Journal of Applied Polymer Science

Development and characterization of gelatin–polysaccharide based phase-separated hydrogels for prevention of sexually transmitted diseases

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ABSTRACT: Bacterial vaginosis is a sexually transmitted vaginal infection prevalent in sexually active women. Gelatin–polysaccharide phase-separated hydrogels were prepared and characterized as vaginal delivery systems for the treatment of bacterial vaginosis. Sodium carboxymethyl cellulose, maltodextrin, and dextran were used as the representative polysaccharides. The developed gels were characterized by impedance spectrometer, X-ray diffraction (XRD) studies, and differential scanning calorimetry. The mucoadhesivity, water absorption, and hemocompatibility of the gels were also studied. The gels were electrically conductive in nature and showed a concentration-dependent behavior. XRD study suggested the amorphous nature of the hydrogels. An increase in the polysaccharide content increased the water holding capacity of the gels. The gels were found to possess mucoadhesive property and were hemocompatible. Metronidazole, a commonly used drug for treating bacterial vaginosis, loaded gels showed diffusion-mediated drug release. The drug-loaded hydrogels showed good antimicrobial activity. In gist, the developed hydrogels may be tried as matrices for vaginal delivery of antimicrobials. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41785.

KEYWORDS: biocompatibility; differential scanning calorimetry (DSC); drug delivery systems

Received 20 July 2014; accepted 19 November 2014
DOI: 10.1002/app.41785

INTRODUCTION

Sexually transmitted diseases are considered as serious health problem caused by various pathogenic bacteria, parasites, and viruses.[1] There are ~20 types of commonly occurring sexually transmitted diseases, which include trichomoniasis, chlamydia, genital herpes, gonorrhea, syphilis, bacterial vaginosis, and human immunodeficiency virus (HIV). Many of the diseases are treatable, but effective cures are lacking for diseases (not limited to) like HIV, HPV, hepatitis B, and hepatitis C.[2] As per World Health Organization (WHO), >1 million people across the globe acquire sexually transmitted infection every day. Most of these diseases affect both men and women, but in many cases, the health problems are only associated with women.[3] Bacterial vaginosis is one such disease. The disease is often associated with the overgrowth of pathogenic bacterias like *Escherichia coli, Mobiluncus curtisii* and *Gardnerella vaginalis*.[4] Bacterial vaginosis is often identified with a smelly vaginal discharge.[5–7] Metronidazole and clindamycin are the drug of choices in treating bacterial vaginosis.[2,8] Though oral medications are available and are convenient to administer, the oral antimicrobial delivery is associated with many side effects. Local administration of the antimicrobials has been suggested to reduce the side effects of the drugs. But the locally administered drugs (in vagina) are washed away due to the increased vaginal discharge during bacterial vaginosis. Mucoadhesive gel-based formulations are being tried for delivering antimicrobials directly inside the vagina with improved bioavailability.[9–11]

Hydrogels have been defined as polymer matrices which can absorb large quantities of water without disintegration/dissolution.[12–20] The stability of the hydrogels may be increased by crosslinking either with natural crosslinkers (e.g., genipin) or with synthetic crosslinkers (e.g., glutaraldehyde).[21–24] The composition and the concentration of the crosslinkers play an important role in tailoring the physical properties (e.g., swelling and pore size) of the hydrogel matrices.[25,26] This has been greatly explored in designing controlled drug delivery systems.[27] The rate of release of the drugs can be easily tailored simply by altering the composition and the crosslinking density (dependent on the

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crosslinker concentration) of the hydrogel matrices. Incorporation of mucoadhesive polymers into the hydrogels have resulted in developing mucoadhesive drug delivery vehicles. These kinds of (mucoadhesive) hydrogels have been well studied to deliver drugs in areas with increased flow of fluids and movement of the surrounding tissues (as in the cases of vaginal lumen, buccal cavity, and ocular sac). The use of mucoadhesive delivery vehicle lowers the chances of washing off of the drugs and the extrusion of the delivery vehicles from the site of action. Such delivery vehicles have shown improved bioavailability of the drugs at the site of action.

Gelatin (GH, molecular weight: 95 kDa) is a protein-based natural polymer which is derived from the denatured collagen of fish and bovine. It has been extensively studied for designing mucoadhesive hydrogels for controlled delivery applications. Gelatin–polysaccharide blend hydrogels have been well documented. Sodium carboxymethyl cellulose (SCMC, molecular weight: 90 kDa), maltodextrin (MD, molecular weight: 10 kDa), and dextran (DX, molecular weight: 40 kDa) were used as representative polysaccharides in this study. SCMC is an anionic semi-synthetic cellulose derivative.[28] MD is a neutral polysaccharide obtained from enzymatic degradation of the starch molecules.[29] Both SCMC and MD are linear polysaccharides. Dextran is a neutral non-linear polysaccharide.[30] These polysaccharides are abundantly used to develop formulations for pharmaceutical, cosmetic, and food applications. Gelatin–polysaccharide systems have been reported to form phase-separated biphasic hydrogels. This may be explained by the fact that two different phases, external phase is rich in gelatin and the dispersed phase is rich in polysaccharides, are formed.

In a previous study, our group has developed gelatin phase-separated physical hydrogels.[31] The hydrogels were thoroughly characterized for their ability to deliver drugs in vagina. Though the hydrogels showed sufficient properties as vehicles for vaginal drug delivery, the thermal instability of the hydrogels may affect the shelf-life of the formulations. The hydrogels showed gel-to-sol transition at temperatures $< 30°C$. Recent literatures suggest that the suppositories which are solid at body temperature have shown better bioavailability of the drugs.[32] This has been explained by the washing of the drug molecules due to the vaginal discharge, which reduces the bioavailability of the drugs.[33,34] There is an increased occurrence of the vaginal discharge during microbial infection of the vagina due to the protective mechanism of the body. An increase in the vaginal discharge further reduces the bioavailability.

Keeping the above facts in mind, in this study, an attempt was made to develop mucoadhesive gelatin–polysaccharide based hydrogels, with improved thermal stability, for controlled delivery of antimicrobials. An improvement in the thermal stability was achieved by crosslinking the phase-separated hydrogels with glutaraldehyde. The effect of the ionic nature and the branching of the polysaccharides on the electrical, swelling, mucoadhesive, mechanical, and drug diffusion properties have been studied in-depth. The effect of the composition of the hydrogels on the properties of the hydrogels has been studied. The suitability of these hydrogels for vaginal delivery was tested using wash-off test. Metronidazole was incorporated within the prepared hydrogel matrices and tested for their *in vitro* drug release and antimicrobial efficiency.

MATERIALS AND METHODS

Materials

Gelatin, MD, dextran, dialysis tubing (MW cutoff: 60 kDa), and nutrient agar were procured from Himedia, Mumbai, India. SCMC was obtained from RFCL, New Delhi, India. Glutaraldehyde and hydrocholric acid (35% pure) were purchased from Merck Specialties Private Limited, Mumbai, India. Microbial cultures of *Bacillus subtilis* (NCIM 2699) were purchased from NCIM, Pune, India. Metronidazole was obtained as a gift sample from Aarti drugs, India.

