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Gelatin-Carbohydrate Phase-Separated Hydrogels as Bioactive Carriers in Vaginal Delivery: Preparation and Physical Characterizations

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ABSTRACT: The current study focuses on the alteration of properties of the gelatin hydrogels using polysaccharides (e.g., maltodextrin, dextran, and sodium carboxymethyl cellulose) for probable use in vaginal delivery of antimicrobials. The hydrogels were prepared by varying the proportions of gelatin and polysaccharides and were characterized by microscopy, mechanical testing, and impedance spectroscopy. Metronidazole (MZ), drug of choice for the treatment of bacterial vaginosis, was incorporated within the hydrogels. *In vitro* release studies of MZ from the hydrogels was studied in-depth using modified Franz's diffusion cell. Antimicrobial efficiency of the MZ-loaded hydrogels was tested against *E. coli* and *B. subtilis*. The results suggested that the incorporation of polysaccharides resulted in the phase-separated hydrogels. The properties of the hydrogels was found be suitable for vaginal delivery. The drug release and antimicrobial efficiency from the hydrogels suggested that the developed hydrogels may be used for the delivery of antimicrobials in the vaginal lumen. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40445.

KEYWORDS: biomaterials; dielectric properties; differential scanning calorimetry (DSC); drug delivery systems; gels

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INTRODUCTION

Bacterial vaginosis (BV) is a pathological condition which is associated with the reduction in the vaginal lactobacilli count, responsible for maintaining the vaginal health, and a subsequent polymicrobial anaerobic overgrowth. Lactobacilli help in maintaining the vaginal pH at ~4.5.[1] The overgrowth of the pathogenic microbes increases the pH of the vaginal lumen, which further complicates the microenvironment of the vagina. BV is a very common disease in women who are sexually active. In many developing countries, the occurrence of BV in women in the reproductive age has been reported to be up to 50%. In pregnant women, if the treatment of BV is not done at an early stage there are chances of preterm birth and in some cases fetal mortality.[2] The local administration of the drugs is a great challenge due to the increased vaginal discharge during BV.[3] This reduces the residence time and the bioavailability of the drugs when administered locally as there is an increased wash-off of the drugs. The residence time of the drugs in the vagina may be improved by delivering the drugs in the vagina using hydrogels.[4,5] This is due to the mucoadhesive property of the hydrogels, which increases the residence time of the drugs in the vagina and hence allows maintaining the concentration of the drug at the site for prolonged periods.

The current study deals with the development of gelatin-polysaccharide based phase-separated hydrogels. The phase-separation happens due to the thermodynamic instability amongst the gelatin and polysaccharide molecules. Because both the phases are aqueous, the gelatin-polysaccharide systems are often regarded as water-in-water emulsions. Gelatin is a protein obtained from the partial hydrolysis of collagen.[6] Because of its unique chemical and physical properties and inherent biocompatibility, gelatin has been widely studied in pharmaceutical, cosmetic, and food industries.[7] Model polysaccharides (e.g., sodium carboxymethyl cellulose (SC), maltodextrin (MD), and dextran (DX)) were used for the preparation of phase-separated hydrogels. SC is an anionic water soluble polymer derived from cellulose. It shows pseudoplastic and thixotropic behavior when dissolved in water and hence has been used to develop self-emulsifying oil-in-water formulations with improved stability. It is also commonly used as food stabilizer and thickener.[8] MD is

Additional Supporting Information may be found in the online version of this article.

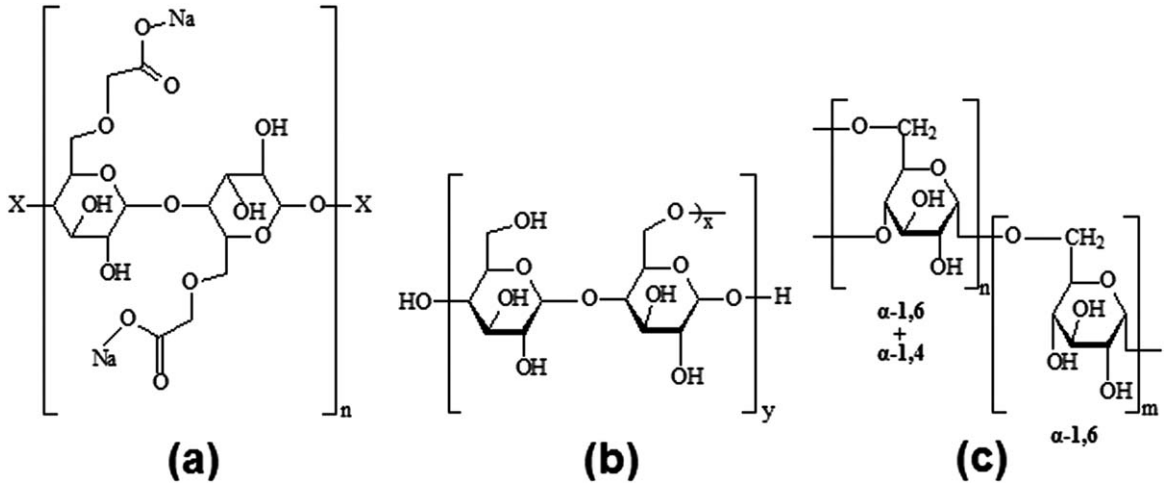

Figure 1. Chemical structure of (a) SC, (b) MD and, (c) DX.

the conversion product obtained by partial hydrolysis of corn starch and is primarily used as a food additive.[9] DX is chemically α-D-(1→6)-linked glucan produced by certain lactic acid producing bacteria. It has the capability to absorb large amount of water to form colloidal solutions with thixotropic nature.[10] DX have also been used for targeted and sustained delivery of drugs, proteins and enzymes.[11] These polysaccharides have been extensively studied for developing pharmaceutical and food formulations. The effect of the variation in the proportions of the polysaccharides on the properties of the hydrogels was studied in details. MD based phase-separated hydrogels have been studied well, but no reports have been found to develop phase-separated hydrogels using SC and DX. SC has a linear cellulosic backbone with the presence of carboxymethyl groups as pendants. Hyperbranching in MD is highest followed by DX and SC, respectively (Figure 1). The current study was designed to develop SC and DX based phase-separated hydrogels and compare the results with the MD based phase-separated hydrogels. The study was done to understand the effect of increase in branching of the cellulosic backbone on the properties of the hydrogels. The hydrogels prepared using gelatin alone have been regarded as GH whereas the hydrogels prepared from gelatin- SC, gelatin-MD and gelatin-DX were regarded as GS, GM, and GD, respectively. The prepared hydrogels were tested as vehicles for antimicrobial drug delivery so as to ascertain their ability to be used for vaginal drug delivery. Metronidazole (MZ) was used as a model antimicrobial agent. MZ is a drug of choice for the treatment of BV.

