REPRINT

ChemPubSoc
Europe

■ **Materials Science inc. Nanomaterials & Polymers**

pH-Responsive Studies of Bacterial Cellulose /Chitosan Hydrogels Crosslinked with Genipin: Swelling and Drug Release Behaviour

Joachim E. Arikibe,[a] Roselyn Lata,[a] Keiichi Kuboyama,[b] Toshiaki Ougizawa,[b] and David Rohindra*[a]

Semi interpenetrating hydrogels (semi-IPN) of bacterial cellulose (BC) and chitosan (Ch) crosslinked with genipin were prepared and characterised using Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). Infrared spectra showed amide I and II absorption peaks at 1632 and 1554 cm^{-1} and C–N stretching absorptions at 1250 and 1020 cm^{-1}, respectively, for crosslinked hydrogels. The fibrous structure of BC and the porosity in the hydrogels were revealed by SEM. Swelling of hydrogels was sensitive to pH, and maximum at pH 1.0. Swelling of non-crosslinked samples decreased as the pH increased. However, with crosslinked hydrogels, swelling increased as chitosan ratio increased at low pH, as well as with increase in BC ratio at high pH. Free, bound and intermediate water types in hydrogels were revealed by DSC. The release kinetics of Quetiapine fumarate (QF), an antipsychotic drug used for the treatment of Schizophrenia was studied using UV spectrometry, followed predominantly, the Higuchi model at all pH for crosslinked hydrogels. The transport mechanism of hydrogels was a combination of non-Fickian and Super Case II. Crosslinked hydrogels showed controlled drug release behaviour. These hydrogel systems possess potential application in pharmaceutical field as all chemicals used in the development of the hydrogels are non-toxic, with BC–Ch 60:40 showing most promising potential.

Introduction

The synthesis of hydrogels consisting of natural polysaccharides and the search for properties suitable for different applications in biomedicine, pharmaceutics have gained considerable interest recently.

Cellulose, a water insoluble polysaccharide,[1,2] is regarded as the most abundant organic compound in nature. Cellulose, which is often derived from plants, can also be synthesized by a variety of microorganisms such as bacteria, algae and fungi.[3–5]

Bacterial cellulose (BC) is a biosynthetic exopolysaccharide considered to be a purified form of cellulose.[6,7] It is produced by various strains of bacteria of the *Acetobacter* genera: *Agrobacterium*, *Aerobacter*, *Achromobacter*, Azotobacter, *Rhizobium, Sarcina and Salmolla*.[1,2,8] In recent times, most studies have used the Gram Negative bacteria, *Gluconacetobacter xylinus* (previously known as *Acetobacter xylinum*). It utilizes

glucose as the substrate which is the most efficient producer of cellulose.[3] This bacteria is able to produce cellulose within a temperature range of 25–30 °C and pH range of 4.5 – 7.0.[9] Giving adequate consideration to its ultra-fine structure, BC exhibits unique physico-chemical properties such as high tensile strength, crystallinity and high water absorption capacity. The superior features of BC have enhanced its utilization in various applications.[7] BC has also been found to have received considerable attention in the field of biomedicine,[3,10] because of its good biocompatibility properties.[11] It may be employed in the manufacture for artificial blood vessels for microsurgery,[12] scaffolds for tissue engineering,[13,14] and wound dressing for burns or wound repair.[7,15–17] BC exhibits high stability, low toxicity, non-allergenicity and can be easily and safely sterilized.

However, the production of BC has been adversely affected by high cost and low yield. Researchers are searching for low cost nutrient with high yield production of BC.

Chitosan, a linear polysaccharide made up of β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine, is a deacetylated form of chitin (> 60%). Chitin is a structural element that is a component of the exoskeleton of insects and crustaceans and found to be the second most abundant natural biopolymer after cellulose.[18] The most exploited sources of chitin are the shells of crabs and shrimps. Because of its non-toxicity, stability, biodegradability, and similarity to the internal tissues of the body, chitosan has found itself as a very versatile material with extensive application in the biomedical and biotechnological fields.[19–21] The swelling properties of chitosan have been

[a] *J. E. Arikibe, R. Lata, Dr. D. Rohindra*
School of Biological and Chemical Sciences,
Faculty of Science Technology and Environment,
The University of the South Pacific, Suva, Fiji.
Tel.: +679 3232867
Fax: +679 323 1512
E-mail: rohindra_d@usp.ac.fj
[b] *Dr. K. Kuboyama, Prof. T. Ougizawa*
Department of Materials Science and Engineering,
Tokyo Institute of Technology, 2–12-1-S8-33, O-okayama, Meguro-ku, Tokyo 152-8552, Japan.

controlled by crosslinking using crosslinking agents such as glutaldehyde,[22] and genipin.[23] These attractive properties also make the polymer an ideal candidate for controlled release formulations.

Research in BC and chitosan hydrogels is an emerging area. BC-chitosan hydrogels have been developed and tested for miscibility,[24] as cell scaffold material,[25] anti-bacterial feature which could be used in tissue engineering,[26] and degradation by *Candida rugosa* lipase.[27]

Biodegradable polymers such as chitosan need to be crosslinked in order to modulate their general properties and to last long enough for delivering drug over a desired period of time such as the BC–Ch system in the present study. Cross-linkers such as glutaraldehyde, tripolyphosphate,[28] ethylene glucol, diglycidyl ether and diisocyanate have been used. However, these synthetic crosslinking reagents are all more or less cytotoxic, may impair the biocompatibility of hydrogels delivery systems and their crosslinked products have been found to show limited stability.[29] Thus, there is a need for a natural crosslinking agent that is nontoxic and that forms stable and utterly biocompatible crosslinked products. An alternative choice is genipin, a natural product with low cytotoxicity. Its use in hydrogels eliminates the effects of cytotoxicity and inflammatory responses.[30,31]

Genipin-crosslinked chitosan system and chitosan-cellulose blends and hydrogel systems have been extensively studied. However, based on our literature search, there is no reported work on genipin-crosslinked bacterial cellulose-chitosan hydrogel which is completely biocompatible, nontoxic, with improved properties and behaviour in terms of swelling andstability for the kinetic release study of Quetiapine fumarate at different physiological pH's. The purpose of this study was to prepare semi-IPN hydrogels consisting of BC and chitosan, crosslinked with genipin, a non-toxic compound, and investigate the swelling properties and drug release behaviour of Quetiapine fumarate at different pH's.

Results and Discussion

Microbial screening of the BC

The BC formed was removed from the fermenting container and washed with distilled water. To ascertain the safety of the produced BC, microbial screening was conducted. The overall number of bacteria, number of molds and yeast and the different types of bacteria; *Enterobacteriaceae* and other negative bacilli (-), *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* were all below the required levels or absent.

Physical appearance of non-crosslinked BC-Ch samples and crosslinked BC-Ch hydrogels

As shown in Figure 1, the BC–Ch hydrogel crosslinked with genipin was dark-blue in colour due to exposure of the material to air which is associated with oxygen-induced polymerization of genipin as well as the reaction of genipin with the
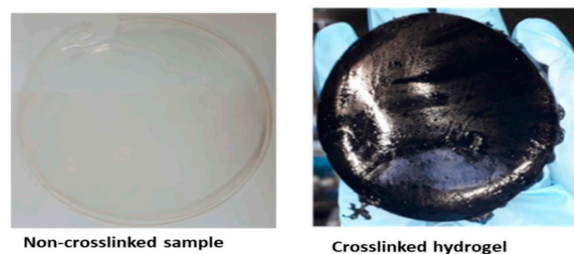

Figure 1. Physical appearance of the non-crosslinked sample and crosslinked hydrogel

nucleophilic amino (NH$_2$) groups of chitosan.[21,32] However, the non-crosslinked sample was transparent in appearance.

The ring-opening reaction results in the formation of new N–H and C–N linkages. The system prepared is a semi interpenetrating network, where the BC incorporates itself into the crosslinked chitosan network by physical entanglements and intermolecular forces.. Figure 2 shows the reaction scheme for the formation of the semi-IPN hydrogels.