Methods

Preparation of Hydrogel. About 20 g of gelatin was dissolved in 70 g of water (50°C), which was made to 100 g by adding water (50°C) in sufficient quantity to prepare 20% (wt/wt) gelatin stock solution. Similarly, 2% (wt/wt) polysaccharide solutions were made. Hydrogels were prepared by varying the compositions of the gelatin and the polysaccharide solutions. The gelatin and the polysaccharide solutions were mixed at 50°C and homogenized using an overhead stirrer at 800 RPM for 15 min. About 1.1 mL of crosslinking solution (0.5 mL glutaraldehyde + 0.5 mL ethanol + 0.1 mL of 0.01*N* hydrochloric acid) was added to the 20 g of the gelatin–polysaccharide blends. The blends were further homogenized for 30 sec, poured into culture bottles/petri-plates and kept at room temperature (25°C) for 1 h to allow crosslinking. Table I shows the compositions of the prepared gelatin–polysaccharide blend hydrogels. The prepared hydrogels were kept under refrigeration for further studies. A 1% (wt/wt) metronidazole (model antimicrobial drug) was dispersed in the gelatin–polysaccharide blends for preparing drug-loaded hydrogels. The rest of the procedure remained same.

The organoleptic properties (e.g., color, texture, and appearance) of the developed hydrogels were observed. The conductivity of the hydrogels was measured using an impedance analyzer (PSM 1735, Numetriq, UK) as per the reported literature.[35]

Microscopic Studies. The surface topologies of the hydrogels were studied under scanning electron microscope (Jeol JSM-6480LV, Japan). The hydrogels were converted into xerogels by incubating the hydrogels at 45°C for 12 h in a hot air over. The xerogels were sputter coated with platinum before analysis.[7,36]

X-ray Diffraction Analysis. The diffraction profile of the hydrogels was obtained using X-ray diffractometer (XRD-PW 1700, Philips, Rockville, USA). The experiment was performed using Cu-Kα as X-ray radiation source in the diffraction angle range of 5°–50° 2θ at a rate of 2° 2θ per min.[37,38]

Test for Mucoadhesion. ***Mucoadhesive strength by wash-off method.*** Wash-off method is one of the commonly used methods to understand the mucoadhesive properties of hydrogels. The method employs USP disintegration apparatus and helps determine the time required to detach the hydrogels from the mucosal surface.[38] The inner surface of the freshly excised and

Table I. Composition of the Hydrogels

Formulations	Gelatin solution (20% wt/wt) (mL)	Polysaccharide solution (2% wt/wt) (mL)			Metronidazole (1%wt/wt)
		SC sol	MD sol	DX sol	
GH	20.0	–	–	–	–
GHM	19.8	–	–	–	0.2
SC1	16.0	4.0	–	–	–
SC1M	15.8	4.0	–	–	0.2
SC2	12.0	8.0	–	–	–
SC2M	11.8	8.0	–	–	0.2
SC3	8.0	12.0	–	–	–
SC3M	7.8	12.0	–	–	0.2
MD1	16.0	–	4.0	–	–
MD1M	15.8	–	4.0	–	0.2
MD2	12.0	–	8.0	–	–
MD2M	11.8	–	8.0	–	0.2
MD3	8.0	–	12.0	–	–
MD3M	7.8	–	12.0	–	0.2
DX1	16.0	–	–	4.0	–
DX1M	15.8	–	–	4.0	0.2
DX2	12.0	–	–	8.0	–
DX2M	11.8	–	–	8.0	0.2
DX3	8.0	–	–	12.0	–
DX3M	7.8	–	–	12.0	0.2

cleaned goat intestine was used as the mucosal surface for the study. The intestinal lumen was longitudinally cut open and attached to a glass slide, such that the mucosal surface is exposed, using acrylate adhesive. Rectangular pieces (10 mm × 10 mm) of the hydrogels were cut and placed on the exposed mucosal layer. 5 g weight was kept over the hydrogels for 5 min. The glass slides were then transferred to the disintegration apparatus, containing 900 mL of phosphate buffer (pH = 7.2) maintained at 37°C.[39] The study was conducted for 24 h and was monitored regularly for the detachment of the hydrogels from the intestinal lumen.[39]

Mucoadhesion by texture analyzer. The mucoadhesive property was further investigated by texture analyzer (Stable Microsystems, TA-HD plus, UK). The hydrogels (3 mm × 3mm) were attached at the surface of the cylindrical probe (30 mm diameter) using double-sided acrylate tape. The goat intestine was longitudinally cut open, cut into rectangular pieces (5 mm × 5 mm) and subsequently attached onto the aluminum platform (base) of the texture analyzer using double-sided acrylate tape such that the mucosal surface was exposed. The probe (attached with hydrogels) exerted a force of 20 g at a rate of 1 mm/sec for 1 min onto the intestinal mucosa. Thereafter, the probe was retracted back and the maximum force required to detach the hydrogels from the mucosal surface was determined.[40]

Gel Strength Studies. The strength of the hydrogels was determined by texture analyzer (Stable Microsystems, TA-HDplus, UK). The study was performed using a 3 mm diameter cylindrical probe under button mode. The pre test and test speed were kept at 1.0 mm/sec and the post test speed was kept at 10.0 mm/sec.

Thermal Properties. The thermal properties of the hydrogels were analyzed using differential scanning calorimeter (DSC-200 F3 Maia, Netzsch, Germany). The hydrogels (~15 mg) were kept in aluminum pans and hermetically sealed. The thermograms were recorded in the temperature range of 20–150°C at a heating rate of 2 °C/min under inert nitrogen environment.[41,42]

Water Uptake Studies. The swelling behavior of the hydrogels was carried out to understand the water uptake ability of the hydrogels. Rectangular pieces of hydrogels (10 mm × 10 mm) were accurately weighted (W_o) and immersed in 100 mL of water. The samples were taken out at regular intervals of time, wiped, and weighed accurately (W_t). The test was conducted until the swelling equilibrium condition was reached. The swelling ratio (SR) was calculated using the formula:[43]

$$SR = \frac{(W_t - W_0)}{W_0}$$

Biocompatibility Studies. The biocompatibility of the hydrogels was evaluated as per the hemocompatibility protocol reported elsewhere.[44] The cytocompatibility of the hydrogels was determined using HaCaT cell line by solvent extraction method. In short, 1 g of the hydrogels was put into the dialysis tubing and was subsequently dipped into 25 mL of phosphate buffer saline (PBS). About 200 μL of the leachate was added to a well of a

