delivery systems that can minimize the pharmacological side effects. Some of the delivery systems include: application of chemotherapeutic prodrugs [35–37], polymers [38,39], liposome [40,41], carbon nanotubes [42] and bioerodibles [43].

Presently, synthetic polymers are used for encapsulation of bisphosphonates and most of them do not exhibit good cell affinity. There is presently no research record to our knowledge that has utilized the combination of natural and synthetic polymers for encapsulation of bisphosphonate. The aim of this research is to utilize the advantages of synthetic and natural polymers by preparing hydrogels from a combination of natural and synthetic polymers in selected ratio. The effect of gum acacia, a natural polymer in hydrogels, on the release pattern of BP was investigated. The hydrogels were synthesized using gum acacia, *N,N*-methylenebisacrylamide, acrylamide and ammonium persulphate as initiator. The hydrogels were characterized by XRD, FTIR, SEM and the release study was performed using UV–Visible spectroscopy. The release profile and mechanism was determined by using selected mathematical release model such as: zero-order and Korsmeyer–Peppas.

2. Experimental

2.1. Materials

*N,N*-methylenebisacrylamide (MBA) and acrylamide (AM) were obtained from Fluka (South Africa). Gum acacia was obtained from Hopkins and Williams (London). Ammonium persulphate (APS) was purchased from S.D. Fine Company (Mumbai, India). Distilled water was used for the preparation of the hydrogels.

2.2. Synthesis of sodium hydrogen (5-amino-1-hydroxy-1-phosphonopentyl) phosphate

Sodium hydrogen (5-amino-1-hydroxy-1-phosphonopentyl) phosphate (Fig. 1A) was synthesized by the reaction of 5-amino pentanoic acid with phosphoric acid and phosphorus trichloride using methane sulphonic acid, according to Kieczykowski et al. [44]. The work-up process was performed by cooling the reaction solution to 20 °C, the pH was adjusted to 4.3 with 50% NaOH (80 mL) and the resulting suspension was aged for 2 h at 0–5 °C. The product was collected by filtration followed by washing with cold water (2 × 50 mL) and 95% ethanol (100 mL) and then dried (air-drying in vacuo at 40 °C) to afford white crystalline salt. The molar mass of the compound is 285.1045 g/mol and the solubility in water is 10 mg/10 mL. The compound synthesized was characterized by 1H NMR, 31P NMR, 31P NMR and FTIR to confirm the isolation of the compound. NMR spectroscopy was performed on Bruker 400 MHz NMR spectrometer using D2O solvent.

2.3. Preparation of the gum acacia hydrogel

Gum acacia hydrogels were prepared by copolymerization of acrylamide and *N,N*-methylene-bisacrylamide onto gum acacia via an initiator system of ammonium persulphate (APS) (Fig. 1B). They were prepared by dissolving gum acacia in 3 mL of 0.05 M of sodium hydroxide solution followed by addition of acrylamide (1 g) and MBA solution (1 mL) (Table 1). The mixture was thoroughly stirred so as to obtain a homogenous mixture before the addition of APS (1 mL) (Table 1). The hydrogels were formed at 40–60 °C on a hot plate. Blank hydrogel was prepared by the same method as above without the addition of gum acacia. The hydrogels prepared were soaked in distilled water overnight in order to get rid of unreacted amine, before drying at ambient temperature for 3 days.
2.4. Preparation of bisphosphonate-loaded hydrogel

A stock solution of 40 mg of BP dissolved in distilled water (80 mL) was prepared. Fifteen millilitres of the stock solution was added to 80 mg of each to the blank, GA-1 and GA-2 dry hydrogels. The hydrogels were left in the stock solution overnight at ambient temperature because the GA hydrogels reached equilibrium swelling over a period of 24 h. The hydrogels were removed from BP solution, rinsed with water so as to get rid of excess BP at the surface of the hydrogels and they were allowed to dry at room temperature for 3 d.