FTIR Analysis of the BC-Ch hydrogels

The infrared spectra of the BC, chitosan and BC–Ch non-crosslinked samples and crosslinked hydrogels are shown in Figure 3. Both BC and chitosan have almost similar IR spectra due to their similarity in chemical structure. However, the regions of interest for this study were around 3500, 1600 and 1500 cm^{-1} that showed some characteristic peaks for BC and chitosan. The band around 3342 cm^{-1} is due to the stretching of the OH group. After blending BC with chitosan, the OH peak remained quite unaffected (not pronounced shift in wave-number) but crosslinking with genipin shifted it to lower wavenumbers. The peak at 1625 cm^{-1} in BC was due to OH bending of absorbed water molecules.[33] This peak shifted to higher wavenumbers to around 1643 cm^{-1} when blended with chitosan. This shift can be attributed to the peak overlapping and interactions occurring between OH bending of water molecules with OH groups in BC and that of chitosan. In chitosan, the peaks at 1649 and 1579 cm^{-1} are attributed to the amino (NH$_2$) and N–H bending vibrations respectively. After crosslinking, the emergence of the peaks at 1632 and 1554 cm^{-1} confirmed the formation of the amide I and amide II representing the moieties of the amino group. Furthermore, the peak observed at 1025 cm^{-1} is distinctly attributed to the C–N stretching resulting from the crosslinking interaction between genipin and chitosan. This peak was absent in BC and the non-crosslinked samples. Considering that BC and chitosan share similar structures and mutually complementary qualities (Figure 2), their combination results in reinforced molecular interaction between the polysaccharides via hydrogen bonding and other physical entanglements. This confirmed that cross-linking the chitosan in the presence of BC resulted in a semi interpenetrating network hydrogel.
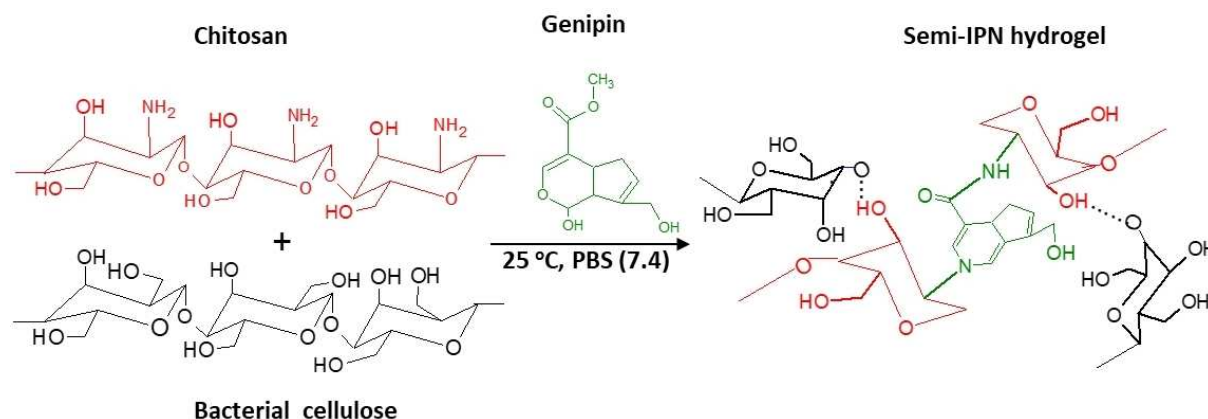
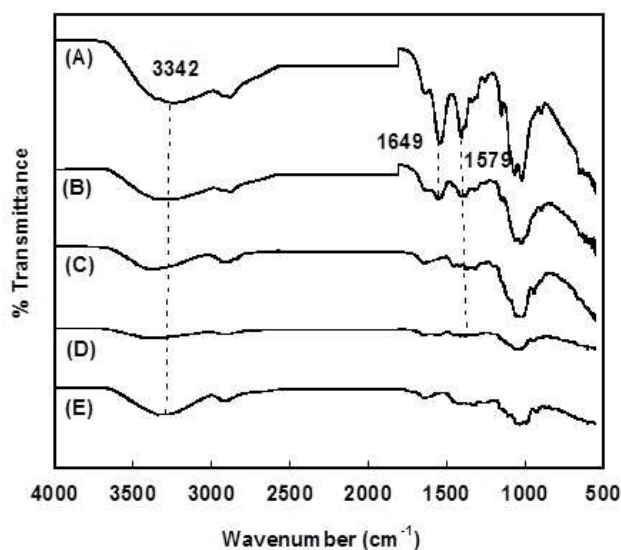**Figure 2.** Reaction scheme for the formation of the semi-IPN hydrogels.

Figure 3. FT-IR absorption peaks for BC, chitosan and BC—Ch (% w/w) hydrogels. (A) Ch UCL (B) Ch CL (C) 60:40 UCL (D) 60:40 CL (E) BC. First value is BC and last value is chitosan. (NB: CL and UCL represent crosslinked and non-crosslinked, respectively)

Figure 4. The chemical structure of Quetiapine fumarate
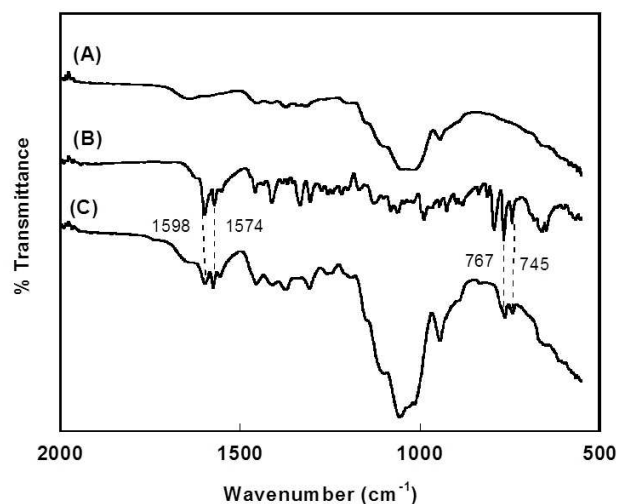
Figure 5. FTIR spectra for (A) BC—Ch 60:40 (% w/w) hydrogel without QF; (B) QF and (C) BC—Ch 60:40 (% w/w) hydrogel loaded with QF. (First value is BC and last value is chitosan)

QF is an antipsychotic drug for treating schizophrenia and the chemical structure is shown in Figure 4. QF is administered twice a day and the problem of patients skipping doses has been reported in most cases.[34,35] Hence, the need for a delivery system for QF that could offers extended release (ER), minimize side effects due to over-dosage with improve compliance in schizophrenia patients and in which QF does not have any chemical interactions with the hydrogel matrix.