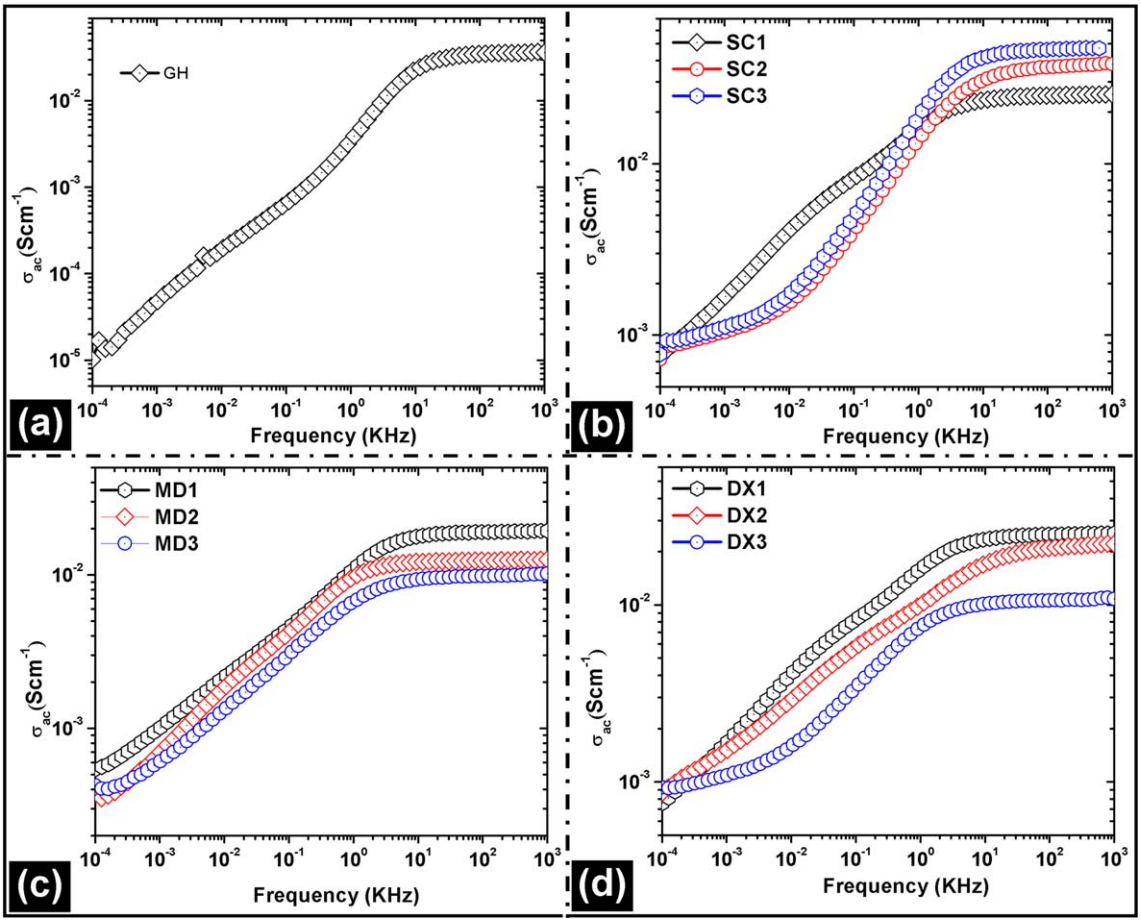

Figure 1. Frequency dependent a.c. conductivity of the hydrogels: (a) GH, (b) SC, (c) MD, and (d) DX. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

96-well plate. The plate was previously seeded with 5×10^4 cells/well and subsequently incubated (37°C, 5% carbon dioxide) for 12 h to allow adherence of the cells. After the addition of the leachate, the plate was further incubated for 48 h. After incubation, the viability of the cells was assessed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.[45,46]

Table II. D.C. Conductivity of the Hydrogels

Formulations	Conductivity (S cm^{-1}) ($\times 10^{-2}$)
GH	2.56
SC1	2.04
SC2	2.77
SC3	3.87
MD1	1.58
MD2	1.12
MD3	0.8
DX1	2.13
DX2	1.69
DX3	0.9

***In Vitro* Drug Release Studies.** The release profile of metronidazole from the hydrogels was studied using 1-basket USP dissolution test apparatus. The hydrogels were cut into rectangular pieces (2 cm × 2 cm), accurately weighed, and put into the dissolution basket containing 900 mL of water (37°C; 100 rpm using paddle). About 5 mL of the aliquot was withdrawn at predetermined regular intervals of time and was replaced with 5 mL of fresh water to maintain sink conditions. The experiment was conducted for 8 h. The collected aliquot were analyzed spectroscopically (UV-3200 Double Beam Spectrophotometer, Labindia Instruments, India) at 321 nm.[47]

Antimicrobial Study. The antibacterial efficacy of the metronidazole-loaded hydrogels was carried out against model gram positive microorganism, *B. subtilis*, according to the reported literature.[48] About 1% metronidazole solution in water and blank hydrogels were taken as a positive and negative control, respectively. The petri-plates were incubated at 37°C for 24 h and the zone of inhibition was measured using a scale.[49]

RESULTS AND DISCUSSIONS

Preparation of Hydrogels

Table I shows the compositions of different gelatin–polysaccharide blend hydrogels. The hydrogels were light golden yellow to dark golden yellow in color depending on the proportions of

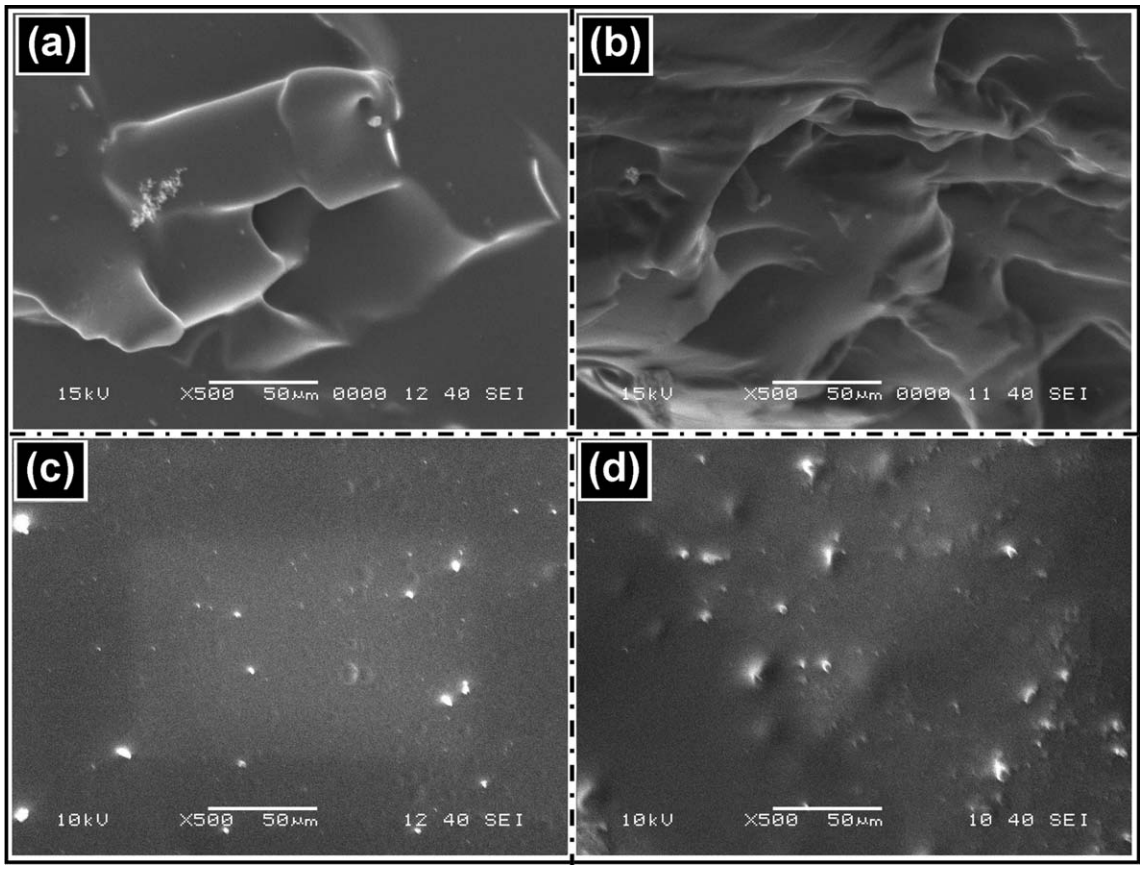

Figure 2. Scanning electron micrographs of representative hydrogels: (a) GH, (b) SC2, (c) MD2, and (d) DX2.

the gelatin present in the hydrogel.[50] The intensity of the yellow color was lowered with the decrease in the gelatin proportion. The hydrogels containing higher polysaccharide content were more transparent.