2.5. Swelling studies

Equilibrium swelling studies of the dried hydrogels were performed at ambient temperature over a period of 24 h. Selected buffer solutions were used such as: pH 1.2 (simulating gastric pH), pH 7.4 (simulating intestinal pH) and pH 10 (basic). The hydrogel sensitivity to selected pH value was observed at the selected buffer solutions. The hydrogels were made to swell in the selected buffer solutions until the equilibrium swelling was reached (after 24 h). They were removed and blotted gently with blotting paper to remove the overloaded water on the surface and weighed. The immersion time and drying of the hydrogels were repeated until the masses of the swollen hydrogels were constant. The swelling ratio at equilibrium (ESR) was calculated from Eq. (1):

\[
ESR = \frac{M_e - M_d}{M_d}
\]  

(1)

The swelling ratio (SR) measurements for the hydrogels were performed after every 30 min at pH 7.4. After 30 min, the hydrogels were removed from the buffer solution and blotted gently with blotting paper and weighed. This is calculated as (SR) from Eq. (2):

\[
SR = \frac{M_e - M_d}{M_d}
\]  

(2)

where \(M_e\) is the weight of the hydrogel at equilibrium, \(M_t\) is the weight of the hydrogel at time \(t\) and \(M_d\) is the weight of the dry hydrogel before swelling.

2.6. FTIR

FTIR analysis was performed so as to identify the functional groups present on the bisphosphonate analogue and to indicate the presence of these functionalities in the hydrogels encapsulated with the bisphosphonate analogue. It was performed on GA-1, GA-1 loaded with BP and pure BP in the range of 500–4000 cm\(^{-1}\). The FTIR spectroscopy was performed on (Perkin Elmer Spectrum 100 FTIR spectrometer), USA.

2.7. Drug loading

The drug loading process of BP onto the hydrogels was performed by placing 80 mg each of GA-1, GA-2 and blank hydrogels in 15 mL of 1.75 mM solution of BP overnight at room temperature. The hydrogels were allowed to reach equilibrium swelling in the drug solution at ambient temperature. They were then removed from the drug solution, rinsed with distilled water to remove any excess drug present on the surface of the hydrogels and left to dry at room temperature for 4 days. The drug loading efficiency (B) was calculated based on the ratio of amount of drug entrapped in the hydrogel to the amount of drug used in the loading process. It was determined by using UV–Visible spectroscopy (Perkin Elmer LAMDA 750S UV/VIS Spectrometer), USA. The Spectrophotometric determination of BP was performed using iron(III) chloride in perchloric acid according to the method by Kuljania et al. [45]. The percentage encapsulation efficiencies for the hydrogels were calculated by using Eq. (3):

\[
B = \frac{\text{% actual amount of drug loaded onto the hydrogels}}{\text{% theoretical amount of drug}} \times 100
\]  

(3)

where B is the % encapsulation loading efficiency. The results are depicted in Table 1.

2.8. SEM

The hydrogels were sputtered with gold nanoparticles before SEM analysis. SEM analysis was performed at an accelerating voltage of 2 kV on JEOL-JSM 7500F Scanning Electron Microscope, USA. This was done in order to examine the surface morphologies of BP, hydrogels before and after loading with the BP.

2.9. XRD

The XRD was performed on the hydrogels using (PANalytical X’Pert PRO, the Netherlands). It was performed at (Cu Kα radiation, \(\lambda = 0.1546 \text{ nm}\) running at 45 kV and 40 mA. This analysis was performed so as to evaluate the state of BP and the hydrogels (amorphous or crystalline) and BP interaction with the hydrogel network.