For the drug QF, FT-IR analysis showed the characteristic peaks at 1600, 766 and 745 cm^{-1} which are attributed to presence of substituted benzene ring. Figure 5 shows the IR spectra of QF, BC—Ch hydrogels with and without QF. The emergence of the characteristic QF peaks in the hydrogel confirmed the presence of the drug.
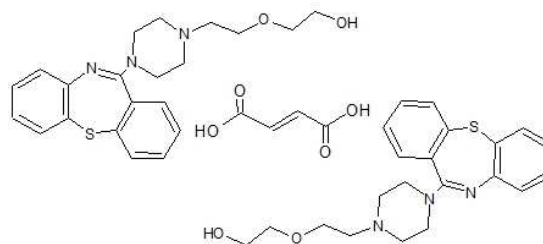
Furthermore, the unchanged positon of the QF's distinct absorption peaks in the hydrogel matrix suggested QF maintained its distinct identity and did not interact chemically with the biopolymers (matrix) but only via intermolecular hydrogen bonding.
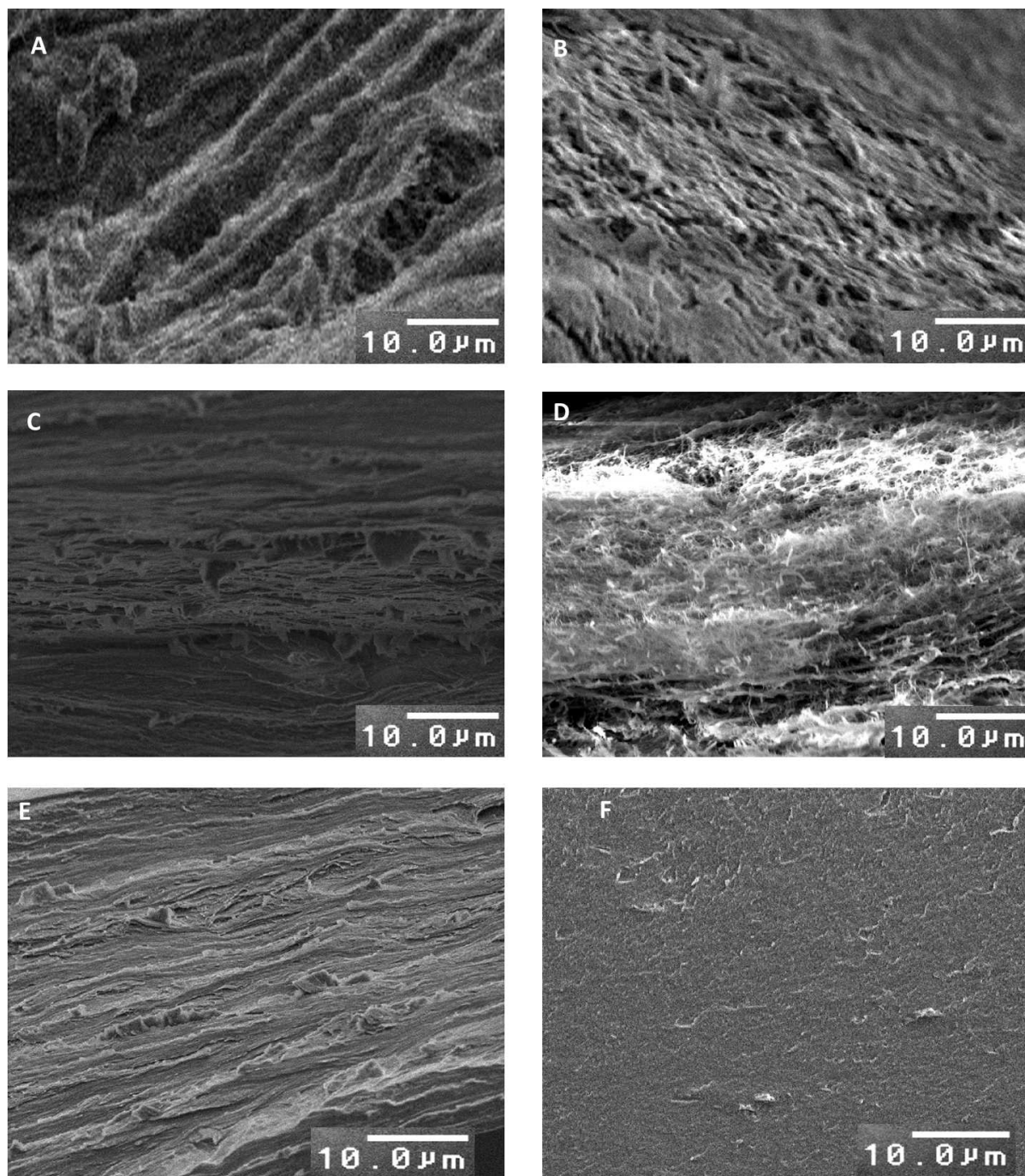
Figure 6. SEM images of BC, chitosan and BC–Ch (% w/w) hydrogels (A) BC, (B) 80:20 UCL, (C) 80:20 CL (D) 60:40 UCL, (E) 60:40 CL and (F) chitosan (Magnification 1000x). (First value is BC and last value is chitosan).

Scanning Electron Microscopy

The SEM images of pure BC and BC–Ch non-crosslinked samples and crosslinked hydrogels are presented in Figure 6A – F. BC membrane was found to possess a network of fibrils. The BC structure observed here agrees with that reported in literature.[36] The SEM revealed the extent of compactness in the structure of the crosslinked hydrogels (Figure 6C and E) due to the crosslinking of chitosan by genipin within the semi-

IPN leading to a decrease in the observed pore sizes of the hydrogels as opposed to the non-crosslinked samples (Figure 6B and D) which showed little compactness and are similar to that observed for pure BC.

Gel Fraction of hydrogels

Figure 7 shows the gel fraction of the hydrogels containing different amounts of BC and chitosan. Gel fraction increased
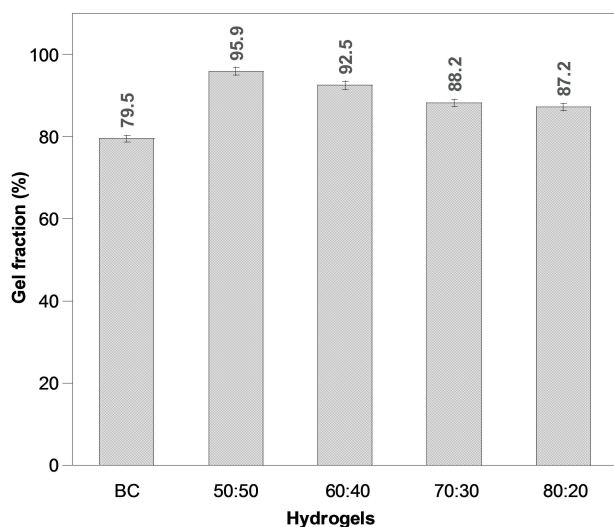
Figure 7. Gel fraction of BC–Ch (% w/w) hydrogels. (First value is BC and last value is chitosan).

with increasing chitosan content in the hydrogel as a result of the availability of sites for crosslinking to occur. This implies that, as the content of chitosan increased in the hydrogels, crosslinking became more pronounced and the amount of unreacted components in the hydrogels decreased since chitosan is the crosslinked component in the hydrogel network. Also, in combination, there might have been an increase in the water holding capacity and gelling property of the combined polymers, resulting to the observed high value of gel fraction as reported by Wahid et al.[26] This observed behaviour explains why the gel fraction in the present study was maximum in BC-Ch 50:50 hydrogel. Similar behavior has been reported for carboxymethyl chitosan/poly (acrylonitrile) semi-IPN hydrogels.[37]

Swelling Behaviour of the Hydrogels

Sensitivity to water and other biological fluids remains the most important property of hydrogels that determine their practical applicability in diverse areas. The swelling behavior of BC, chitosan and BC–Ch (80:20 and 60:40) crosslinked hydrogels and non-crosslinked samples in different artificial gastro-intestinal media of varying pH are presented in Figure 8A–D. All the hydrogels exhibited high swelling in the different media. Noticeably, BC maintained the highest swelling in all the media, because of the interactions between the OH groups of BC and water molecules, making BC high in water permeability. Although BC and plant cellulose have the same chemical structures, however, BC possesses macromolecular properties different from plant cellulose. The thin microfibrils of BC are significantly smaller than those in plant cellulose, making BC much more porous than plant cellulose.[38] The high aspect ratio of BC fibrils provided BC with high swelling ability, which resulted in high water holding capacity.[29,38,39]

At pH values (1.0 and 4.5), the non-crosslinked samples recorded the highest swelling compared to the crosslinked hydrogels. This is because the polymer chains were flexible and relaxed due to the absence of crosslinking,[22] allowing more fluid to go into the polymer networks. Hydrogels with high chitosan content showed increased swelling at low pH. This is because the NH_2 groups of chitosan got protonated resulting in polymer-polymer chains repulsion, thereby making chitosan a water-soluble cationic polyelectrolyte which resulted in allowing more fluids into the hydrogels network. At low pH, the amount of protonated positive charges – (NH_3^+) in chitosan is observed to be highest because there are more extensions of the chains,[37] increasing the hydrodynamic volume of the hydrogels. For the crosslinked hydrogels, a decrease in swelling was observed and is attributed to the effect of genipin crosslinking the chitosan within the hydrogel networks which reduced the water permeability of the hydrogels. The cross-linking of chitosan with genipin results in both hydrophilic and hydrophobic interactions. These interactions involve the hydro-phobic methyl group, dihydropyran ring as well as hydrophilic groups such OH, amine, amide, and the amphiphilic polymers behaviour within the hydrogels, thereby bringing about a reduction in water absorbency.[33,40] In this context, the fluid content of the hydrogels may have altered the chemical potential as a result of elastic forces, which are responsible for change of volume fraction density of the polymer chains in the crosslinking process.