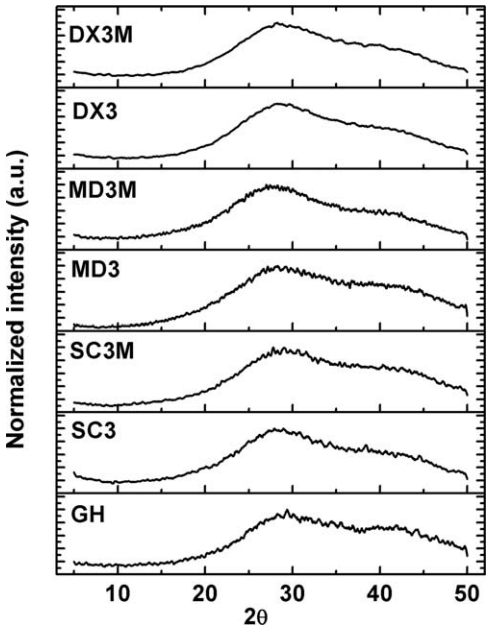

Figure 3. XRD diffractograms of representative hydrogels and their drug-loaded hydrogels: (a) GH, (b) SC2, (c) MD2, and (d) DX2.

The electrical conductivity of any system depends on various factors. The major factors include the amount of moisture present, the polymer concentration, ionic nature of the polymer, and the temperature of the hydrogel matrices.[51] In the current study, the conductive nature of the hydrogels can be attributed mainly to the presence of high amount of moisture within the gelled matrix. The amount of moisture in the gelled system may also affect the mobility of the polymer and the ions within the matrix system.[52] Usually, the amount of moisture is directly proportional to the overall bulk conductivity of the hydrogels.

Table III. Test for Mucoadhesion

Formulations	Mucoadhesion time (h)	Work of adhesion (g cm)
GH	> 24	4.08 ± 0.50
SC1	20.4 ± 0.75	1.84 ± 0.33
SC2	10.2 ± 0.50	0.69 ± 0.29
SC3	9.7 ± 0.50	0.59 ± 0.20
MD1	15.4 ± 0.62	1.34 ± 0.35
MD2	>24	2.57 ± 0.45
MD3	>24	2.94 ± 0.41
DX1	9.1 ± 0.41	0.88 ± 0.30
DX2	9.5 ± 0.34	0.94 ± 0.11
DX3	11.2 ± 0.57	1.13 ± 0.26

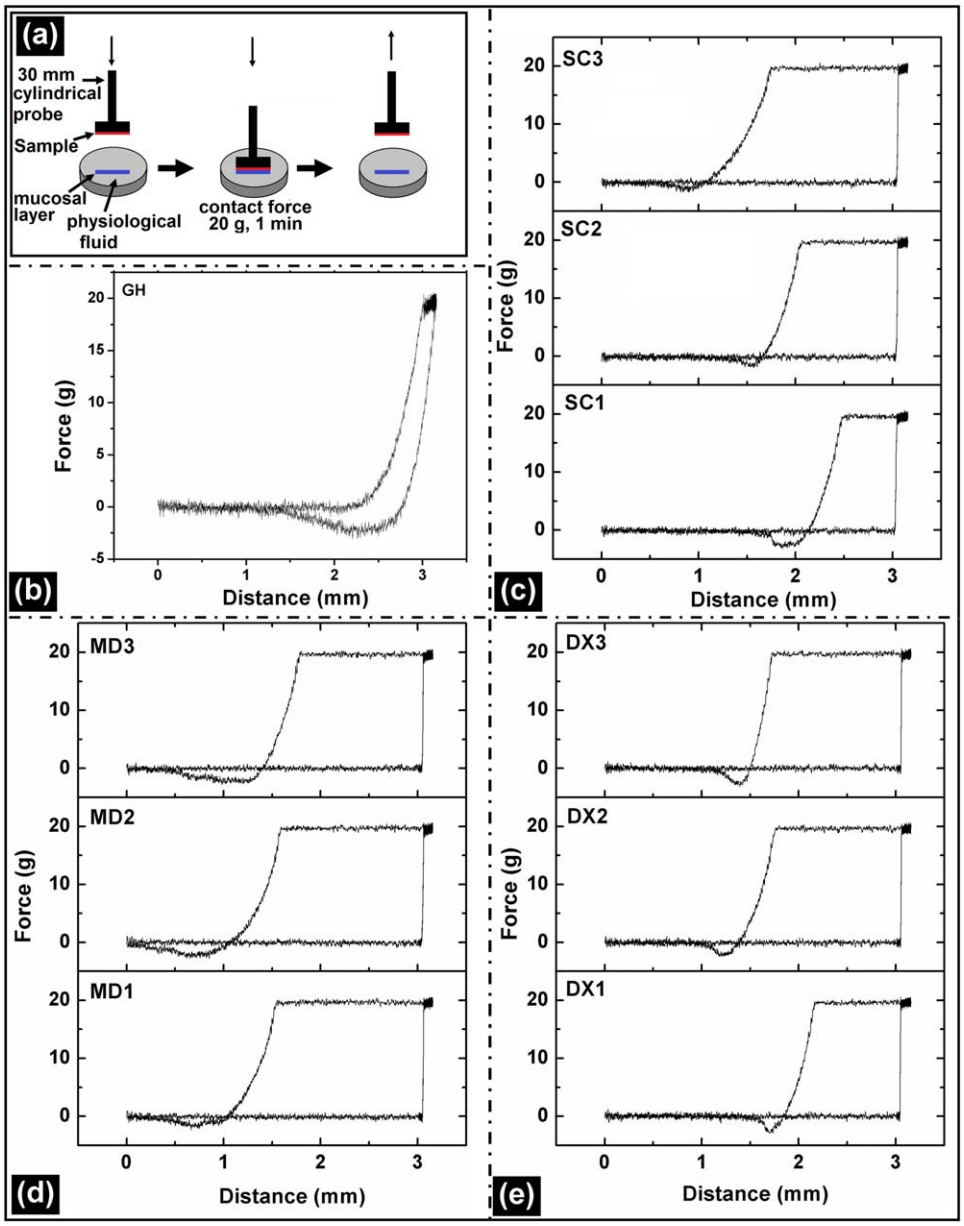

Figure 4. Mucoadhesivity of hydrogels by texture analyzer: (a) pictorial representation of the mucoadhesion test; force–distance graph showing work of adhesion of hydrogels: (b) GH, (c) SC, (d) MD, and (e) DX. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

But, it has been reported recently that after a critical concentration of the moisture, bulk conductivity does not increase.[53] Hence, to have an understanding about the composition of the hydrogel matrices, the electrical conductivity of the prepared hydrogels were analyzed. The conductivity profile of the hydrogels showed the presence of two distinct regions: one being a dispersion region in low frequency and another being an independent plateau in the high frequency region (Figure 1). The dispersion observed in the low frequency region is due to the polarization effects at the electrode and the gel interface.[54] Accumulation of charges occurs at the electrode–hydrogel interface in the low frequency region, commonly known as polarization effect. This resulted in the drop in the conductivity.[55] The low frequency dispersion region showed two distinct linear zones when the concentration of the polysaccharides was high. The slope of the first linear zone was lower as compared to the slope of the second linear zone. At low concentrations of polysaccharides (e.g., SC1, MD1, DX1) only one dispersion region was observed. Gelatin only gels also showed one dispersion region. This indicated that an increase in the polysaccharide content altered the microstructure of the hydrogels to a great extent which, in turn, was responsible for the altered conductivity profile. The frequency independent plateau gives indication about the true conductivity of the hydrogels. This is due to the fact that at high frequencies the polarization effect gets minimized (or is completely absent).[56] The results suggested that the