2.10. In vitro release studies

In vitro release study was performed by placing each BP-loaded hydrogel (0.08 g) in 30 mL of selected buffer solutions (1.2 and 7.4) at 37°C. A shaker BS-06 (Lab Companion, USA) was used at 100 rpm. The drug release profiles were obtained using UV–Visible spectrophotometry. The release study was performed over a period of 24 h by collecting 4 mL of the sample solution and replacing it with equivalent amount of buffer solution. The release study was performed over a period of 24 h because the hydrogels containing gum acacia were found to reach equilibrium swelling over a period of 24 h. The formation of complex between BP and iron(III) chloride in perchloric was used to determine the amount of BP released from the hydrogels. For the calibration graph, six standard solutions were prepared by dilution of the corresponding stock solution to obtain a concentration of 0.00027–0.84 mM in perchloric acid solution. The stoichiometric ratio of the bisphosphonate to Fe(III) ions in the chromophoric complex was 1:1.5.

3. Results and discussion

The gum acacia containing hydrogels prepared were hard, flat gels with glassy network. The main reason for the addition of gum acacia in the hydrogels is due to its biodegradable, non-toxic, present of hydrophilic groups and pharmaceutical applications. The
swelling ability and drug release of the hydrogels were studied at pH values of 1.2, 7.4 and 10.

3.1. Synthesis of sodium hydrogen (5-amino-1-hydroxy-1-phosphonopentyl) phosphonate

Sodium hydrogen (5-amino-1-hydroxy-1-phosphonopentyl) phosphonate was synthesized according to an improved procedure for the preparation of 1-hydroxy-1,1-bisphosphonic acid by Kieczykowski et al. [44]. This improved method was used so as to isolate BP as a white crystalline salt in excellent yield and purity. NMR analysis was performed using NaOH/D₂O as solvent. ¹H NMR spectrum showed signals for the alkyl CH₂ at 1.28–1.33, 1.45–1.47, 1.73–1.77 and 2.52 ppm, respectively. The CH₂ signals peak were found to all integrate for two protons each, thereby confirming the compound. Further analysis was performed on the compound by ³¹P NMR spectroscopy which was performed using NaOH/D₂O. The signal for the phosphonate group was visible at 19.02 ppm as a singlet, which further confirmed the compound [44].
1H NMR (NaOH/D₂O) 2.52 ppm (t, J = 7.2 Hz, 2H), 1.73–1.77 ppm (m, 2H), 1.45–1.48 ppm (m, 2H), 1.28–1.33 ppm (pent, J = 7.2 Hz, 2H), and 1H NMR (NaOH/D₂O): 19.02 ppm. 31P NMR (NaOH/D₂O): 77.4, 41.3, 36.7, 33.7 and 22.3 ppm.

3.2. Spectral studies

3.2.1. SEM analysis

SEM analysis was performed so as to determine the morphology of the BP and the hydrogels. The SEM analysis was performed on the BP, GA-1 hydrogel before loading, after drug loading and after drug release. The SEM images of BP were found to exhibit a stack of rod-shaped morphology (green arrows point to the morphology of the BP) (Fig. 2A). SEM images of the GA-1 hydrogel was found to be a combination of a smooth surface and spherical swollen topologies as represented in (Fig. 2B). The morphology of GA-1 hydrogel loaded with bisphosphonate was found to be a combination of a smooth surface, swollen spherical topologies and stack of rod-shaped morphologies believed to signify the presence of BP loaded on to the hydrogel (Fig. 2C). The SEM images of the dried hydrogel (GA-1) after 24 h drug release exhibited large holes and cracks (yellow arrows point to the holes found on the surface of the hydrogel after drug release). This observation suggests the degradation of the hydrogel and confirms the biodegradable nature of the hydrogel (Fig. 2D). Similar observation was reported by Gong et al. where composite hydrogel containing Pluronic F127 exhibited large pores and cracks after drug release indicating degradation [46].