At pH values (6.8 and 7.0), the swelling of the hydrogels increased with increasing BC content in both the crosslinked hydrogels and non-crosslinked samples. The non-crosslinked samples showed higher swelling compared to the crosslinked hydrogels. At these pHs the swelling was minimum in hydro-gels with high chitosan content. This is because the cationic property of chitosan is diminished, thereby leaving chitosan less protonated resulting in smaller hydrodynamic volume. The swelling is suggested to be due to the hydrophilicity provided by the higher BC contents in the hydrogels. When pH is raised to a value higher than 6, the NH_2 groups in chitosan are deprotonated and there is loss of charge on the polymer, thus becoming insoluble. The soluble-insoluble transition takes place at pH values near the pKa value of chitosan, which is around 6–6.5.[41] This deprotonation of the amino groups in chitosan recede repulsion in polymer chains which brings about shrinking of the hydrogel.[22] The variations in the swelling behaviour of the hydrogels indicated that the swelling is pH sensitive with respect to chitosan but independent with respect to BC. Swelling decreased significantly in the non-crosslinked samples as the pH increased. However, with the crosslinked hydrogels, blends with increasing chitosan content swelled more at low pH's (1.0 and 4.5) while blends with higher BC content swelled more at high pH's (6.8 and 7.0). The swelling behaviours of non-crosslinked samples and crosslinked hydrogels 70:30 and 50:50 were similar to that of 80:20 and 60:40.
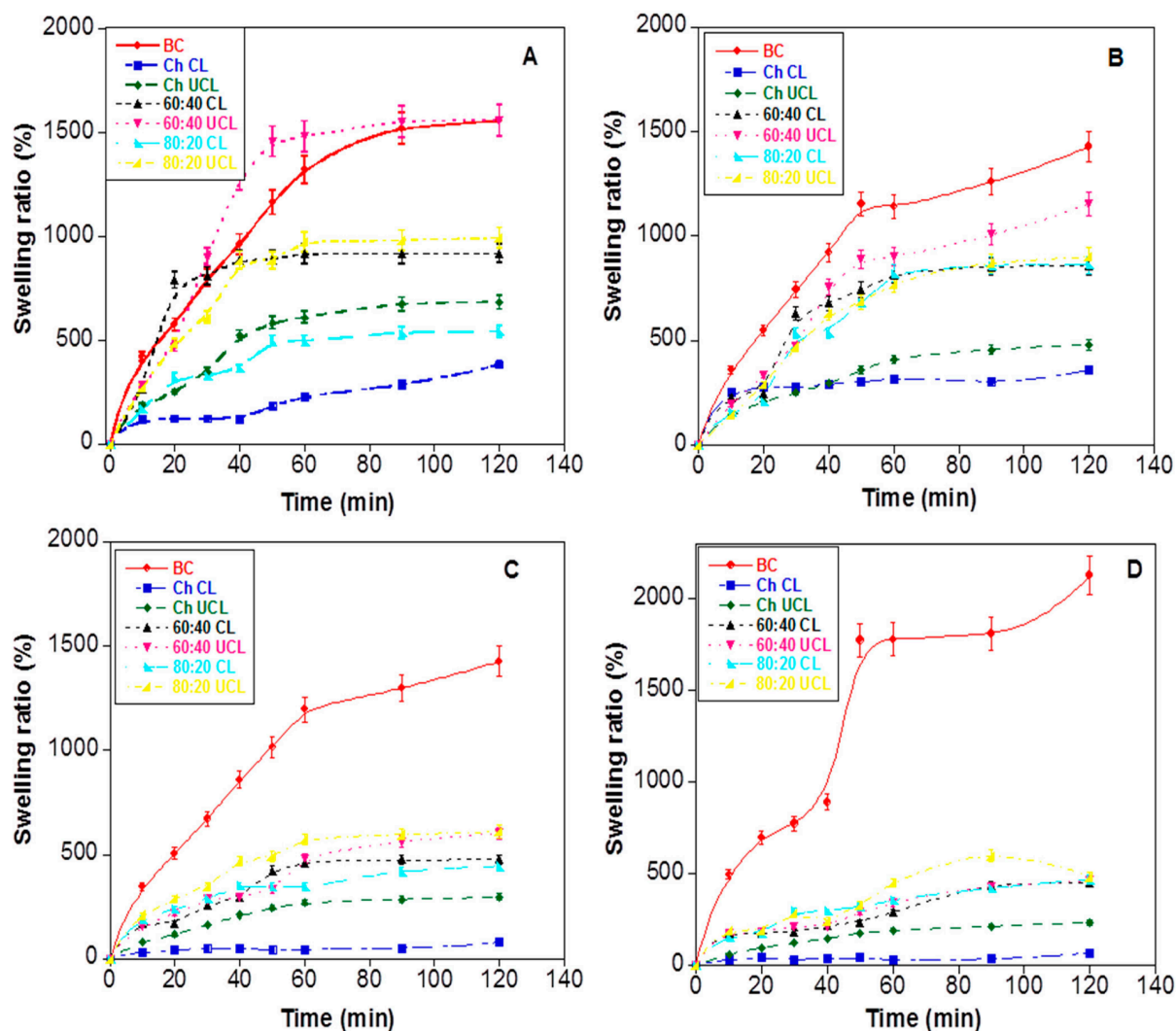
Figure 8. Swelling ratio of non-crosslinked (UCL) and crosslinked (CL) BC–Ch (% w/w) hydrogels at (A) pH 1.0 (B) pH 4.5 (C) pH 6.8 and (D) pH 7.0. (First value is BC and last value is chitosan).

Thermal behavior of the water in the swollen hydrogels

Figure 9 A and B shows the normalized heating profiles of the swollen non-crosslinked samples and crosslinked hydrogels, respectively, after being cooled to $-50\,°C$ and heated at a rate of $10\,°C\ min^{-1}$. In the non-crosslinked samples, only one endothermic peak was observed around $0\,°C$ while for the crosslinked hydrogels, two endothermic peaks were observed, one at lower temperature around $-10\,°C$ and the other around $0\,°C$.

It has been reported,[22] that three types of water exist in hydrogels and are referred to as free, intermediate and bound water. Free water freezes upon cooling to low temperatures and shows a melting endotherm in the DSC heating scan at $0\,°C$. Intermediate water represents water molecules that form weak interactions with the polymer molecules of the hydrogels and the frozen segment shows a melting endotherm less than $0\,°C$. Bound water also referred to as non-freezing water is the

type that forms strong interactions with the hydrogel and does not freeze upon cooling, consequently shows no melting endotherm. Non-crosslinked samples showed a single endothermic peak which ranged from 0 to $10\,°C$ for chitosan to BC, respectively. Crosslinking is basically responsible for the 3-D networks of hydrogels and other entanglements of the polymers and it is the dominant factor that impacts on all features of hydrogels, including the nature or types of water that can be found within a hydrogel system. The non-crosslinked BC–Ch systems showing only one type of water may not be unconnected to the absence of a 3-D network typically found in hydrogels and other entanglements brought about by crosslinking that result from interaction between BC and chitosan. As a result, non-crosslinked systems do not possess the network for the water structured in the "ice-cage" conformation.[42,43] However, the onset temperature of the endothermic peaks was at $0\,°C$. Interestingly, crosslinked hydrogels showed the presence of both intermediate and free water.
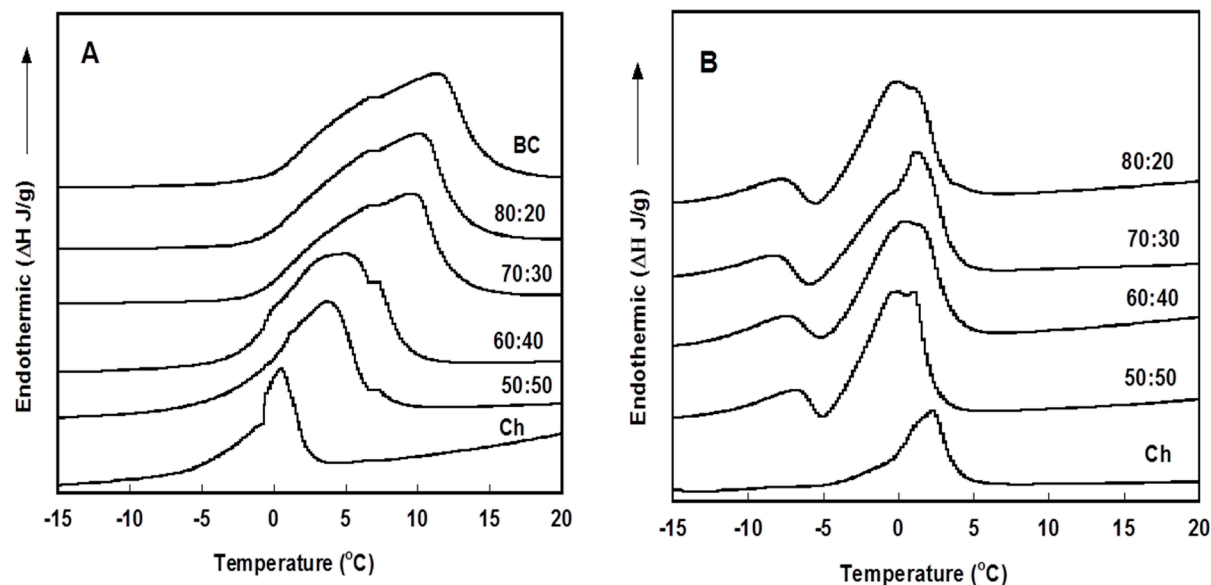
Figure 9. The heating profiles of BC–Ch (% w/w) (A) non-crosslinked and (B) crosslinked hydrogels. (First value is BC and last value is chitosan).