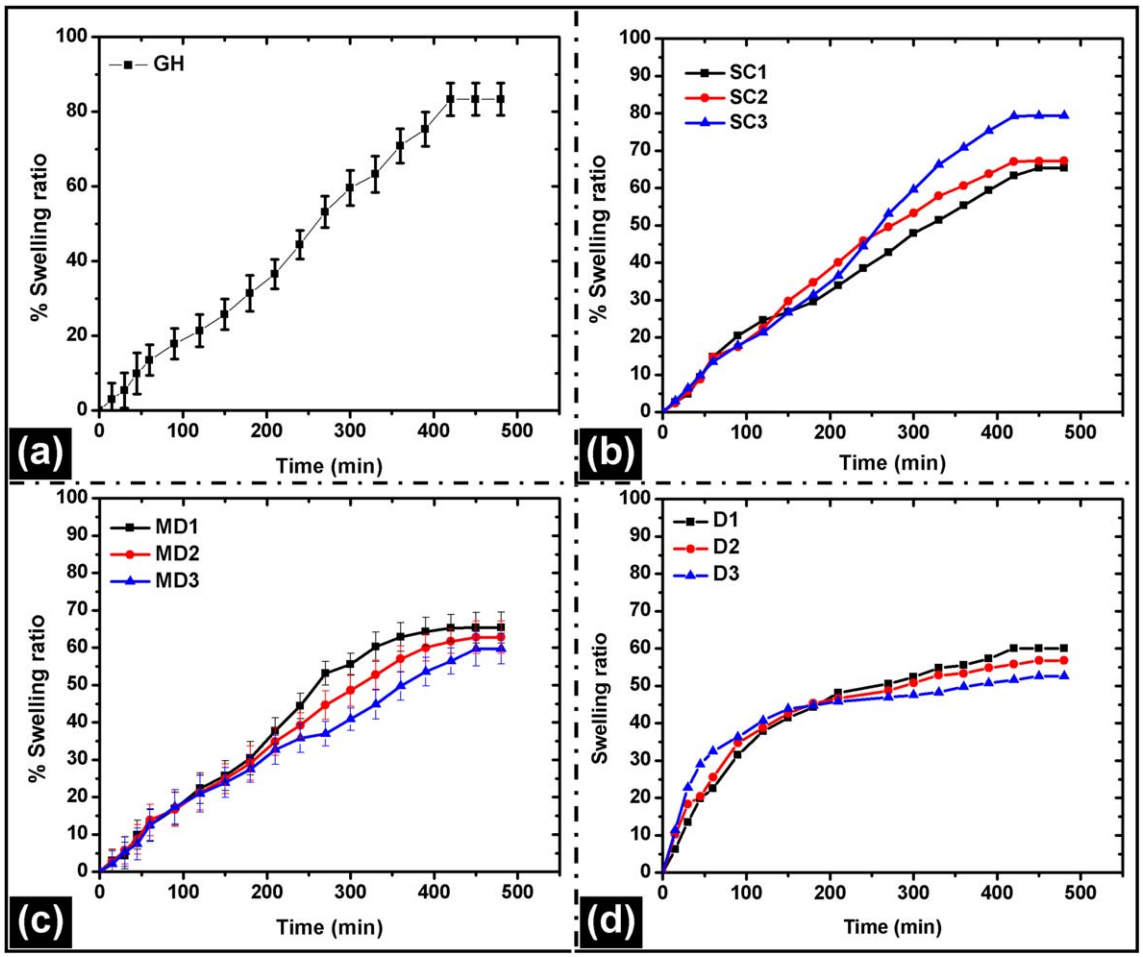

Figure 5. Percent swelling ratio of the hydrogels: (a) GH, (b) SC, (c) MD, and (d) DX. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

conductivity of the gels was in the order of SC > GH > MD > DX. With the increase in the concentration of MD and dextran in the hydrogels, there was a subsequent decrease in the conductivity. On the other hand, at low concentrations of SCMC, the conductivity of the hydrogel (SC1) was lower than GH. An increase in the concentration of SCMC resulted in the increase in the conductivity, which were higher than GH. This was quite expected as SCMC is an anionic polyelectrolyte and an increase in the concentration resulted in the increase in the conductivity. The d.c conductivity of the hydrogels was in the same order as the a.c. conductivity (Table II).

Microscopic Studies

The surface topologies of GH, SC2, MD2, and DX2 were studied under scanning electron microscopy as xerogels (Figure 2). The xerogels were prepared by incubating the hydrogels at 40°C under vacuum for 12 h. This ensured the complete evaporation of water from the matrices. The micrographs of GH and SC2 showed a smooth texture. This suggested that SCMC formed homogenous blends in gelatin matrices. On the other hand, the micrographs of MD2 and DX2 showed the presence of small spherical structures in a smooth matrix. This can be explained by the formation of phase-separated gelatin–polysaccharide hydrogels.[31] Gelatin–polysaccharide phase-separated hydrogels have been extensively reported. Phase-separated systems are generally formed due to the thermodynamic incompatibility amongst gelatin and polysaccharides during the preparation of the hydrogels.

XRD Studies

The X-ray diffractograms of the representative hydrogels showed a broad hump at ~30° 2θ (Figure 3). The presence of a broad peak may be explained by the predominant amorphous nature of the hydrogels.[57] The shift in the peak of the diffractograms toward higher 2θ values indicated a change in the crystal structure (Supporting Information Table S1).[58] The differences in full width at half maximum (FWHM) values of the hydrogels were insignificant in comparison to GH.

Test for Mucoadhesion

The mucoadhesive properties of the hydrogels were studied by *in vitro* wash-off method and texture analyzer (Table III). The mucoadhesive property of a material is not only dependent on the composition of the hydrogel matrices but also the crosslinking density of the hydrogels.[59] The *in vitro* wash-off method results showed that the mucoadhesion time was in the order of GH > MD > SC > DX. The presence of carboxyl group in gelatin may be responsible for higher mucoadhesive property of the

