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CONTROLLED RELEASE OF VANCOMYCIN HYDROCHLORIDE FROM BIOPOLYMER BASEDHYDROGELS

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Abstract- **Gelatin based hydrogelsareprepared withpoly(acrylic acid). Gelatin and poly(acrylic acid) were cross-linked with glutaraldehyde and with N,N'-methylenebisacrylamide, respectively at various cross-linker concentrations.Decreasing the cross-linking concentration of cross-linkers *viz*N,N'-methylenebisacrylamide from 0.5to 0.2 mole% and glutaraldehyde from 4 w% to 0.5 w%, reduced the *in vitro* degradation time from 38±2 days to 8±1 days in water(pH ~5.9) and from 35±2 days to 7±1 days in phosphate buffer (pH 7.4). Full inter penetrating networks hydrogels (IPNs) demonstrate steady swelling followed by irregular loss of weight till study time of 35 days. Semi IPNs, where acrylic acid was cross linked show higher swelling and lower degradation as compared to semi IPNs where gelatin was cross linked. *In-vitro* degraded products analyzed by MALDI, demonstrated low molecular weight products, which can be either phagocytes or easily filtrated via kidney. Drug release was mainly governed by diffusion and erosion of polymer matrix. Scanning electron micrographs gave an insight about drug distribution in hydrogel and degradation of hydrogel.**

Keywords: Hydrogel, Interpenetrating polymer network, Vancomycin hydrochloride, Biodegradation, Gelatin.

I. INTRODUCTION

The osteomyelitis is bone infection caused by *Staphylococcus* [1] which require 7 weekstreatment. Due to low circulation of blood in bone, high dose of antibiotics required for oral or injection form drug delivery. For localized chronic infection like osteomyelitis, use of site-specific biodegradable polymeric systems as an implantable controlled release drug delivery system in combination with surgery is a recognized treatment [2,3]. Such treatment may be more acceptable to the patients, reducing the period of hospitalization, cost of treatment, and provides high concentration of drug at the site of infection with a low systemic toxicity [7]. Various localdrug delivery systems for antibiotics delivery at the site of infection have been attempted [8-10]. Many inorganic [11-13] and organic biodegradable materials such as, collagen/fibrin [14,15], peptide [16], and, hydroxy apatitecomposite with organic materials [17],etc. were attempted. Collagen antibiotics sponge appears to be equally as popular as PMMA beads for the treatment of bone infection but*in vitro* antibiotics release from collagen sponges was short lived. Hydroxy apatite, due to similar chemical composition to natural bone was considered as an ideal candidate for long-term local antibiotics or drug delivery [18]. However, the brittleness and poor strength of hydroxy apatite limited its use. Ideal strategies for the management of osteomyelitis by implant device should aim at the restoration of bone congruity and maintenance of the high concentration of antibiotics at the site of infection for prolong period of time [19-21]. There

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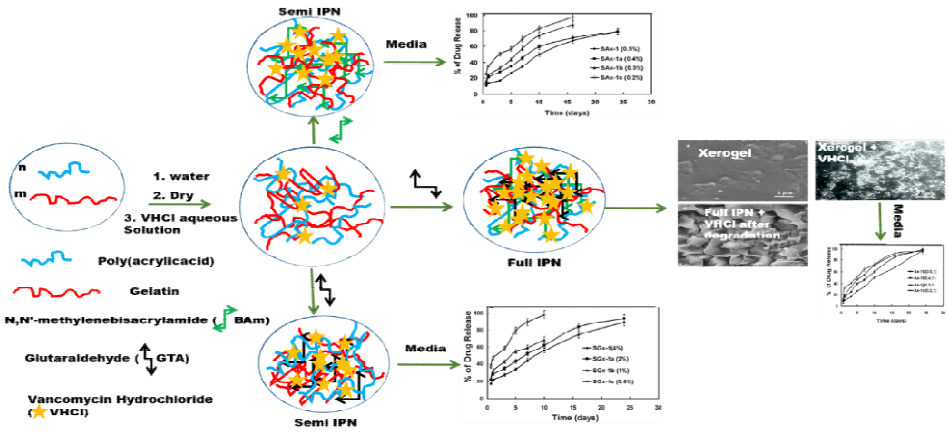
has been continuous research for development of effective, biodegradable and biocompatible drug delivery system [22-24].

Hydrogels are three-dimensional natural or synthetic hydrophilic polymer network structure which has capacity to swell by absorbing large volumes of water without disturbing original structure [25,26]. However, swelling behavior of hydrogelsare sensitive to surrounding environmental condition, such astemperature, pH, ionic strength, electric fields, and magneticfields, [27,28]. Due to presence of water, surfaces ofthe hydrogels arewet and malleable and as a result significantly reduces irritation to the surrounding tissues. In an area of biomedical science, hydrogels are a new classof smart material with enormous potential inbiotechnology [29-31].