EXPERIMENTAL

Materials

Gelatin (GH), maltodextrin (MD), and dextran (DX) were purchased from Himedia, Mumbai, India. Sodium carboxy methyl cellulose (SC) was brought from RFCL, New Delhi. Trisodium citrate was procured from Loba Chemie, Mumbai, India. Nutrient agar and dialysis tubing (MW cutoff: 60 kDa) were purchased from Himedia, Mumbai, India. Microbial cultures of *Bacillus subtilis* (NCIM 2699) and *Escheratia coli* (NCIM 2563) were obtained from NCIM, Pune, India. Metronidazole (MZ) was a kind gift from Aarti drugs, India. Hydrochloric acid (HCl) was purchased from Merck Specialist, Mumbai, India. Ethanol was purchased from Honyon International, Hong Yang Chemical Corporation, China. Goat blood was collected from the local butcher shop. Double distilled water (DW) was used to carry out the experiment.

Methods

Preparation of Physical Gels. The phase-separated hydrogels were prepared by varying the proportions of the polysaccharides (SC, MD and DX) sols and gelatin solution (Table I).

Gelatin stock solution was prepared by dissolving 20 g of gelatin powder in 70 g of DW, maintained at ~50°C. The final weight was made to 100 g using warm DW. Polysaccharide sol was prepared by dispersing 2 g of the polysaccharide (SC, DX, or MD) in 90 g of DW, maintained at ~50°C. The final weight was made to 100 g using warm DW. The phase-separated hydrogels were prepared by mixing the warm solution of gelatin and warm polysaccharide sol in varied proportions. The mixture was homogenized using an overhead stirrer (Remi, Model RQ-126/D) at 500 rpm for 10 min. The temperature during homogenization was maintained at 50°C. The homogenized mixtures

Table I. Composition of the Hydrogels

		Volume (mL)			
S. no.	Sample code	Gelatin solution	SC sol	MD sol	DX sol
1	GH	10	0	-	-
2	GS1	8	2	-	-
3	GS2	6	4	-	-
4	GS3	4	6	-	-
5	GM1	8	-	2	-
6	GM2	6	-	4	-
7	GM3	4	-	6	-
8	GD1	8	-	-	2
9	GD2	6	-	-	4
10	GD3	4	-	-	6

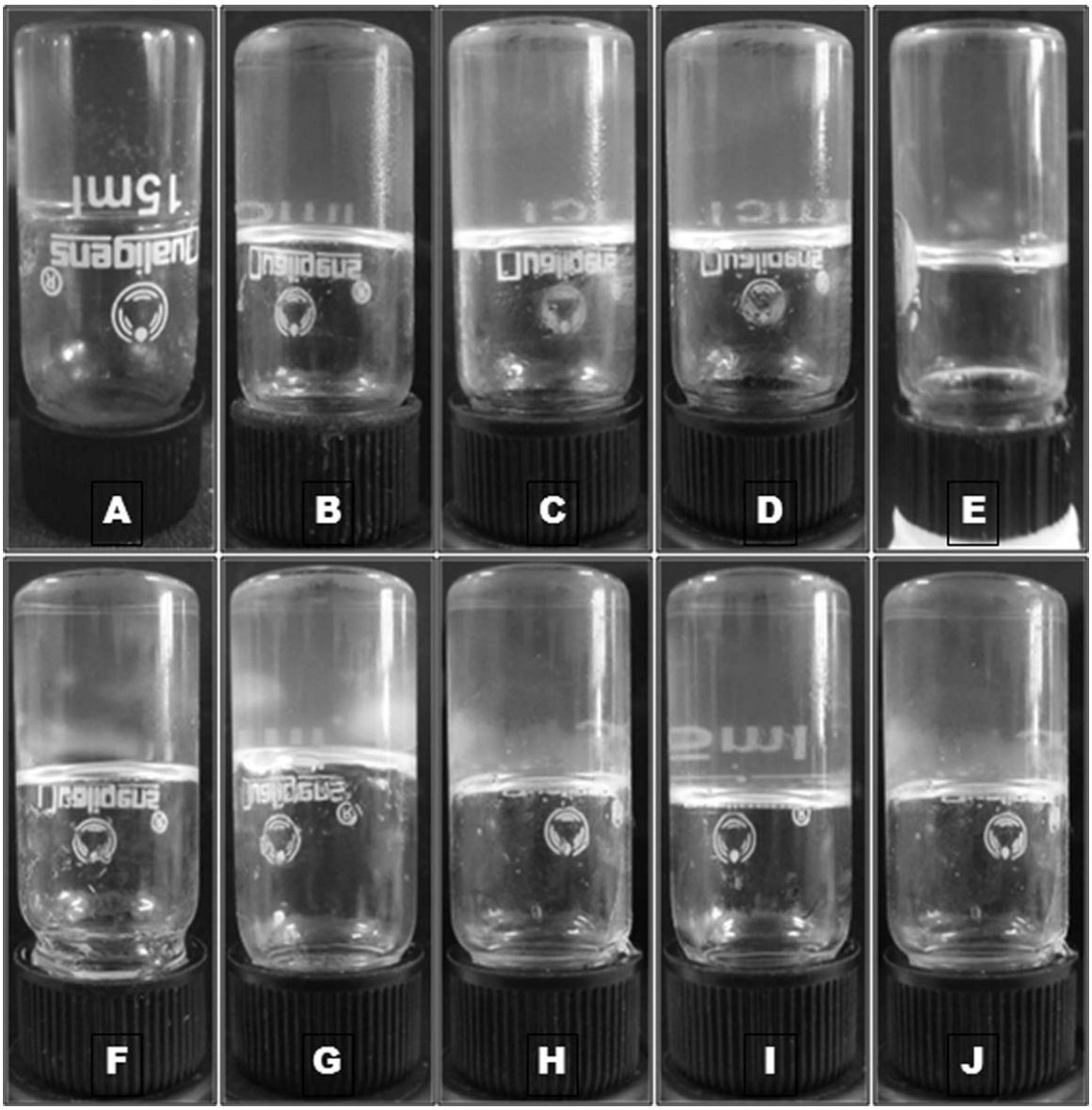

Figure 2. Confirmation of hydrogel formation by inverting tube method. (a) GH, (b) GS1, (c) GS2, (d) GS3, (e) GM1, (f) GM2, (g) GM3, (h) GD1, (i) GD2, and (j) GD3.

were then allowed to cool for 30 min in a temperature controlled cabinet, maintained at 4°C ± 1°C, to induce gellation of the liquid mixtures. The prepared hydrogels were stored in refrigerator for further analysis. About 1% (w/w) MZ drug loaded samples were also prepared in the similar manner. The biocompatibility of the hydrogels was tested by hemocompatibility test.[12] In short, 0.5 mL of leachant of the gels was mixed with 0.5 mL of diluted blood which is referred as test sample. The positive control was prepared by mixing 0.5 mL of diluted blood with 0.5 mL of 0.01*N* HCl. However, negative control was prepared by mixing 0.5 mL of diluted blood with 0.5 mL of normal saline. All the test samples, including controls, were diluted to 10 mL with normal saline and kept for incubation at 37°C for 1 h. After 1 h of incubation, the samples were centrifuged at 3000 rpm for 15 min and the absorption of the supernatant was determined using UV–visible double beam spectrophotometer (UV-3200, LABINDIA, Mumbai, India) at 545 nm. The percentage hemolysis of the RBCs was calculated by using the formula.[13,14]

$$\%\,\text{Hemolysis} = \frac{OD_{test} - OD_{Negative}}{OD_{positive} - OD_{Negative}} \times 100$$

where,

OD_{test}—Absorbance of the test sample

$OD_{positive}$—Absorbance of the positive control

$OD_{negative}$—Absorbance of the negative control

The temporal stability of the developed hydrogels was determined by storing the samples at 5°C for 12 months.