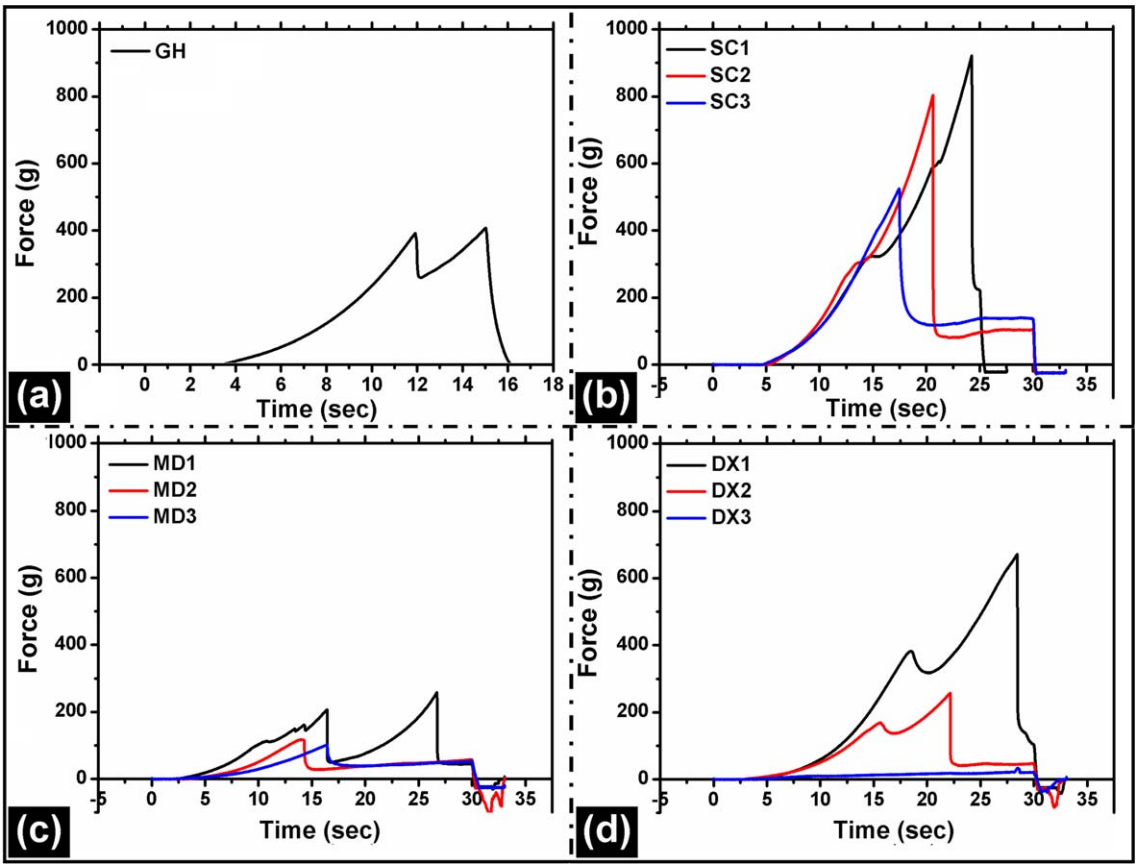

Figure 6. Gel strength of the hydrogels: (a) GH, (b) SC, (c) MD, and (d) DX. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

GH.[60] In general, the mucoadhesive property of the hydrogels reduced with an increase in the polysaccharide content except MD gels which showed an initial decrease followed by an increase in the mucoadhesive property of the hydrogels. The mucoadhesion time of the hydrogels was found to be ~10 h or more. The results indicated that the developed hydrogels may be effectively used for controlled vaginal drug delivery. During bacterial vaginosis, there is an increased vaginal discharge which results in the washing off of the administered drugs and also the migration of the delivery vehicles. This reduces the efficiency of the treatment. Since the mucoadhesion times of the hydrogels were ~10 h or more, it is expected that the use of the prepared hydrogels will prevent the migration of the delivery vehicle within the vaginal lumen and hence will help in maintaining the sufficient drug concentration at the site of action.

The work done for the separation of the hydrogels from the mucosal surface was determined using a static mechanical tester. The area under the force–distance curve during the separation of the hydrogels from the mucosal surface is regarded as the work of adhesion (Figure 4). The results indicated that the work of adhesion of GH was much higher than the gelatin–polysaccharide hydrogels. An increase in the mucoadhesive force was observed with the increase in the concentration of MD and dextran in the hydrogels. On the other hand, SCMC containing hydrogels showed a decrease in the mucoadhesion as the concentration of the polysaccharide was increased. The results suggested that the hydrogels had sufficient mucoadhesive property to be used as mucoadhesive drug delivery vehicles.

Water Uptake Studies

The swelling of the hydrogels is a direct indication of the amount of water uptake by the hydrogels (Figure 5). Water uptake studies showed highest swelling in GH, which showed ~80% of water uptake. An increase in the concentration of MD and dextran in the gelatin–polysaccharide hydrogels resulted in the reduction in the water uptake. On the contrary, an increase in the concentration of the SCMC resulted in the corresponding increase in the water uptake. The results can be explained by the conductivity of the hydrogels where it was observed that an increase in the proportion of the SCMC resulted in the increase in the conductivity of the hydrogels whereas an increase in the proportion of MD and dextran resulted in the decrease in the conductivity of the hydrogels.

Gel Strength Studies

The gel strength of the formulations was evaluated using mechanical tester (Figure 6). The positive peak of the force–time graph may be regarded as the strength of the formulation. It was in the order of SC > GH > DX > MD. Individually, SCMC containing hydrogels showed an increase in gel strength with the increase in the SCMS concentration (SC1 < SC2 < SC3). MD and dextran containing hydrogels showed a reverse trend. The gel strength of the hydrogels decreased with increased concentration

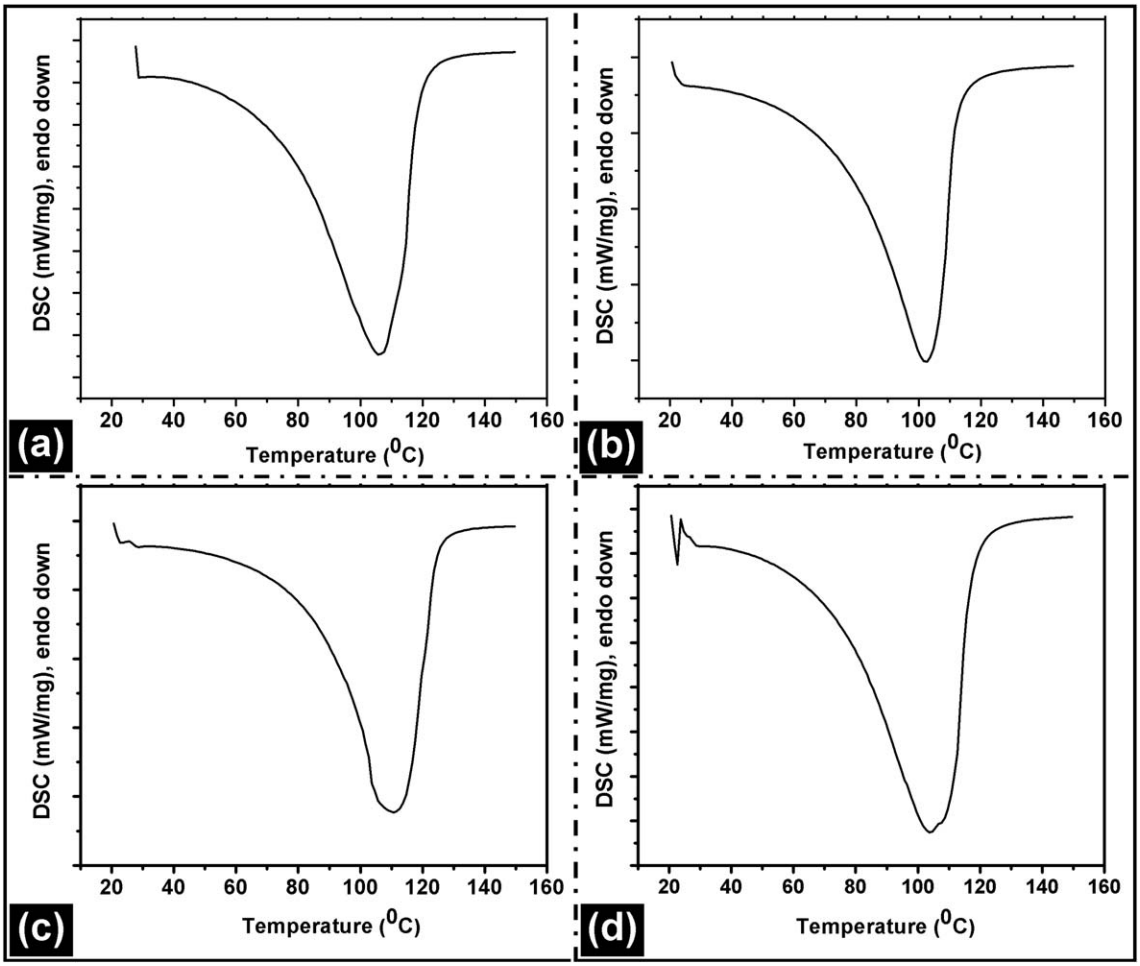

Figure 7. Differential scanning calorimetry of the representative hydrogels: (a) GH, (b) SC2, (c) MD2, and (d) DX2.

of MD and dextran (MD1 > MD2 > MD3 and DX1 > DX2 > DX3). This may be explained by the microscopic observation where it was found that the SCMC-based hydrogels formed blend hydrogel, whereas MD- and dextran-based hydrogels formed phase-separated hydrogels. The formation of the phase-separated systems might have resulted in the introduction of defects within the gelatin matrix, thereby resulting in the decrease in the gel strength of the MD- and dextran-based hydrogels.