For implantable drug delivery system, controlled release of drug, degradation, systemic toxicity of implantable delivery system, and degraded products play significant role for its application as implantable device. Thereis considerable attention were given to study the effect of cross-linker concentration on degradation of hydrogels without considering loaded drugs. Also, a sperate study on systemic toxicity of degraded products is required to develop implantable biomaterials. Theobjective of the present study is to investigate the synergic effect of loaded vancomycin hydrochloride (VHCl) and cross-linking concentration on degradation and drug release behavior of gelatin and acrylic acid-based semi and full interpenetrating network hydrogel (Scheme 1).The molecular weight of depredated products (m/z) was determined by MALDI technique.



Scheme 1:Model for hydrogel synthesis, characterization, drug loading/release, and degradation

II. MATERIALS AND METHOD

Materials

Acrylic acid (AA) (G. S. Chemicals) was purified by distillation under reduced pressure. Gelatin (Ge) (Type B), glutaraldehyde (25% AR), and sodium metabisulphite, areall obtained from s. d. fine chem. Ltd, India. N,N'-methylenebisacrylamide(BAm) and ammonium presulfate from SISCO research lab, Mumbai, Muellar Hinton Agar and Muellar Hinton broth from Titan BiotechLimited (Bhiwandi, India) and vancomycin hydrochloride (Lilly Pharma Fertigung und Distribution GmbH & Co.KG 35387, Giessen Germany) were used as obtained.

Hydrogel Synthesis

Hydrogels were prepared as per method described in reported work [32]. Full and semi-interpenetrating polymer networks (IPNs) based on poly(acrylic acid) and gelatin, which were cross-linked selectively using N,N'-methylenebisacrylamide(BAm) (at 0.2 to 0.5 mole % concentration) and glutaraldehyde (0.5 to 4 w %),respectively. Table 1, shows the sample designation and composition of the various hydrogels used in this study.

Vancomycin Hydrochloride Loading in Hydrogels

Vancomycin hydrochloride was loaded by swelling10 mg of dry hydrogel sample in 10 mL of vancomycin hydrochloride solution (2 mg/mL) in phosphate buffer (pH 7.4) at 37 ± 0.1 °C under nitrogen atmosphere. It was seen that nitrogen atmosphere prevented the fungal contamination on polymer during loading of the drug. For observing the leaching of polymer component from polymer network, the xero gel was immersed in phosphate buffer (pH 7.4) under nitrogen atmosphere at 37 °C. After immersing the polymer sample for 24 h, it was taken out, dried and reweighed. The increase in the weight of the polymer was taken as the amount of drug loaded, whereas negligible change in weight was observed in neat polymer. Furthermore, for the confirmation of percentage of drug

loading in hydrogels, the amount of vancomycin hydrochloride left in the loading medium, was determined by microbial method using disk diffusion technique [33].

In-vitro Degradation

15 milligram of cylindrical shaped hydrogel placebo devices (l~3mm, d~2 mm), were immersed in 20 mL of water (pH ~5.8) / phosphate buffers (pH 7.4) at 37± 0.1°C, respectively. The change in weight of the hydrogel at pre-determined time intervals was noted for required period of time. After each 24 hours, the degradation medium was changed with fresh solvent of 20mL.

Evaluation of Degraded Product by Matrix Assisted Laser Desorption Ionisation (MALDI)

Matrix assisted laser desorption ionisation mass spectra of degraded sample in phosphate buffer (pH 7.4) were recorded using Kratose Kompact MALDI 4 with α-cyano-4-hydroxy-cinnamic acid as a matrix from irradiation with a very brief pulse (3 ns) of nitrogen laser light (λ= 337nm).

In-vitro Vancomycin Hydrochloride Release from Hydrogels

For the drug release studies, 12 mg of cylindrical shaped (3x2 mm), 80 percent drug loaded polymer (polymer: drug :5:4 w/w) and 7 mg of placebo devices were immersed in 10 mL water (~ pH 5.8) and phosphate buffer (pH 7.4), respectively and were left in a shaking water bath at 37±0.1°C. Samples were withdrawn at regular intervals. With each sampling, release media was changed with fresh medium, maintaining the total volume constant. Quantitative analysis of vancomycin hydrochloride was carried out by microbiological assay.

Estimation of Vancomycin Hydrochloride

The minimum inhibitory concentration of vancomycin hydrochloride to *Staphylococcus aureus (A*TCC 259523) was established by using an antibiotic tube dilution method in cation supplemented Mueller–Hinton broth, containing 5:0 x 10^5colonies forming units per milliliter using Walker and Kopp et al process [34,35]. After the minimum inhibitory concentration was determined, quantification of vancomycin hydrochloride to *Staphylococcus aureus* (ATCC 259523) was determined by using an antibiotic disk diffusion method in the Mueller–Hinton agar. For this purpose, 6mm wells were punched into agar disc inoculated with *Staphylococcus aureus* (ATCC 259523, 10^6 CFU/mL) and they were filed with 20 µL of aqueous vancomycin hydrochloride solution as standard. After incubation (18h), the zones of inhibition were measured. The experiments were repeated five times. The standard straight line was obtained by plotting the mean dose logarithms as a function of associated inhibition zone diameters. This straight line was used to calculate the antibiotics concentration release medium.