3.2.2. FTIR analysis

Another piece of evidence for the formation of drug, gum acacia hydrogel and gum acacia hydrogel containing BP was obtained from the FTIR spectra. The FTIR spectrum for BP displayed characteristic peaks at 797 cm⁻¹ for P–C stretching, at 1175 cm⁻¹ for P–OH stretching, at 3425 cm⁻¹ for N–H stretching, 1100 cm⁻¹ for C–N stretching and at 1139 cm⁻¹ for P=O stretching which confirmed the successful isolation of BP (Fig. 3A). The characteristic peaks for the BP namely: P–OH, P=O and P–C stretching were absent in the FTIR spectrum of hydrogel without BP. FTIR spectrum for the GA-1 (hydrogel without BP) exhibited sharp amide peaks for C=O stretching at 1663 cm⁻¹, N–H stretching at 3414 cm⁻¹ and C–O–C diaryl peak was present at 1000–1080 cm⁻¹ signifying the presence of gum acacia in the hydrogel (Fig. 3B). The characteristic peak for GA in hydrogels crosslinked with GA at 1080 was also reported by Juby et al. and Dostatek et al. [47,48]. This further confirmed the successful crosslinking of gum acacia with the polymer chain. The spectrum for GA-1 hydrogel loaded with BP is depicted in Fig. 3C and it displayed the characteristic peaks for BP at 778 cm⁻¹ for P–C stretching, 1176 cm⁻¹ for P–OH stretching, amide peaks were found at 3409 cm⁻¹ for N–H stretching, 1664 cm⁻¹ for C=O stretching and C–O–C diaryl peak was present at 1000–1080 cm⁻¹ confirming the presence of gum acacia. The characteristic peaks of BP and gum acacia on GA-1 loaded with BP confirmed the successful incorporation of gum acacia and the loading of the BP onto the hydrogel.

3.2.3. XRD analysis

XRD was used to evaluate the interaction of BP with the hydrogel network. The diffraction pattern for BP was found to be sharp due to its crystalline nature, whereas the GA-1 hydrogel diffraction pattern was found to be very broad. This is as a result of the copolymerization reaction of the monomers which resulted in amorphous copolymer [49]. The characteristic peaks for BP were observed at 2θ = 8.43°, 10.23°, 10.30°, 10.34° and 10.47°, respectively. These peaks were present and broad with low intensity in the hydrogel loaded with the BP but were absent in the hydrogel without BP (Fig. 4). The characteristic peak for gum acacia was visible but broad at 2θ = 20.97° in the GA-1 hydrogel confirming the successful crosslinking of GA with the hydrogel network. Similar finding was reported by Almuslet et al., where the characteristic peak for GA was visible at 19.895° and when grafted onto acrylamide the peak shifted to 20.4° [50]. The presence of the characteristic peaks for BP in the XRD diffraction pattern for hydrogels loaded with BP further confirmed the successful loading of BP onto the hydrogel network.
Table 2
Statistical analysis of swelling properties of the hydrogels at pH 7.4.

<table>
<thead>
<tr>
<th>Hydrogel</th>
<th>K</th>
<th>D (cm²s⁻¹)</th>
<th>n</th>
<th>R²</th>
<th>r</th>
<th>Probability value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>1.71</td>
<td>0.3711</td>
<td>0.62</td>
<td>0.984</td>
<td>0.992</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GA-1</td>
<td>1.80</td>
<td>0.5450</td>
<td>0.74</td>
<td>0.980</td>
<td>0.990</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GA-2</td>
<td>1.75</td>
<td>0.4655</td>
<td>0.70</td>
<td>0.992</td>
<td>0.996</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