DSC Studies

GH, SC2, MD2, and DX2 were used as the representative hydrogels for studying the thermal properties (Figure 7). The hydrogels showed a broad endothermic peak in the temperature range of 100–110°C. The endothermic peaks may be related to the loss of water content present in the hydrogel matrices. The change in the enthalpy (ΔH_m) and the change in the entropy (ΔS_m) were calculated from the area under the endothermic curves (Table IV). ΔH_m and ΔS_m values of the gelatin–polysaccharide blend hydrogels were higher than GH. This suggested higher water holding capacity of the blend hydrogels as compared to GH, which showed higher water imbibing capacity.[61]

Table IV. Thermal Properties of the Representative Hydrogels

Formulations	Peak (°C)	ΔH_m (J/g)	ΔS_m (J/g/K)
GH	106.8	1835.67	17.19
SC2	101.3	2514.22	24.82
MD2	110.2	2417.80	21.94
DX2	103.2	1927.59	18.68

Biocompatibility Studies

All the hydrogels showed <5% hemolysis when tested in the goat blood [Figure 8(a)]. This indicated hemocompatible nature of the prepared hydrogels.[62] Previous report indicates that hemocompatible hydrogels are usually non-irritant in nature.[49] Hence the developed hydrogels may be considered for vaginal drug delivery.

Cytocompatibility of the hydrogels was checked against HaCaT cells. The leachants of the hydrogels were used in this study. MTT assay indicated that the relative proliferation of the cells in the presence of the leachants and the control was statistically insignificant ($P > 0.05$) thereby suggesting the cytocompatibility of the prepared hydrogels [Figure 8(b)]. The growth in the presence of leachate has been shown in Supporting Information Figure S1. *In vitro* cell cytocompatibility studies have confirmed that the developed formulations were biocompatible in nature and can be employed for the *in vivo* applications.

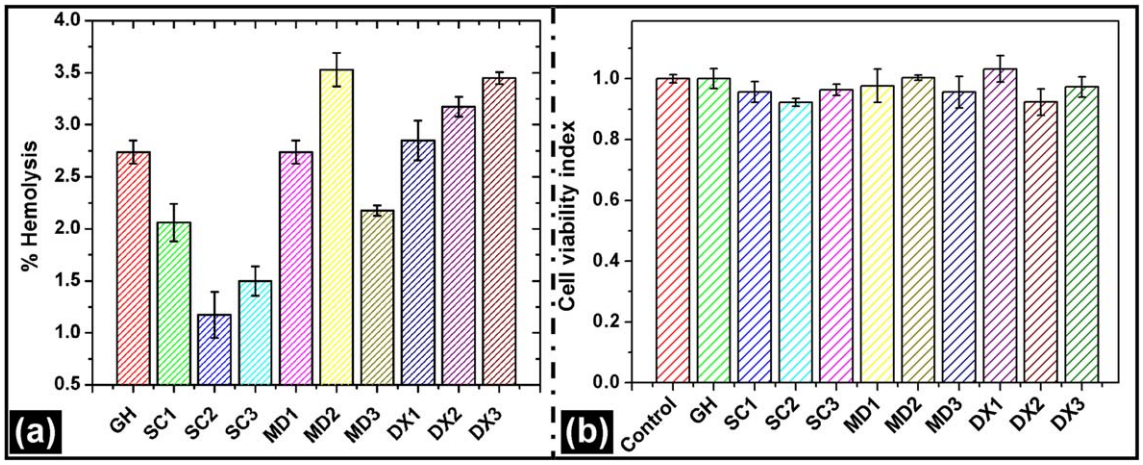

Figure 8. Biocompatibility study of the hydrogels: (a) hemocompatibility and (b) cell viability studies. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

In Vitro Drug Release Studies

The release of metronidazole from the hydrogel matrices was dependent on the composition of the hydrogel (Figure 9). GH showed highest release of the drug in comparison to the gelatin–polysaccharide blend hydrogels. In general, the amount of drug released at the end of 8 h from the hydrogels was in the order of GH > SC > MD > DX. An increase in the concentration of MD and dextran resulted in the decrease in the amount of drug released. A reverse trend was observed in SCMC hydrogels, where an increase in the concentration of SCMC resulted in the

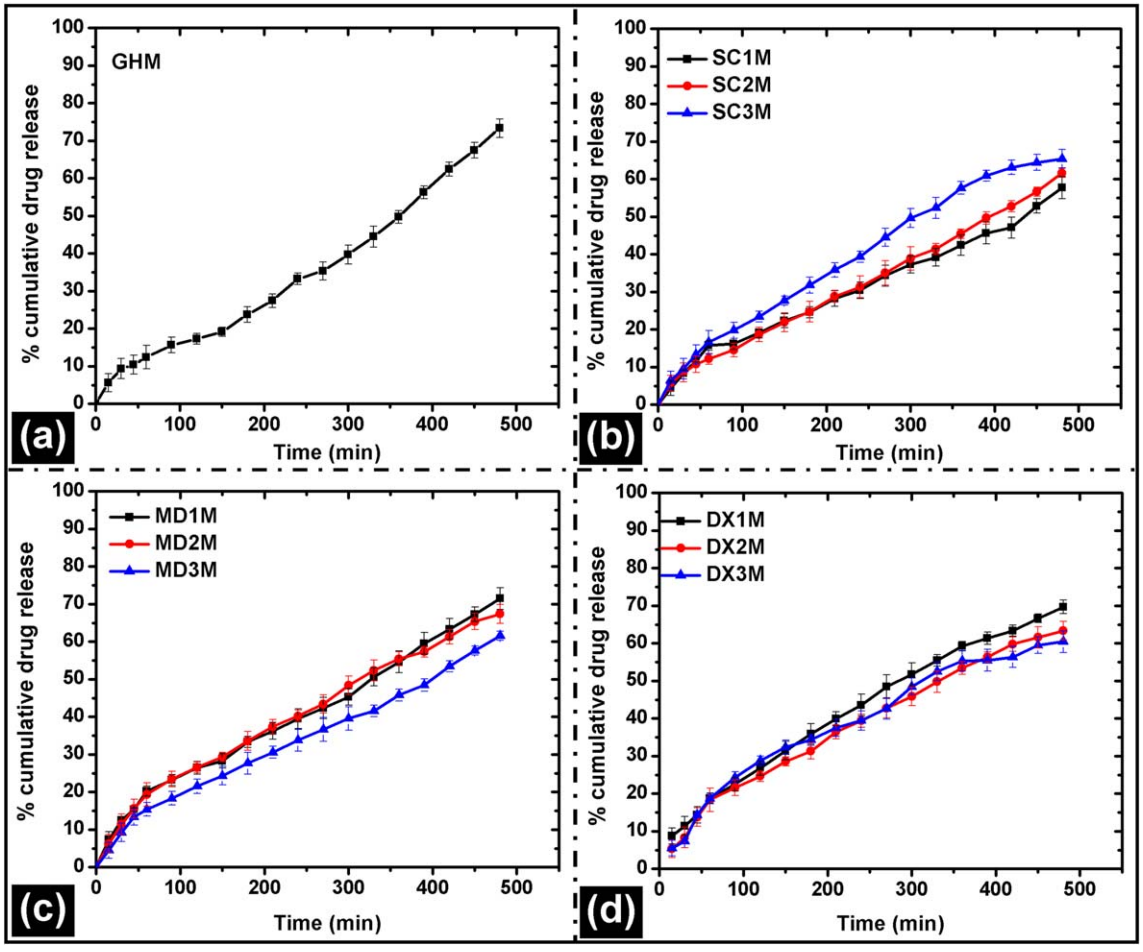

Figure 9. % cumulative drug release of metronidazole-loaded hydrogels: (a) GH, (b) SC, (c) MD, and (d) DX. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