The free water melted at 0 °C. The size of the endothermic peak corresponding to the intermediate water was found to increase with increasing chitosan content and is suggested to be due to the entrapment of water molecules in the network and forming weak hydrogen bonds.

The different types of water present in non-crosslinked samples and crosslinked hydrogels of different ratios are listed in Table 1. The equilibrium water content (ECW) (%) of non-

Table 1. Different states of water in the different BC–Ch (% w/w) hydrogels. (First value is BC and last value is chitosan).

Sample	EWC (%)	Free water (%)	Bound water (%)	Intermediate water (%)
BC	95.10	91.50	3.60	-
Ch UCL	39.13	31.73	7.40	-
50:50 UCL	70.20	57.90	12.30	-
60:40 UCL	87.30	88.70	Deviation observed	-
70:30UCL	88.10	93.30	Deviation observed	-
80:20 UCL	88.20	96.41	Deviation observed	-
Ch CL	27.24	8.16	19.08	-
50:50 CL	72.50	42.70	25.10	4.70
60:40 CL	73.10	45.00	23.80	4.30
70:30 CL	75.70	56.20	16.90	2.60
80:20 CL	77.80	62.00	14.50	1.30

crosslinked samples was higher than those of the crosslinked hydrogels. An increase in ECW (%) was observed in both crosslinked hydrogels and non-crosslinked samples as the amount of BC increased and is suggested to be due to the hydrophilic contribution of BC and its porous nature. Only the

crosslinked hydrogels contained intermediate water in their matrix and was observed to increase as the amount of chitosan increased. This is possibly due to the entrapment of water within the entanglement created between genipin crosslinked chitosan and BC and the hydrophilic groups of the polymer chains forming intermolecular forces with water. Free water in crosslinked hydrogels increased as the amount of BC increased while bound water increased as ratio of chitosan increased. However, an unusual phenomenon was observed in the BC–Ch 60:40, BC–Ch 70:30 and BC–Ch 80:20, where the calculated amount of free water was found to be higher than the ECW. The suggestion to this observation may be during freezing, as the volume of water expanded (ice has higher volume than liquid water), the polymer chains also rearranged due to stress imposed by the expanding water. Upon heating, the endothermic peak at 0 °C contains both the heat required for melting ice and for polymer chain relaxation. The combined heat could be the reason for the observation of this phenomenon.

In vitro Drug release kinetic and transport mechanism

Kinetic models (Higuchi, First Order, Zero order, Hixson) are used to show forms of drug release and transport mechanism involved in matrice of diverse geometries and compositions. These models are based on different mathematical functions. The Higuchi model describes the drug release as a diffusion process based on Fick's law. The first order model shows that the rate of drug released from matrice depends on the concentration of drug within the dissolution medium. For zero order model, drug release occurs rapidly after the drug dissociates, which implies that the rate of release is, in fact, independent of the concentration. Another model, the Korsmeyer-Peppas describes the release behaviour of drug from hydrophilic matrix and shows whether transport mechanism is
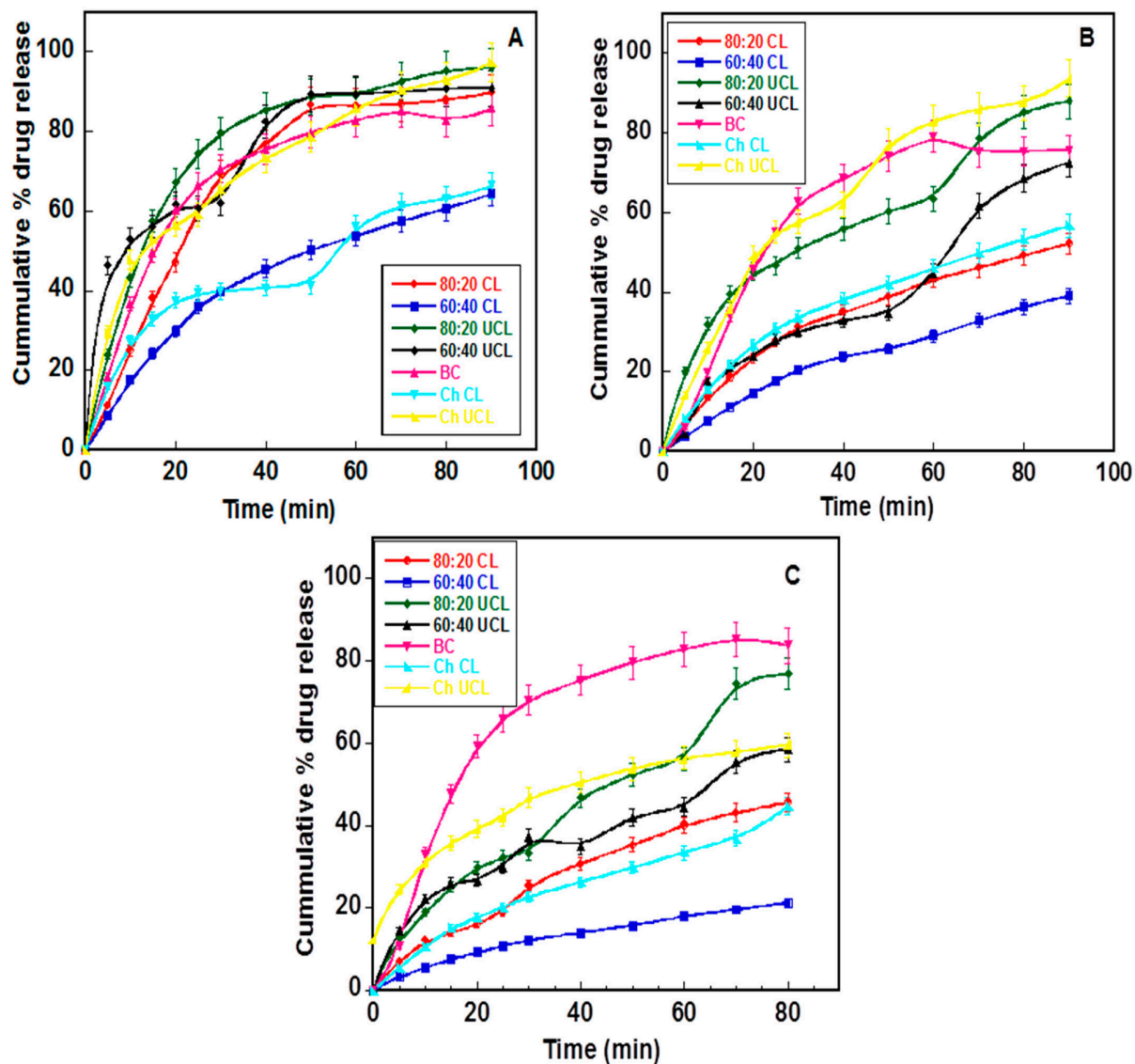
Figure 10. Drug release profile of BC, chitosan and BC-Ch(% w/w) hydrogels (n = 3) at (A) pH 1.0 (B) pH 4.5 and (C) pH 6.8. (first value is BC and last value is chitosan).

according to Fickian, non-Fickian, Case II or Super Case II. For any of the models to be valid, the plot of the drug release data with time should be linear. These models have been discussed in detail in the literature.[44–46]

The drug release profiles from the hydrogels were obtained over a 90 min period by measuring the UV absorbance of the drug in the different media used. The amount of drug released from the matrice was calculated from the calibration plot of UV absorbance against drug concentration. The cumulative % drug release was calculated using Equation 9.

$$Cumulative\ release\ (\%) = \frac{volume\ of\ sample\ withrawn}{bath\ volume}\ x\ P\ (t-1) + Pt \quad (9)$$

Where: Pt = Percentage release at time, t and P (t – 1) = Percentage release previous to 't'.