Microscopic Studies. The microstructure of the hydrogels was studied using phase contrast microscopy (PCM; Carl Zeiss,

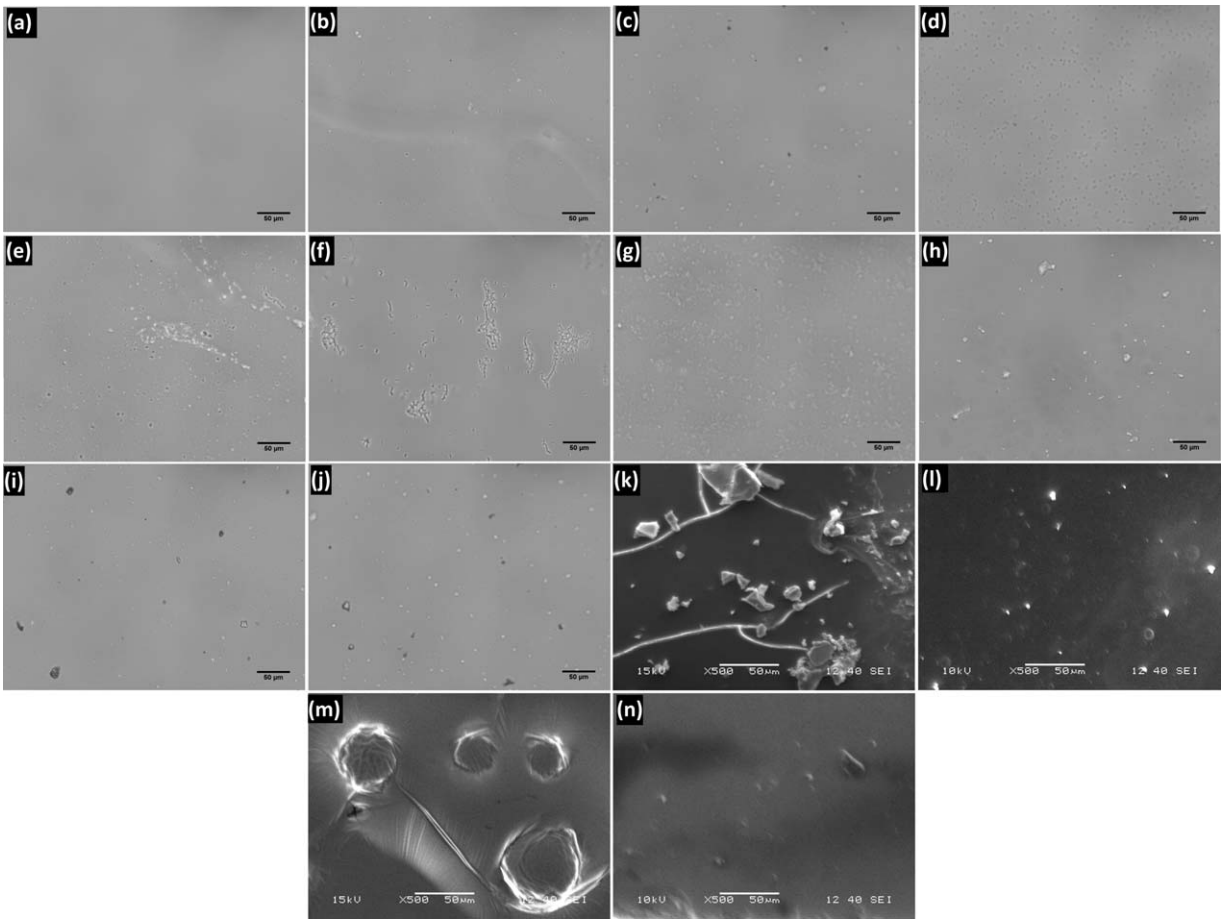

Figure 3. Micrographs of the hydrogels. Phase contrast micrographs of: (a) GH, (b) GS1, (c) GS2, (d) GS3, (e) GM1, (f) GM2, (g) GM3, (h) GD1, (i) GD2, and (j) GD3; and Scanning electron micrographs of: (k) GH (l) GS2, (m) GM2, and (n) GD2.

Model HBO 50, Germany).[15,16] The hydrogels were also analyzed using scanning electron microscopy (SEM; JEOL, JSM-6390, Japan). The hydrogels were converted to xerogel and were subsequently sputter coated with platinum before analyzing under scanning electron microscope.[17]

Mechanical Properties. The mechanical properties of the hydrogels were studied using a texture analyzer (Stable Microsystems, TA-HD plus, UK).[18] Freshly prepared hydrogels were used for measuring the mechanical properties (gel strength, stress relaxation, and spreadability) of the hydrogels (Supporting Information Table SI). The gel strength parameters (hardness, brittleness, and rigidity) were determined using a 3 mm SS cylindrical probe.[19] The study was conducted at 4°C. A 45° perpex conical probe (spreadability fixture) was used for stress relaxation (SR) and spreadability studies of the hydrogels.[18] The compressive strength of the hydrogels was determined using samples molded in cylindrical shapes (height 2 cm; diameter 1.5 cm).[20]

Thermal Properties. The melting point of the developed hydrogels was determined by the falling ball method as per the reported literature.[21] Nearly 2 g of the molten hydrogels were transferred in 10 mL test tubes. The test tubes were incubated in a temperature controlled thermal cabinet, maintained at 4°C, for 30 min. A stainless steel ball (weight: 110 mg, diameter: 1 mm) was put on the surface of the hydrogels. The samples were subsequently heated at a rate of 1°C min^{-1} in a temperature controlled oil-bath. The temperature at which the ball reached the bottom of the test tubes was regarded as melting point (T_m) of the hydrogels.