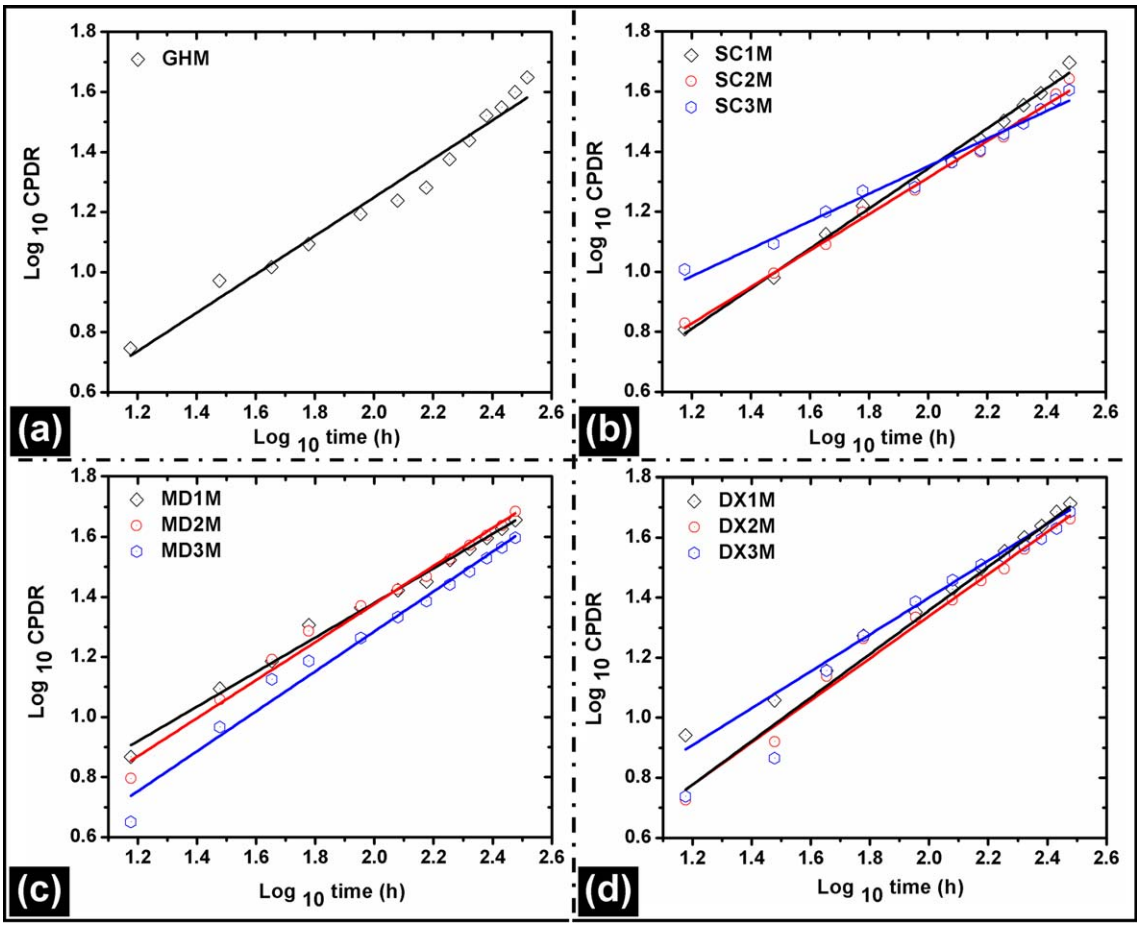

Figure 10. Korsmeyer–Peppas model fitting of metronidazole-loaded hydrogels: (a) GH, (b) SC, (c) MD, and (d) DX. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

increase in the amount of the drug released. The trend of drug release was found to be in direct relation with the electrical conductivity and swelling behavior. This may be accounted for the water imbibing capacity of the hydrogels.[63] The release kinetics of the drugs was estimated by fitting various models, viz. zero-order, first-order, and Higuchi models. Zero-order kinetics was found to be the best fit model. This suggested that the release of metronidazole was concentration independent. Hence, the developed hydrogels may be used as controlled release matrices. The *n*-value (Fickian value) was calculated using Korsmeyer–Peppas model (Figure 10). The *n*-value was found to be in the

Table V. Drug Release Kinetics of the Hydrogels

Formulations	Zero-order (r^2)	KP model		
		(r^2)	*n*	Type of flow
GHM	0.98	0.97	0.64	Non-Fickian
SC1M	0.98	0.99	0.66	Non-Fickian
SC2M	0.99	0.99	0.60	Non-Fickian
SC3M	0.98	0.98	0.46	Non-Fickian
MD1M	0.98	0.99	0.57	Non-Fickian
MD2M	0.98	0.98	0.63	Non-Fickian
MD3M	0.98	0.98	0.66	Non-Fickian
DX1M	0.98	0.99	0.61	Non-Fickian
DX2M	0.98	0.98	0.70	Non-Fickian
DX3M	0.95	0.96	0.72	Non-Fickian

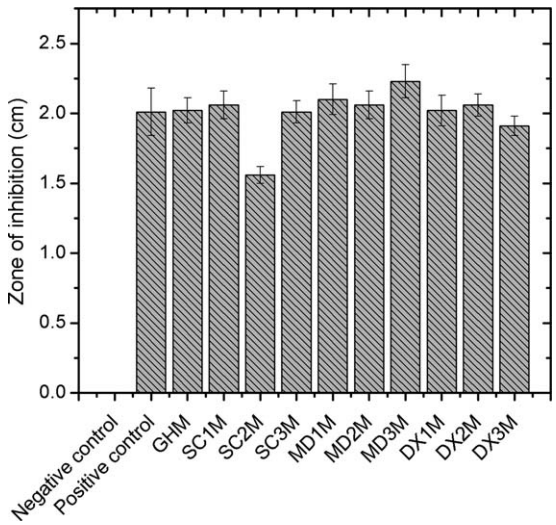

Figure 11. Antimicrobial study of the hydrogels.

range of 0.45–0.75 which indicated non-Fickian type diffusion of the drug from the hydrogels (Table V).

Antimicrobial Studies

The zone of inhibition of the microbes was found to be nearly equal for all the drug-loaded hydrogels (Figure 11). The differences in the zone of inhibitions were found to be insignificant. The antimicrobial efficiency was found to be comparable to the positive control. The results suggested that the drug-loaded hydrogels showed sufficient antimicrobial efficiency against *B. subtilis*.

CONCLUSION

The current study successfully deciphers the development of gelatin–polysaccharide based hydrogels. The properties of the hydrogels were found to be dependent on the composition of the hydrogels. The conductivity and water absorption properties of the hydrogels were found to tailor the release performance of the drug from the hydrogels. The hydrogels were found to be biocompatible and had sufficient mucoadhesive properties for vaginal drug delivery applications. The release of metronidazole was diffusion-mediated and followed zero-order kinetics. The antimicrobial assay showed good activity against *B. subtilis*. In conclusion, the developed hydrogels can be used as matrices for controlled delivery of metronidazole in treating bacterial vaginosis.

ACKNOWLEDGMENTS

The authors acknowledge National Institute of Technology, Rourkela for providing logistic support and Department of Biotechnology, Govt. of India for the financial support from the project (BT/220/NE/TBP/2011) for successful completion of this work.

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