The drug released by BC matrix shown in Figure 10 A–C was observed to follow the Higuchi model indicating the drug release was via diffusion. The release exponent (n) from Peppas power law showed that the drug release followed the anomalous transport mechanism with $0.5 < n < 1$ (n = 0.94) at pH 4.5 while the transport mechanism was a super case-II at pH 1.0 and 6.8 with n > 1 (n = 1.03 and 1.40, respectively). This suggests that the transport mechanism of BC becomes complex at low and high pH's. Interestingly, it can be observed that BC swelled more than other samples at pH 6.8 but recorded not much difference in drug release when compared to other samples at pH 6.8 (Figure 10 C). This is because swelling of BC was dependent on the solvent penetration into

Table 2. Parameters of kinetic release for QF for BC–Ch (% w/w) hydrogels in different biological fluids. First value is BC and last value is chitosan.										
Hydrogels	Zero order		Kosymer-Peppas		First Order Model		Higuchi Model		Hixson Model	
	R²	m	R²	m	R²	m	R²	m	R²	m
pH 1.0										
BC_Ch:80_20 CL	0.798	0.954	0.919	0.955	0.925	-0.112	0.931	10.874	0.892	0.029
BC_Ch:60_40 CL	0.904	0.657	0.944	0.870	0.970	-0.005	0.987	7.25	0.951	0.014
BC_Ch:80_20 UCL	0.706	0.849	0.818	0.879	0.967	-0.015	0.906	10.16	0.900	0.031
BC_Ch:60_40 UCL	0.713	0.747	0.732	0.807	0.897	-0.012	0.987	8.855	0.860	0.026
BC	0.711	0.784	0.953	0.962	0.886	-0.009	0.905	9.341	0.835	0.023
Ch CL	0.864	0.597	0.852	0.793	0.933	-0.005	0.959	6.64	0.916	0.013
Ch UCL	0.977	0.848	0.802	0.8458	0.960	-0.015	0.954	9.721	0.927	0.031
pH 4.5										
BC_Ch:80_20 CL	0.923	0.536	0.960	0.836	0.968	-0.003	0.990	5.865	0.955	0.011
BC_Ch:60_40 CL	0.961	0.412	0.990	0.817	0.982	-0.002	0.990	0.981	0.976	0.008
BC_Ch:80_20 UCL	0.906	0.812	0.859	0.846	0.948	-0.099	0.982	8.924	0.958	0.023
BC_Ch:60_40 UCL	0.959	0.749	0.961	0.906	0.933	-0.006	0.920	7.741	0.949	0.017
BC	0.747	0.820	0.813	0.839	0.832	-0.008	0.896	9.482	0.809	0.205
Ch CL	0.916	0.568	0.945	0.835	0.970	-0.004	0.992	6.235	0.955	0.012
Ch UCL	0.997	0.954	0.915	0.922	0.985	-0.012	0.980	10.579	0.978	-0.029
pH 6.8										
BC_Ch:80_20 CL	0.960	0.52	0.975	0.82	0.983	-0.003	0.987	5.536	0.977	0.01
BC_Ch:60_40 CL	0.957	0.233	0.996	0.686	0.97	-0.001	0.992	2.505	0.966	0.004
BC_Ch:80_20 UCL	0.969	0.84	0.905	0.990	0.965	-0.007	0.964	8.845	0.975	0.026
BC_Ch:60_40 UCL	0.921	0.572	0.951	1.102	0.961	-0.004	0.978	6.224	0.952	0.012
BC	0.719	0.831	0.986	1.028	0.894	-0.010	0.898	9.804	0.842	0.024
Ch CL	0.972	0.486	0.980	0.813	0.983	-0.003	0.972	5.135	0.981	0.009
Ch UCL	0.812	0.556	0.878	0.812	0.978	-0.010	0.965	6.417	0.873	0.012

the BC porous network (matrix) while the drug release is influenced by BC matrix in terms of swelling as well as the drug's solubility and diffusion in the solvent at that pH.

The release from the non-crosslinked chitosan revealed a zero order model at pHs 1.0 and 4.5, suggesting a case of rapid or burst release because of chitosan being soluble in acidic media. At pH 6.8, first order model was followed, suggesting the release was dependent on the drug concentration within the matrix. The release exponent showed an anomalous transport mechanism at all pHs. For the crosslinked chitosan, drug release followed the first order model at all pH and the release exponent showed an anomalous transport mechanism at pH 6.8 (n = 0.81-0.87) while a super case-II transport mechanism (n = 1.18 and 1.3) was recorded at pH's 4.5 and 1.0.

There was no significant difference (p < 0.05) seen in the drug release for non-crosslinked BC–Ch samples. The drug release initially showed fast release at all pH (Figure 10A–C) which was highest at pH 1.0 followed by pH 4.5 and pH 6.8, respectively. This is due to the interpenetration of polymer chains, their physical entanglements as well as the porosity in hydrogels,[47] which was further enhanced by high hydrophilicity contribution from both BC and chitosan, respectively.

In the crosslinked BC–Ch hydrogels, the crosslinking reaction involving chitosan and genipin brought about the formation of hydrophilic and hydrophobic interactions,[48] within the entire hydrogel network. The coexistence of these two interactions (introduction of hydrophobic unit) resulted in a decrease in drug release due to decrease in the total porosity (initial porosity plus porosity due to drug dissolution) of the matrix. The drug released from all the crosslinked hydrogels

matrix was slow at all pH, except at pH 1.0 for BC–Ch 80:20 which was significantly different (p > 0.05). This is attributed to high amount of BC which may have hindered, to some extent, adequate crosslinking interaction between chitosan and genipin via masking effect.

The drug release kinetics data obtained from the different models showed that all crosslinked hydrogels (with the exception of first order kinetic model shown by BC–Ch 80:20 at pH 6.8) followed the Higuchi model (Figure 11 A), suggesting that matrixes were diffusion controlled as plots of the cumulative % drug release versus square root of time (SQRT) were found to be linear, and the correlation coefficient (R²) higher than those of other models as shown in Table 2. Non-crosslinked BC–Ch 80:20 at pH 1.0 followed the first order kinetic model (Figure 8 B) while BC–Ch 60:40 UCL at pH 4.5 was found to be of zero order (Figure 11 C and Table 2).

From Korsmeyer-Peppas power law, all crosslinked hydrogels and non-crosslinked samples at pH 4.5 recorded release exponents (n) 0.5 < n < 1 (n = 0.82-0.92). This is a case of non-Fickian or anomalous transport,[44,49–51] and the drug release is brought about by the processes of diffusion and swelling within the hydrogels matrice. The slow rearrangement of the polymeric chains and the diffusion process simultaneously caused the time-dependent anomalous effects. This anomalous transport mechanism indicates that the velocity of the buffer (pH 4.5) diffusing into the matrix and the polymer relaxation processes are similar in magnitudes.