The thermal profiles of the selected hydrogels were analyzed using differential scanning calorimeter (DSC-200 F3 Maia, Netzsch, Germany). GH, GS2, GM2, and GD2 were selected as the representative hydrogels for thermal analysis. Accurately weighed (~15 mg) of the hydrogels were sealed in aluminum pans with pierced lids. The thermal properties were studied in the temperature range of 20–150°C at a scan rate of 2°C min^{-1} under N_2 environment.[22]

Electrochemical Impedance Spectroscopy. The electrochemical properties of the hydrogels were studied using a computer controlled impedance analyzer (PSM 1735, Numetriq, UK). All the measurements were done by injecting an AC voltage of 100 mV in the frequency range of 0.1 Hz–1.0 MHz at RT.[23]

In Vitro Drug Release Studies. The objective of this test was to study the release pattern of MZ from the drug loaded hydrogels. The study was carried out in a modified Franz's diffusion cell as

Table II. Textural Properties of the Hydrogels

Formulations	Gel strength			Stress relaxation	Spreadability		Compressive strength	
	Hardness (g)	Brittleness (mm)	Rigidity (g mm^{-1})	% SR	Firmness (kg)	Cohesiveness (kg s^{-1})	Maximum load (kg)	Work of shear (kg s^{-1})
GH	279.5 ± 41.2	0.150 ± 0.004	41.78 ± 1.79	19.79 ± 0.81	8.45 ± 0.34	16.08 ± 0.42	4.31 ± 0.17	19.58 ± 0.86
GS1	82.8 ± 3.12	0.113 ± 0.002	9.39 ± 0.42	17.79 ± 0.69	6.09 ± 0.21	11.52 ± 0.43	4.19 ± 0.19	15.44 ± 0.67
GS2	54.4 ± 2.61	0.125 ± 0.007	6.79 ± 0.34	17.91 ± 0.76	2.57 ± 0.11	3.72 ± 0.10	2.11 ± 0.07	6.08 ± 0.25
GS3	46.6 ± 2.06	0.120 ± 0.002	5.63 ± 0.26	74.70 ± 2.63	1.27 ± 0.09	1.95 ± 0.06	1.86 ± 0.04	5.03 ± 0.22
GM1	37.7 ± 1.96	0.138 ± 0.008	5.22 ± 0.21	17.83 ± 3.12	6.67 ± 0.26	13.22 ± 0.51	1.98 ± 0.06	8.46 ± 0.28
GM2	25.3 ± 1.16	0.127 ± 0.005	3.23 ± 0.14	17.62 ± 0.82	3.29 ± 0.16	4.49 ± 0.19	1.40 ± 0.02	4.38 ± 0.21
GM3	11.3 ± 0.42	0.133 ± 0.004	1.51 ± 0.11	18.00 ± 0.93	2.43 ± 0.09	5.91 ± 0.39	0.79 ± 0.03	2.26 ± 0.11
GD1	57.3 ± 2.57	0.156 ± 0.003	8.92 ± 0.37	16.39 ± 0.58	6.16 ± 0.29	10.36 ± 0.49	3.75 ± 0.11	16.35 ± 0.48
GD2	34.8 ± 1.68	0.170 ± 0.006	5.92 ± 0.34	6.84 ± 0.37	4.47 ± 0.18	8.67 ± 0.32	2.14 ± 0.08	8.09 ± 0.37
GD3	11.7 ± 0.39	0.147 ± 0.002	1.72 ± 0.12	47.12 ± 2.18	2.80 ± 0.12	5.26 ± 0.28	0.50 ± 0.01	1.80 ± 0.05

per the reported literature.[24] In short, accurately weighed (~1.5 g) MZ loaded hydrogels were placed in the donor. The receptor contained 50 mL of DW, maintained at 37°C ±1°C and kept under stirring at 100 ± 2 rpm using a magnetic stirrer. The donor was lowered so as to touch the receptor fluid. The contents of the donor and the receptor were separated by the previously activated dialysis membrane. The contents of the receptor were changed with fresh DW at regular intervals of time. The replaced receptor fluid was tested for the drug, to estimate the amount of the drug released, at 321 nm using double beam UV–visible spectrophotometer (UV-3200, LABINDIA, Mumbai, India).[25] The study was carried out for 8 h. Cumulative percent drug release (CPDR) was calculated from the release profile. The release mechanism for MZ was predicted by fitting various release kinetic models [e.g., zero order, first order, Higuchian kinetics, and Korsameyer–Peppas (KP)].[26]

The release of the drug was also evaluated by performing antimicrobial studies. The antibacterial activities of the drug loaded hydrogels were determined against *Bacillus subtilis* and *Escherichia coli* using a bore-well method.[27] Nutrient agar medium was used as the growth medium for the culture of bacteria. About 1 mL of suspension culture (10^6-10^7 cfu mL^{-1}) was spread over the solid nutrient agar medium. The efficiency of the MZ loaded hydrogels were compared with Metrogyl®, a marketed MZ gel. Disc containing marketed gel (1% MZ) served as the positive control while blank sample served as negative control. The petri-plates were kept for incubation at 37°C for 24 h. The zone of inhibition was measured at the end of 24 h using ruler.[24]

RESULTS AND DISCUSSIONS

Preparation of Physical Hydrogels

The formation of phase-separated hydrogels was confirmed by inverted tube method (Figure 2). The samples were found to be transparent. The transparency of the samples decreased with the decrease in the concentration of gelatin. All the hydrogels were homogenous and had a smooth texture. The color of the hydrogels varied from light brown to brownish-white. The intensity of the brown color increased with the increase in the gelatin concentration. With the increase of the polysaccharide proportion, there was an increase in the whitish tinge in the hydrogels. This may be due to the white color associated with the polysaccharides. The hydrogels were odorless and had a soothing effect when placed in contact with the skin. The hydrogels had a bland taste. The pHs of hydrogels were in the range of 5.0 to 6.0. The hemocompatibility test results have indicated that the developed phase-separated hydrogels were highly hemocompatible in nature. The developed hydrogels have shown good temporal stability till 12 months. There were no significant visual and mechanical changes in the sample properties upon keeping for 12 months.

Microscopic Studies

Bright field microscopy was unable to provide information about the structural organization of the hydrogels. PCM micrographs (as observed under 40× magnification) of the polysaccharide containing hydrogels have shown presence of uniformly dispersed spherical droplets throughout the hydrogel matrices [Figure 3(a–j)]. This observation may be associated to the

Table III. Thermal Properties of the Hydrogels

Formulations	Melting point (°C)	DSC parameters		
		Peak (°C)	ΔH_m (J g^{-1})	ΔS_m (J g^{-1} K^{-1})
GH	29.4 ± 0.31	104.8	1971	9530.235
GS1	25.2 ± 0.54	-	-	-
GS2	27.5 ± 0.26	110.6	1764	8556.844
GS3	29.1 ± 0.23	-	-	-
GM1	19.7 ± 0.18	-	-	-
GM2	18.1 ± 0.22	112.4	2244	10992.08
GM3	16.9 ± 0.41	-	-	-
GD1	20.1 ± 0.27	-	-	-
GD2	19.4 ± 0.41	110.8	1913	9263.209
GD3	17.2 ± 0.51	-	-	-

formation of phase-separated biphasic hydrogels. The occurrence of this type of structures may be attributed to the thermal incompatibility amongst the protein and polysaccharide molecules which results in the formation of water-in-water emulsions.[28] The PCM results were verified by analyzing the selected hydrogels as xerogels under SEM. The micrographs obtained from the SEM [Figure 3(k–n)] also revealed the presence of phase-separated dispersed globules, uniformly arranged in the continuum phase.