3.3. Swelling properties

Swelling capacity of the hydrogels depends on the hydrophilic nature of the polymers, network density, nature of solvent and polymer solvent interaction[51]. Hydrogel swelling is an important factor in drug release, and the mechanism of drug release occurs when diffusion of the drug is faster than the hydrogel swelling [52]. This property is an important phenomenon in the biomedical application of hydrogels as drug-delivery systems. The relationship between the content of gum acacia in the hydrogels and the degree of water sorption was evaluated at selected buffer solution (pH 1.2, 7.4 and 10) simulating gastric, intestinal and basic pH, respectively. Fig. 5A gives an illustration of the equilibrium swelling capacity (ESR) of the hydrogels at pH 1.2, 7.4 and 10. At pH of 1.2, the hydrogels exhibited low degree of swelling because the carboxylate anions are protonated and as such, anion–anion repulsive forces are eliminated. The highest swelling ability of the hydrogels was observed at pH 10 which is attributed to ionize carboxylate groups resulting in mutual repulsion between the carboxylate groups. The general pattern of swelling of these hydrogels was that GA-1 hydrogel which had the least content of gum acacia was found to exhibit the highest swelling ratios at the selected pH values. The swelling pattern suggests that the swelling ratio increased with decrease in the content of gum acacia. When the content of GA is low, it renders the hydrogel network more hydrophilic due to the hydroxyl and carboxyl group, thereby increasing the degree of water sorption and swelling ratio. When the content of the GA was increased from 0.05 to 0.15 g, the swelling ratio decreased because an increase in GA increased the compartment and reduced the mesh size thereby leading to reduced degree of swelling [53]. The statistical analysis for the swelling ability of the hydrogels is depicted in Table 2. The blank hydrogel exhibited the lowest swelling ratios of 8.146 and 7.908 at pH of 1.2 and 7.4, respectively. This observation suggests that the addition of gum acacia, a natural polymer, improved the swelling ability of the hydrogels (GA-1 and GA-2). The swelling patterns of the hydrogels were similar to the research report of GA-based hydrogel by Kaith and Ranjta, Juby et al. and Nagireddy et al. [24,47,54]. The solvent diffusion and polymer matrix relaxation effect [55] was analysed by examining the exponent n from Eq. (4).

\[
\frac{M_t}{M_\infty} = Kt^n
\]  

where \(M_t\) and \(M_\infty\) are the masses of the hydrogel at time \(t\) and at equilibrium, respectively, and \(K\) is the diffusion constant of water into the hydrogel network and \(n\) is the diffusion exponent.

Fig. 4. XRD representation for sodium hydrogen (5-amino-1-hydroxy-1-phosphonopentyl)phosphonate, gum acacia containing hydrogel and hydrogel loaded with sodium hydrogen (5-amino-1-hydroxy-1-phosphonopentyl)phosphonate.

Fig. 5. (A) A graph of equilibrium swelling ratio of the hydrogels at pH 1.2, 7.4 and 10. The error bars represent standard deviation for the three measurements (mean ± SD, n = 3). (B) A graph of Ln swelling ratio versus Ln time. (C) A graph of swelling ratio versus square root of time.
When $n = 0.5$, it indicate case I which is a perfect Fickian process, where the rate of network relaxation is faster than the rate of diffusion. When $n = 1.0$, it indicates a non-Fickian diffusion where water transport is controlled and the rate of diffusion is faster than the network relaxation. When $0.5 < n < 1.0$, it indicates that the rate of penetrant mobility and segmental relaxation are comparable [55]. The swelling exponent $n$ was determined from the slope of the graph of $\ln(M_t/M_\infty)$ versus $\ln t$ for a period of 5 h because more than 60% swelling ratio was obtained during this period (Fig. 5B). Only 60% of the swelling ratio was used and $n$ was found to be in a range of 0.62–0.74 indicating an anomalous non-Fickian process and with a coefficient of determination of 0.984–0.999, indicating good linearity and confidence interval for $n$ are depicted in Table 2.

The hydrogels diffusion coefficients were calculated using Eq. (5):

$$S = 4 \left[ \frac{D}{\pi r^2} \right]^{1/2} t^{1/2}$$

where $D$, $r$, $S$ and $t$ represent the diffusion coefficient of the hydrogel, radius of the hydrogel, fractional swelling and time, respectively. To investigate the diffusion coefficient of hydrogels, $S$ versus $t^{1/2}$ graphs at pH 7.4 were drawn for all the hydrogels and the diffusion coefficients were calculated from the slopes of these graphs (Fig. 5C). The diffusion coefficient was found to be 0.3711, 0.4655 and 0.5450, respectively, for blank, GA-2 and GA-1, respectively (Table 2).