At pH 1.0, all non-crosslinked BC–Ch samples had release exponents (n) of 0.5 < n < 1, (n = 0.81-0.88) which suggests that the drug release mechanism followed a non-Fickian or
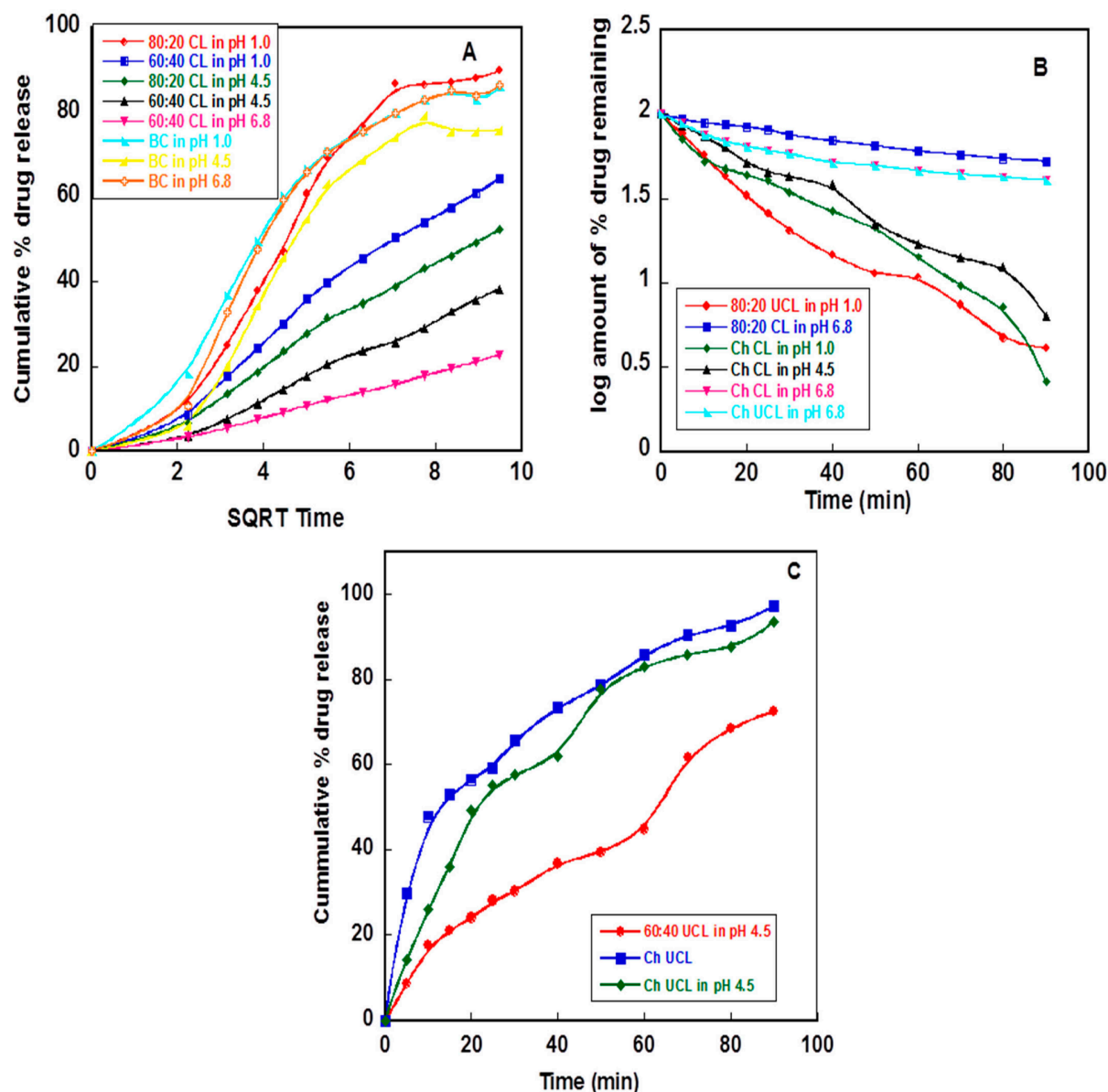
Figure 11. Kinetic models for QF release in artificial gastrointestinal fluids from different matrixes. (A) Higuchi (B) First order (C) Zero order. (For each BC–Ch (% w/w), the first value is BC and the last value is chitosan

anomalous transport in which the release was governed by both diffusion and swelling. However, with the crosslinked hydrogels, the transport mechanism changed from anomalous to a Super Case II (n > 1), with release exponents of 1.03 and 1.36 respectively. This is an extreme form of transport, which implies that the drug release mechanism was both by diffusion and polymer chain relaxation. As stated hitherto, this behaviour could be due to the fact that at the point of the sorption process, tension and relaxation of the polymer chains (solvent crazing),[44] occurred in the hydrogels. Further elucidation may suggest chain rearrangements emanating from the crosslinking of the NH$_2$ group in chitosan by genipin which altered the nature of interpenetration and entanglements compared to that present in the non-crosslinked samples, making the outer

layer of the hydrogels to limit/or prevent axial swelling of the gels, yielding tension of compression on the hydrogel nucleus.[44,45] As the polymeric interface gel-vitreous moved to the nucleus, the tension increased until the nucleus broke. More so, this observed release transport mechanism may be linked to the velocity of the pH 1.0 medium-diffusion which is much higher, causing an acceleration of the solvent penetration due to low pH.

At pH 6.8, the release mechanisms were similar to those at pHs 1.0 and 4.5. The crosslinked hydrogels followed a non-Fickian transport with values 0.67 and 0.82 (0.5 < n < 1); the non-crosslinked samples showed release exponents n > 1 with values 1.10 and 1.12, the so called Super Case II transport mechanism.

The drug release from the hydrogels matrice at different pHs revealed that the percentage of drug released decreased as pH increased for both crosslinked hydrogels and non-crosslinked samples, except for non-crosslinkedd BC–Ch 60:40 which showed a different behaviour at pH 6.8. However, tthrough crosslinking and changing the composition of the hydrogels, the drug release mechanism changed. These hydrogel systems possess potential application in pharmaceutical field as all chemicals used in the development of the hydrogels are non-toxic and are also biocompatible with extracellular tissues.

Low swelling hydrogels have been reported to possess various advantages,[52] due to decrease in the relaxation of polymers chains as a result of crosslinking which results in lesser swelling. In the present study, the hydrogel demonstrate ability to slowly release drug in controlled manner demonstrating it may be suitable for the administration of QF for the treatment of Schizophrenia, with prolonged efficacy and decreased toxicity, especially for the elderly people who normally skip doses because of cognitive problems.

Conclusion

BC was produced from coconut water by fermenting with the bacteria *Gluconacetobacter xylinus*. Semi-IPN hydrogels were prepared by mixing the BC with chitosan and selectively crosslinking the chitosan with genipin. The FT-IR of the semi-IPN revealed shifts in the peak intensity at 1600 cm^{-1} and the emergence of the peak at 1500 cm^{-1} emanating from the crosslinking reaction of chitosan with genipin. The swelling behaviour of crosslinked hydrogels and non-crosslinked samples increased with higher chitosan contents at low pH's while at high pH's, the swelling increased with increasing amount of BC. The swelling of crosslinked hydrogels were lower than those of the non-crosslinked samples. The *in vitro* release kinetic of QF showed that non-crosslinked samples released the drug faster from their matrice. Crosslinked hydrogels showed controlled release. The drug release kinetics from the crosslinked hydrogels followed predominantly, the Higuchi model in all media and the transport mechanism showed that the release exponent of the hydrogels were a combination of non-Fickian and Super Case II transport, indicating a possible case of complex transport mechanism. Crosslinked BC–Ch 60:40 hydrogel showed the most promising potential in controlling the drug release in different media. The results show that BC-Ch semi-IPN hydrogels have potentials as drug carriers.

Supporting Information Summary

Supporting information PDF file contains materials and detailed experimental procedures for the preparation of *G. xylinus* inoculum, synthesis of BC, preparation of BC–Ch hydrogels, characterization of hydrogels using FTIR, DSC, SEM, swelling ratio and gel fraction as well as the drug loading and release for hydrogel matrice.

Acknowledgements

Conflict of Interest

The authors declare no conflict of interest.