Mechanical Properties

The specific properties of the polysaccharides were related to mechanical properties of the phase-separated hydrogels. SC consists of a pendant carboxymethyl group attached to —CH_2OH group of glucopyranose backbone with an ether linkage.[29] The molecular weight of SC is 70–700 kDa.[30] MD is a nonsweet nutritive polysaccharide of D-glucose units with a dextrose equivalent <20 and is primarily used as a food additive. The D-glucose units are linked primarily via 1–4 bonds.[31] The molecular weight of MD is 5–35 kDa.[9] DX is chemically α-D-(1→6)-linked glucan produced by certain lactic acid producing bacteria. It is a homopolysaccharide primarily composed of glucopyranose units (Figure 1). The degree of branching is ~5%. The branches are mostly 1–2 glucose units long. The molecular weight of DX vary distantly, but the DX having MW <100 kDa has only been used as food additive.[32]

The results of the mechanical tests have been compiled in Table II (Supporting Information Figure S1–S4). The maximum force (F_m) required to rupture the hydrogel surface is regarded as the hardness of the hydrogels and the inverse of the distance travelled (D_m) by the probe to rupture the surface is regarded as the brittleness of the hydrogels. The rigidity of the hydrogels is dependent on both hardness and brittleness and is defined as the ratio of F_m : D_m.[33] The hardness of the phase-separated hydrogels was in the order of GS > GD > GM when the gelatin: polysaccharide ratio was same. The hardness of the GH hydrogel was much higher as compared to the phase-separated

Table IV. Release Studies and Antimicrobial Efficacy of the Drug Loaded Hydrogels

Formulations	R^2 value				KP model		Zone of inhibition ± SD (cm)	
	Zero order model	First order model	Higuchi model	Best fit	R^2	"n" value	*B. subtilis*	*E. coli*
GHM	0.990	0.939	0.936	Zero order	0.796	1.257	2.0 ± 0.1	2.2 ± 0.1
GS1M	0.980	0.958	0.968	Zero order	0.971	0.511	2.0 ± 0.1	2.3 ± 0.1
GS2M	0.969	0.986	0.990	Higuchi model	0.957	0.45	1.9 ± 0.11	2.2 ± 0.11
GS3M	0.979	0.981	0.983	Higuchi model	0.976	0.491	2.0 ± 0.11	2.1 ± 0.09
GM1M	0.996	0.982	0.991	Zero order	0.993	0.546	2.0 ± 0.1	2.2 ± 0.1
GM2M	0.993	0.988	0.993	Zero order	0.986	0.640	1.8 ± 0.1	1.9 ± 0.1
GM3M	0.957	0.982	0.983	Higuchi model	0.965	0.684	2.3 ± 0.2	2.0 ± 0.2
GD1M	0.984	0.983	0.984	Zero order	0.994	0.612	2.1 ± 0.1	2.0 ± 0.1
GD2M	0.992	0.976	0.955	Zero order	0.997	0.590	2.0 ± 0.2	1.9 ± 0.2
GD3M	0.979	0.974	0.966	Zero order	0.966	0.447	2.1 ± 0.1	2.1 ± 0.1
Negative control	-	-	-	-	-	-	0	0
Metrogyl ®	-	-	-	-	-	-	2.0 ± 0.21	2.0 ± 0.2

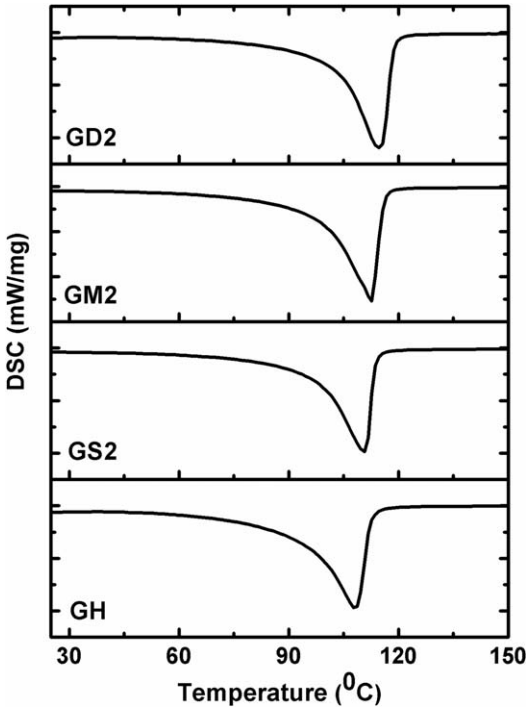

Figure 4. DSC profiles of the selected hydrogels.

hydrogels. The hardness of the hydrogels was lowered as the proportion of gelatin was reduced. In other terms, increase in the polysaccharide concentration reduced the hardness of the hydrogels. This may be attributed to the fact that the strength of the hydrogels is mainly provided by the gelatin molecules. Hence, a reduction in the gelatin concentration resulted in the formation of weaker hydrogels. The brittleness of the hydrogels was in the order of GS> GM > GH > GD. There was a reduction in the brittleness of the hydrogels with the incorporation of polysaccharides. Amongst the polysaccharide containing hydrogels, the brittleness of GD hydrogels was highest followed by GM and GS, respectively (Supporting Information Figure S1). The SR profile provides information about the viscoelastic properties of the materials under study (Supporting Information Figure S2). F_0 (the force after the probe has moved a certain distance) and F_{30} (the force after holding the probe at the said distance for 30 s) were used for the calculation of % SR.[34] In general, % SR increased with the decrease in gelatin concentration and a subsequent increase in the polysaccharide concentration. The results suggested an increase in the spreadability of the hydrogels with the increase in the polysaccharide concentration. The spreadability test provided information about the firmness and cohesiveness of the hydrogels. The maximum force sensed by the probe while penetrating into the female cone is