**3.4. In-vitro release of loaded bisphosphonate**

The % encapsulation of BP onto the hydrogels increased with decrease in the content of GA. The results for the % encapsulation and the statistical analysis mean $(n = 3) \pm SD$ are depicted in Table 1. Sodium hydrogen (5-amino-1-hydroxy-1-phosphonopentyl)phosphonate was selected as a drug of choice because it is a nitrogen containing bisphosphonate similar in structure to alendronate. The release study was performed over a period of 24 h because hydrogels containing gum acacia were found to reach equilibrium swelling over a period of 24 h. The % drug released was calculated from Eq. (6):

$$BR = \frac{M_b}{M_{hi}} \times 100$$

where BR is the % of BP released, $M_b$ is the mass of BP released in the buffer solutions of pH 1.2 and 7.4, and $M_{hi}$ is the mass of BP entrapped in the hydrogel. The release study was performed in triplicate for statistical relevance. For the calibration graph, six standard solutions were prepared by dilution of the corresponding stock solution to obtain a concentration of 0.00027–0.84 mM in perchloric acid solution. The standard solutions were mixed with ferric chloride solution and all measurements were performed against blank perchloric acid solution. A linear graph of absorbance against the standard solution was obtained with coefficient of determination ($R^2$)=0.999 indicating good linearity. The cumulative release profile for blank, GA-1 and GA-2 hydrogels loaded with BP in pH 1.2 and 7.4 is shown in Fig. 6A and B and the error bars represent mean ± SD where $n = 3$. At pH 1.2, the hydrogels were found to release 36–53% of the BP after 24 h but between 28 and 43% was released after 5 h. The rate of release of BP from the hydrogels was found to be faster in the hydrogels containing the least amount of gum acacia. These observations suggest that these hydrogels are potential devices that can control the release of BP at the gastrointestinal region. The content of gum acacia in the hydrogel was found to influence the rate of drug release. The drug release result was in good agreement with the effect of the concentration of gum acacia on the swelling of hydrogel as discussed in section 3.3. At pH 7.4, 40–69% of the BP was released after 5 h and 54–100% of BP was released after 24 h. The hydrogels were found to release the drug faster in pH 7.4 than in pH 1.2 and this is as a result of the sensitivity of the hydrogel to pH. The release profile of BP from the gum acacia hydrogels was dependent on the pH, degree of crosslinking and swelling property. Similar finding was reported by Shaikh et al. in the kinetic release of paracetamol from gum acacia containing hydrogels [56]. The release profile of BP from gum acacia hydrogel was a zero-order release.

**3.5. In vitro release kinetics**

The general recognized release mechanisms of drug release from a polymer matrix can be classified into three main processes namely: (1) diffusion from non-degradable polymers (diffusion-controlled system), (2) enhanced drug diffusion due to polymer swelling (swelling-controlled system) and (3) polymer degradation and erosion (erosion-controlled system) [57]. To determine and understand the mechanism of release of BP from the hydrogels used in this study, % drug release data were fitted to selected mathematical release models. The mathematical models selected for this study were Korsmeyer–Peppas (Eq. (7)) and zero-order (Eq. (8)). These equations were used to understand the mechanism of release of BP form the hydrogels at pH values of 1.2 and 7.4, respectively.