[1] P. R. Chawla, I. B. Bajaj, S. A. Survase, R. S. Singhal, *Food Technol. Biotechnol.* **2009**, *47*, 107–124.
[2] L. Huang, X. Chen, T. X. Nguyen, H. Tang, L. Zhang, G. Yang, *J. Mater. Chem. B* **2013**, *1*, 2976–2984.
[3] H. G. de Oliveira Barud, R. R. da Silva, H. da Silva Barud, A. Tercjak, J. Gutierrez, W. R. Lustri, O. B. de Oliveira, S. J. L. Ribeiro, *Carbohydr. Polym.* **2016**, *153*, 406–420.
[4] A. M. A. Gallegos, S. Herrera Carrera, R. Parra, T. Keshavarz, H. M. N. Iqbal, *BioResources* **2016**, *11*, 5641–5655.
[5] X. Shen, J. L. Shamshina, P. Berton, G. Gurau, R. D. Rogers, *Green Chem.* **2016**, *18*, 53–75.
[6] F. Esa, S. M. Tasirin, N. A. Rahman, *Agric. Agric. Sci. Procedia* **2014**, *2*, 113–119.
[7] W.-C. Lin, C.-C. Lien, H.-J. Yeh, C.-M. Yu, S. Hsu, *Carbohydr. Polym.* **2013**, *94*, 603–611.
[8] M. Shoda, Y. Sugano, *Biotechnol. Bioprocess Eng.* **2005**, *10*, 1–8.
[9] H.-J. Son, M.-S. Heo, Y.-G. Kim, S.-J. Lee, *Biotechnol. Appl. Biochem.* **2001**, *33*, 1–5.
[10] G. F. Picheth, C. L. Pirich, M. R. Sierakowski, M. A. Woehl, C. N. Sakakibara, C. F. de Souza, A. A. Martin, R. da Silva, R. A. de Freitas, *Int. J. Biol. Macromol.* **2017**, *104*, 97–106.
[11] M. Ul-Islam, T. Khan, J. K. Park, *Carbohydr. Polym.* **2012**, *88*, 596–603.
[12] D. Klemm, B. Heublein, H.-P. Fink, A. Bohn, *Angew. Chem. Int. Ed.* **2005**, *44*, 3358–3393.
[13] A. Svensson, E. Nicklasson, T. Harrah, B. Panilaitis, D. L. Kaplan, M. Brittberg, P. Gatenholm, *Biomaterials* **2005**, *26*, 419–431.
[14] S. Torgbo, P. Sukyai, *Appl. Mater. Today* **2018**, *11*, 34–49.
[15] M. Fürsatz, M. Skog, P. Sivlér, E. Palm, C. Aronsson, A. Skallberg, G. Greczynski, H. Khalaf, T. Bengtsson, D. Aili, *Biomed. Mater.* **2018**, *13*, 25014–25025.
[16] J. Kucińska-Lipka, I. Gubanska, H. Janik, *Polym. Bull.* **2015**, *72*, 2399–2419.
[17] I. Sulaeva, U. Henniges, T. Rosenau, A. Potthast, *Biotechnol. Adv.* **2015**, *33*, 1547–1571.
[18] R. A. A. Muzzarelli, C. Muzzarelli, in *Polysacch. I* (Ed.: T. Heinze), Springer-Verlag, Berlin/Heidelberg, **2005**, pp. 151–209.
[19] M. N. V. R. Kumar, R. A. A. Muzzarelli, C. Muzzarelli, H. Sashiwa, A. J. Domb, *Chem. Rev.* **2004**, *104*, 6017–6084.
[20] Q. Li, X. Wang, X. Lou, H. Yuan, H. Tu, B. Li, Y. Zhang, *Carbohydr. Polym.* **2015**, *130*, 166–174.
[21] R. A. A. Muzzarelli, *Carbohydr. Polym.* **2009**, *77*, 1–9.
[22] D. R. Rohindra, A. V. Nand, J. R. Khurma, *S. Pac. J. Nat. Sci.* **2004**, *22*, 32–35.
[23] J. R. Khurma, D. R. Rohindra, A. V. Nand, *Polym. Bull.* **2005**, *54*, 195–204.
[24] Y. Jia, X. Wang, M. Huo, X. Zhai, F. Li, C. Zhong, *Nanomater. Nanotechnol.* **2017**, *7*, 1–8.
[25] M. A. Johns, Y. Bae, F. E. G. Guimarães, E. M. Lanzoni, C. A. R. Costa, P. M. Murray, C. Deneke, F. Galembeck, J. L. Scott, R. I. Sharma, *ACS Omega* **2018**, *3*, 937–945.
[26] F. Wahid, X.-H. Hu, L.-Q. Chu, S.-R. Jia, Y.-Y. Xie, C. Zhong, *Int. J. Biol. Macromol.* **2019**, *122*, 380–387.
[27] H. J. Kim, J. N. Jin, E. Kan, K. J. Kim, S. H. Lee, *Biotechnol. Bioprocess Eng.* **2017**, *22*, 89–94.
[28] S. Papadimitriou, D. Bikiaris, K. Avgoustakis, E. Karavas, M. Georgarakis, *Carbohydr. Polym.* **2008**, *73*, 44–54.

[29] J. Liang, R. Wang, R. Chen, *Polymers* **2019**, *11*, 491–505.

[30] P. Aramwit, T. Siritienthong, T. Srichana, J. Ratanavaraporn, *Cells Tissues Organs* **2013**, *197*, 224–238.

[31] K. Pal, A. T. Paulson, D. Rousseau, in *Handb. Biopolym. Biodegrad. Plast.*, Elsevier, **2013**, pp. 329–363.

[32] H. M. C. De Azeredo, M. F. Rosa, M. De Sá, M. Souza Filho, K. W. Waldron, in *Adv. Biorefineries*, Elsevier, **2014**, pp. 819–874.

[33] A. N. B. Romainor, S. F. Chin, S. C. Pang, L. M. Bilung, *J. Nanomater.* **2014**, *2014*, 1–10.

[34] M. Akhlaq, F. Maryam, A. Elaissari, H. Ullah, M. Adeel, A. Hussain, M. Ramzan, O. Ullah, M. Zeeshan Danish, S. Iftikhar, *Drug Deliv.* **2018**, *25*, 916–927.

[35] M. Taskeen, M. Tirunagari, Husna. K. Qureshi, *ACTA Pharm. Sci.* **2017**, *55*, 21–37.

[36] S. Yano, H. Maeda, M. Nakajima, T. Hagiwara, T. Sawaguchi, *Cellulose* **2008**, *15*, 111–120.

[37] R. R. Mohamed, R. S. Seoudi, Magdy. W. Sabaa, *Cellulose* **2012**, *19*, 947–958.

[38] H. M. C. Azeredo, H. Barud, C. S. Farinas, V. M. Vasconcellos, A. M. Claro, *Front. Sustain. Food Syst.* **2019**, *3*, 1–14.

[39] A. F. S. Costa, F. C. G. Almeida, G. M. Vinhas, L. A. Sarubbo, *Front. Microbiol.* **2017**, *8*, 1–12.

[40] B. Lindman, B. Medronho, L. Alves, C. Costa, H. Edlund, M. Norgren, *Phys. Chem. Chem. Phys.* **2017**, *19*, 23704–23718.

[41] C. N. Costa, V. G. Teixeira, M. C. Delpech, J. V. S. Souza, M. A. S. Costa, *Carbohydr. Polym.* **2015**, *133*, 245–250.

[42] T. Wang, S. Gunasekaran, *J. Appl. Polym. Sci.* **2006**, *101*, 3227–3232.

[43] A. S. Hoffman, *Adv. Drug Deliv. Rev.* **2002**, *54*, 3–12.

[44] M. L. Bruschi, Elsevier, **2015**, pp. 63–86.

[45] R. Gouda, H. Baishya, Z. Qing, *J. Dev. Drugs* **2017**, *6*, 1–8.

[46] N. A. Peppas, J. J. Sahlin, *Int. J. Pharm.* **1989**, *57*, 169–172.

[47] R. . B. Baggi, N. . B. Kilaru, *Asian J. Pharm. Technol.* **2016**, *6*, 223–230.

[48] L. Mengatto, M. G. Ferreyra, A. Rubiolo, I. Rintoul, J. Luna, *Mater. Chem. Phys.* **2013**, *139*, 181–186.

[49] P. Costa, J. M. Sousa Lobo, *Eur. J. Pharm. Sci.* **2001**, *13*, 123–133.

[50] P. L. Ritger, N. A. Peppas, *J. Controlled Release* **1987**, *5*, 23–36.

[51] D. Sahu, *Pharm. Sin.* **2010**, *1*, 48–57.

[52] G. Z. Kyzas, D. N. Bikiaris, N. K. Lazaridis, *Langmuir* **2008**, *24*, 4791–4799.