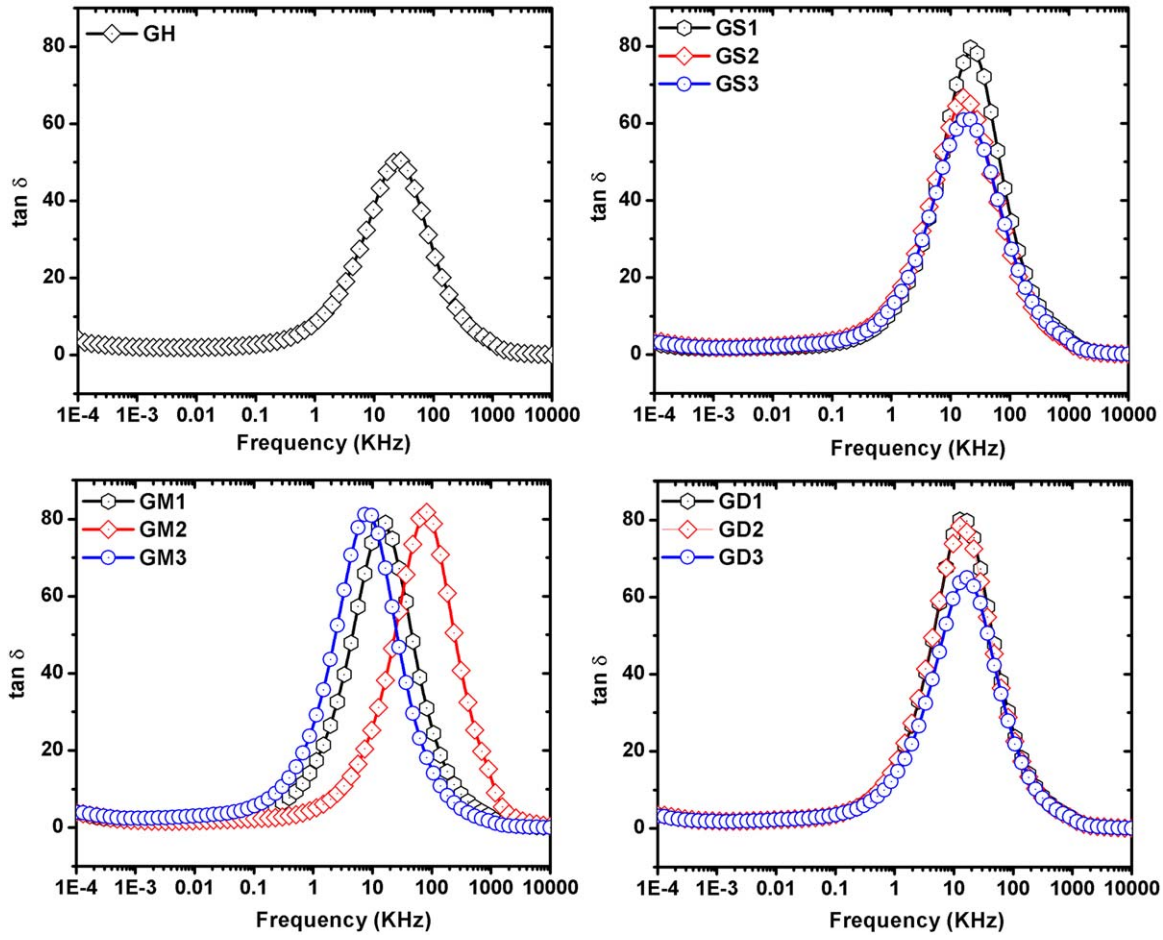

Figure 5. Frequency dependent tangential loss of the hydrogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

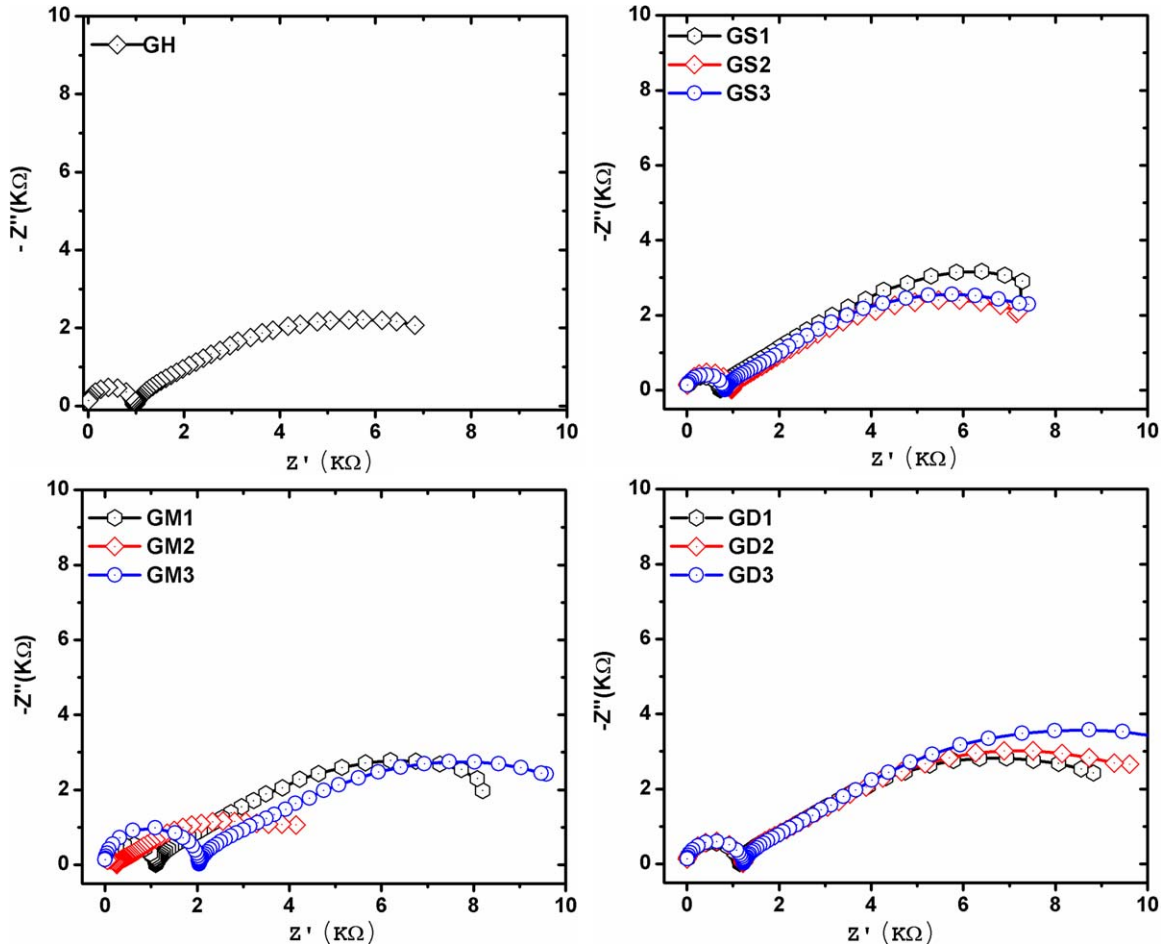

Figure 6. Nyquist plot of the hydrogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

known as the firmness of the hydrogel. The area under the positive peak is defined as the cohesiveness of the hydrogels. GH has shown highest firmness and cohesiveness as compared to the GS, GM, and GD hydrogels (Supporting Information Figure S3). In general, there was a decrease in the firmness and cohesiveness of the hydrogels with the increase in the polysaccharide concentration. The spreadability of the formulations is inversely related to cohesiveness suggesting that incorporation of polysaccharide resulted in the increase in the spreadability of the hydrogels.[35] The maximum compressive strength and work of shear was found to be highest in GH followed by GS, GD, and GM hydrogels, respectively. Similar to the previous results, the compressive study also suggested that the strength of the hydrogels were reduced with the increase in the polysaccharide concentration.