$$F = \frac{M_t}{M_i} = K_m t^n$$

where $F$ is the fraction of drug released at time $t$, $M_t$ is the amount of drug released at time $t$, $M_i$ is the total amount of drug in dosage
form, $K_m$ is the kinetic constant and $n$ is the diffusion or release exponent. Diffusion exponent was estimated from linear regression of $\log(M_t/M)$ versus $\log t$ for the first 60% drug release. When $n = 0.5$, it indicates Fickian diffusion, when $0.5 < n < 1$, it indicates anomalous or non-Fickian diffusion which refers to a combination of diffusion- and erosion-controlled rate release. When $n = 1$, it indicates case II and when $n > 1$, it indicates super case transport 2 [58]. The release behaviour was anomalous for GA-1, GA-2 and blank hydrogels at pH 1.2 with $n = 0.80, 0.83, 0.97$ ($R^2 = 0.998, 0.981$ and 0.993), respectively. This suggests that the drug release was as a result of a combination of diffusion- and erosion-controlled rate release (Table 3). This observation further confirms an interaction between the natural polymer network and BP. Although anomalous release mechanism implies a combination of erosion and diffusion, the release profile from the hydrogels can be affected by various factors such as the network composition (e.g. degree of crosslinking), drug properties and compatibility between the drug and the polymer matrix [58]. Statistical calculations were performed using GraphPad INSTAT software and data including probability level, 95% confidence intervals, mean ± SD were obtained which are depicted in Tables 2–4. A probability value of $<0.05$ was considered to be statistically significant.

The release profile of BP from the hydrogels at pH 7.4 was a zero-order kinetics with $K = 0.1639, 0.2145$ and $0.1861$ ($R^2 = 0.997, 0.999$ and 0.998) for blank, GA-1 and GA-2 hydrogels, respectively. These values were derived from Eq. (7).

$$M_t = M_0 + Kt$$ (8)

where $M_0$ is the initial amount of drug in the hydrogel, $M_t$ is the cumulative amount of drug released at time $t$, $K$ is the zero-order release constant and $t$ is the time in hours. The zero-order equation is used to describe a system in which the release mechanism is independent of the concentration of the dissolved substance [58]. Zero-order dominated the release profile at pH 7.4 which is a unique property in controlled release systems (Table 4).

A graph of % cumulative drug release was plotted against the time in hours for the hydrogels (Fig. 6A and B). The $r$, $R^2$, $K$ values and statistical analysis were determined from the graph as shown (Table 4). The release of BP from the gum acacia containing hydrogel was found affected by the content of gum acacia in the hydrogels and the pH values. This indicates that the degree of crosslinking and pH were major factors although more research is needed to confirm this findings. The percentage of drug released over a period of 24 h from GA-1 (i.e. the less crosslinked) was found to be higher than GA-2 with the highest content of gum acacia. This suggests that increasing the content of gum acacia in the polymer network increases the retention time of the drug in the matrix.

### 4. Conclusion

The presence of gum acacia in the hydrogels prepared was found to improve its swelling ability. Hydrogels containing gum acacia which are potential drug-delivery systems were found to exhibit higher swelling ratio than the blank hydrogel. At pH values of 1.2 and 7.4, GA-1 exhibited the highest swelling ability followed by GA-2, while the blank hydrogel exhibited the lowest swelling ability. The successful loading of BP to the hydrogels was confirmed by SEM by stacks of rod-shaped morphology. This was further confirmed by XRD at $2\theta = 8.43^\circ$, 10.23$^\circ$ and FTIR displayed the characteristic absorption peaks for P–C, P–OH and P=O at 797, 1175 and 1139 cm$^{-1}$, respectively. The drug release profile suggests that the addition of gum acacia, a natural polymer to this type of hydrogels investigated in this research, increases the rate of release of bioactive agents from the hydrogels. This observation is desirable in controlled drug-delivery system and it implies that the rate of drug release can be controlled by either increasing or decreasing the amount of gum acacia in this kind of hydrogels. The release kinetics of BP from the gum acacia containing hydrogels was found to differ with pH values. At pH 1.2, the release profile was found to be anomalous for GA-1 and GA-2 hydrogels with $n = 0.8$ and 0.83, respectively, suggesting that the drug release was as a result of a combination of diffusion and polymer erosion. At pH 7.4, the release kinetic of BP was a perfect zero-order release mechanism. The zero-order kinetics constant was found to be 0.1639, 0.2145 and 0.1861 ($R^2 = 0.997, 0.999$ and 0.998) for blank, GA-1 and GA-2 hydrogels, respectively, at pH 7.4. An in vivo study is needed to confirm the results obtained in this research.

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### References

Antimicrob. length