Thermal Properties

The thermal properties of the hydrogels have been tabulated in Table III. The melting points MPs of the hydrogels were in the order of: GH > GS > GD > GM. In general, the MP of the hydrogels decreased with the increase in the concentration of MD and DX. On the other hand, an increase in the SC concentration resulted in the increase in the MP of the GS hydrogels. The results indicated that the molecular interactions amongst the SC and gelatin molecules were higher as compared to DX/MD and gelatin molecules.

The DSC thermogram has been shown in Figure 4. GH has shown a broad endothermic peak at ~105°C. GS, GM and GD hydrogels have shown a slight shift in the endothermic peak toward higher temperature (~110°C) which may be associated with the alterations in the microstructures of the hydrogels at the molecular level. The presence of broad endothermic peak may be attributed to the evaporation of water molecules at the said temperature. ΔH_m (change in enthalpy) and ΔS_m (change in entropy) values were calculated from the area under the endothermic curve (Table IV). ΔH_m and ΔS_m values were found to be in the order of GM2 > GH > GD2 > GS2. The higher ΔH_m and ΔS_m values indicated corresponding higher thermal stability.[36] Hence DSC studies confirmed that GM2 was thermally most stable whereas GS2 was thermally least stable hydrogels amongst the hydrogels evaluated.

Electrochemical Impedance Spectroscopy (EIS)

EIS study was performed to understand the conductivity profile of the developed hydrogels. Conductivity of the hydrogels helps understanding the release profile of the drugs from the gel matrices. Preliminary studies conducted in our lab has shown

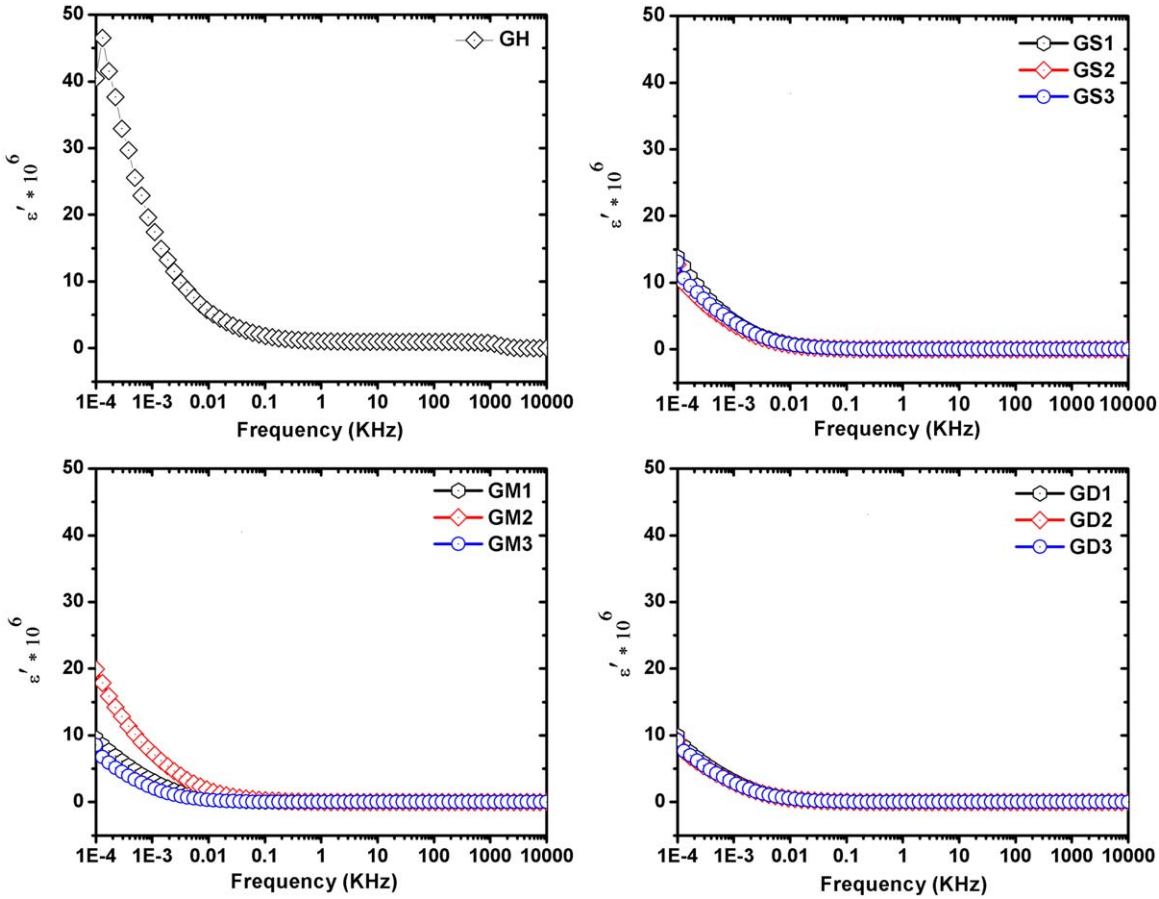

Figure 7. Dielectric constant profiles of the hydrogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

higher the conductivity of the hydrogels, higher was the drug release rate from the gel based formulation. The hydrogel having higher resistance is expected to show lower amount of drug release in a given time period. Hence, to have an understanding about the release rate, EIS studies were conducted. Also, EIS studies help understanding whether a formulation may be used as matrices for iontophoretic drug delivery.

The frequency dependent tangential loss has been shown in Figure 5. GH has shown lowest dielectric loss as compared to the polysaccharide containing hydrogels. All the hydrogels have shown tan δ peaks in the range of 1–10^2 KHz. In general, the dielectric loss profile of GD and GS hydrogels suggested decrease in the dielectric loss profile with the increase in the concentration of the DX and CS polysaccharides and a subsequent increase in the concentration of gelatin. GM hydrogels have shown almost similar loss pattern but loss peak for GM2 was found to be in the frequency range of 10–10^3 KHz. The higher the tangent loss, the higher is the viscoelastic properties. Hence it was confirmed from the loss profile that the formulations containing higher concentration of gelatin possessed higher viscoelastic properties.[37]

The Nyquist plots (Z'' vs. Z') of the hydrogels have been shown in Figure 6. The Nyquist profile suggested formation of two semicircles. The low frequency region semicircle was incomplete due to the measuring limitation of the impedance analyzer used for the measurement. The intersection of the semicircle with the real axis gives the bulk resistance (R_b). The formation of semicircle in the high frequency region may be associated to the bulk resistance and bulk capacitance whereas the low frequency semicircle formation may be attributed to the grain boundary resistance and grain boundary capacitance.[38,39] In general, the R_b of the hydrogels were in the order of GD > GM > GH > GS. The R_b values of the MD based hydrogels were found to be in the order of GM2 < GM1 < GM3. GM1 has shown almost similar R_b value to blank GH. GM2 showed lowest R_b. The change in the composition always does not show a linear trend in the properties of the gels. Quite often, it has been reported that after a critical concentration, a reverse trend in the properties of the gels occurs. Like electrical properties, mechanical properties (reported in section Mechanical properties) of GM gels also showed a similar trend. Similar types of results were also report by Anis et al.[40]

The frequency dependent dielectric constant (ε') of the hydrogels has been shown in Figure 7. The ε' values were found to be higher in the low frequency region and exponentially decreased to a lower constant value above 10^2 Hz as there was an increase in the frequency of the electric current. This phenomenon may be explained due to the deposition of the charges at the sample-electrode interface at low frequencies and quick reversal

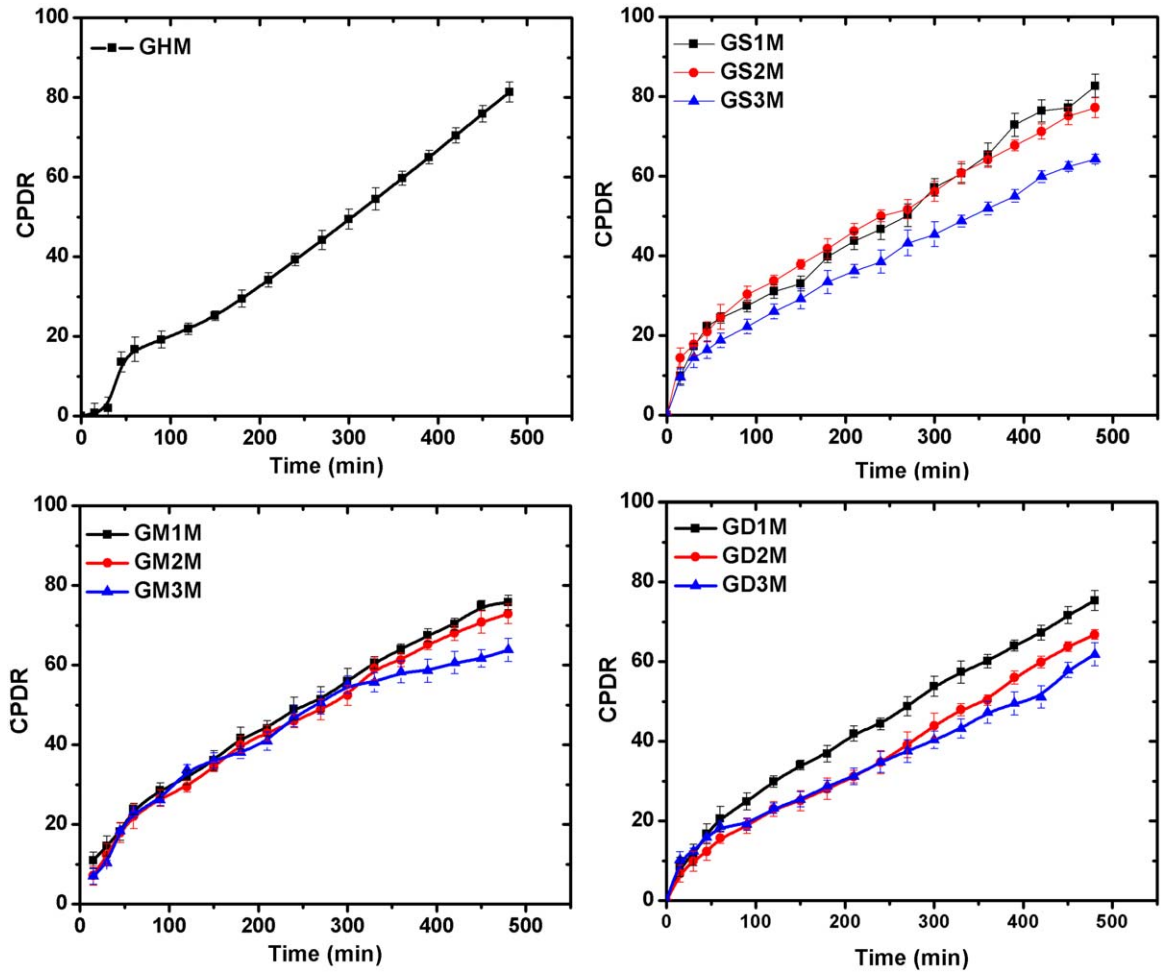

Figure 8. *In vitro* drug release profiles from the drug loaded hydrogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of the electric field across the sample-electrode interface at high frequencies.[39] Similar patterns were observed in all the hydrogels. GS hydrogels have shown higher ε' values as compared to the other hydrogels, with the exception of GM2. In general, there was a decrease in the ε' values with the decrease in the gelatin concentration. GH has shown least ε' value in comparison to the gelatin-polysaccharide phase-separated hydrogels. The results were in exact association to the Nyquist plot and tangential loss results. The ε' profiles of the hydrogels have suggested a capacitive dominant properties of the hydrogels.[18]

In Vitro Drug Release Studies

The release rate was highly dependent on the composition of the hydrogels (Table IV). The GS hydrogels have shown higher drug release as compared to GM and GD hydrogels. The drug release was higher in GH compared to the phase-separated hydrogels. The CPDR was in the range of GS> GH> GM> GD which was directly proportional to the AC conductivity of the hydrogels. In short, the hydrogels with higher conductivity have shown higher drug release. The amount of the drug release was lower in hydrogels with lower proportions of gelatin (Figure 8).

The release of the drug followed either zero-order or Higuchian kinetics. As the proportion of the polysaccharide was increased, there was a tendency for the hydrogels to follow Higuchian release kinetics. The results suggested that the release of MZ, when the proportion of polysaccharide was low, was constant rate process and was independent of concentration of the MZ incorporated within the hydrogels. As the proportion of the polysaccharide was increased in GS and GM, the phase-separated systems behaved as matrix type delivery vehicle. The n-value was predicted using KP model to understand the diffusion pattern of MZ during release process. The n-value >0.45 indicated non-Fickian diffusion of the drugs from the phase-separated hydrogel.[41,42]

The developed hydrogels have shown similar or better antimicrobial efficacy as compared to the marketed formulation against *B. subtilis* whereas the antimicrobial efficacy was similar against *E. coli*.

CONCLUSION

The study reports the successful development of gelatin/polysaccharides based phase-separated hydrogels. The physico-chemical properties of the hydrogels were studied in-depth. The results suggested that the mechanical and electrical properties of the developed hydrogels varied based on the variation of

composition of gelatin/polysaccharide composition. Altering the composition of the hydrogels affected the release mechanism of the incorporated drug molecules. In general, increase in the hyperbranching of the polysaccharides resulted in the decrease in the mechanical properties and lower release rates of the drugs from the matrices. The hydrogels were found to possess smooth texture and were highly hemocompatible (results not shown). Generally during bacterial vaginosis, the pH of the vaginal lumen is >4.5. It is expected that the developed gels will be able to maintain its integrity under bacterial vaginosis conditions as the pH of the gels found to be in between 5.0 and 6.0. The preliminary results suggested that the hydrogels may be used as carriers for controlled drug delivery of MZ in the vaginal lumen, without compromising the antimicrobial activity of the drug, as was evident from the antimicrobial studies.

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