Morphological Studies

The morphology of *in vitro* degraded sample was investigated using Cambridge Stereoscan Model SGX1-10 Scanning electron microscope. The samples were mounted on the base plate and coated with gold using vapor deposition techniques. Cross-section of VHCl loaded hydrogel was usedto study the drug distribution in hydrogel.

III. RESULTS AND DISCUSSION

In-vitroDegradation

Effect of decreasing cross linker concentration of poly (acrylic acid) and gelatin chain (AA: Ge: 1:1 w/w) on rate of degradation of full and semi IPNs hydrogels.In our previous work [36], we have reported the effect of acrylic acid and gelatin concentration on the degradation of hydrogels. We observed that full IPNs, Ax-3 (AA: Ge: 5:1 w/w), did not degrade even after 130 days, whereas samples Ax-1 (AA:Ge: 1:1 w/w) and Ax-2 (AA: Ge: 2:1 w/w) degraded within 60 days at 0.5 mole % BAm and 4 wt % glutaraldehyde concentration.In the present study, we are reporting the effect of decreasing cross-linking concentration of BAm and glutaraldehyde on *in-vitro* degradation of full and semi IPNs. Percent change in weight of the hydrogels at 37± 0.1°C in water (pH~ 5.8) and phosphate buffer (pH 7.4) was investigated as a function of time. Table 2 summaries the effect of cross-linking concentration of poly(acrylic acid) and gelatin chain on the degradation of full and semi IPNs. The rate of degradation was statistically identical in phosphate buffer and water. It has been noticed that reduction in the concentration of BAm and glutaraldehyde, increases the rate of degradation of both full and semi IPNs. The above generalization was supported by considering the following three cases. In the first case, the concentration of glutaraldehyde was kept constant (2 wt %) and the concentration of BAm was varied from 0.5 to 0.2 mole % (Ax-1a, Ax-1e, Ax-1i and Ax-1m), the degradation time was reduced from 35±2 days to 14±1 days in phosphate buffer (pH 7.4) (Figure 1a) at 37°C. In second case; the concentration of glutaraldehyde was fixed at 1 wt%, degradation time reduced by one third (Figure 1b) as the BAm

concentration reduced from 0.5 to 0.2 mole % in full IPNs. In third case, at 0.5 w/w % of glutaraldehyde concentration, the hydrogels degraded within 7 days leading to an opaque solution (Figure1c).

All the full IPNs demonstrate a steady swelling up to maximal and then loss of weight was observed from day 5 andtill 30 days depending on the cross-linker concentration in acrylic acid and gelatin chain. Effect of cross-linker concentration on degradation pattern of semi IPNs and simple hydrogels is depicted in Figures 2a, b, and c, respectively. Semi IPNs in which acrylic acid is cross-linked and gelatin is free (AxG) (Figure 2a), showed much higher swelling than semi IPNs, in which acrylic acid is free and gelatin is cross-linked (AGx). Semi IPNs of AGx class, degraded much faster than semi IPNs of AxG (Figure2b). Both types of the semi IPNs devices (AxG/ AGx) exhibit swelling, followed by instantaneous loss of mass, depicting the solubility of un-cross linked, short length linear chains of poly(acrylic acid)/ gelatin in degradation medium and contribute to lesser swelling of semi IPNs than corresponding full IPNs[36,37]. The degradation pattern of simple hydrogel showed in Figure 2c. Simple hydrogel Axa (0.3mole%) and Axb (0.2 mole%) demonstrated very high swelling of about 12000% wt change up to 4^{th} day and changed the physical state from devices to viscous mass. Whereas, Gxa and Gxb showed much lower swelling as compared to Ax. Swelling of simple hydrogel of Ax class was much higher than full IPNs and semi IPNs.

Table 1: Preparation of hydrogels, feed composition and sample designation.

Network Composition	Sample Designation	Crosslinking concentration of acrylic acid(AA) chain (mole %)	Crosslinking concentration of gelatin chain (Ge)(mole%)	Monomer Ratio (AA:Ge) w/w
Simple Hydrogels→				
Ax	Ax	0.5	-	1:0
	Axa	0.3	-	
	Axb	0.2	-	
Gx	Gx	-	4.0	0:1
	Gxb	-	3.0	
	Gxc	-	2.0	
Full IPNs→				
AxGx	Ax-1	0.5	4.0	1:1
	Ax-1a	0.5	2.0	
	Ax-1b	0.5	1.0	
	Ax-1c	0.5	0.5	
	Ax-1d	0.4	4.0	
	Ax-1e	0.4	2.0	
	Ax-1f	0.4	1.0	
	Ax-1g	0.4	0.5	
	Ax-1h	0.3	4.0	
	Ax-1i	0.3	2.0	
	Ax-1j	0.3	1.0	
	Ax-1k	0.3	0.5	
	Ax-1l	0.2	4.0	
	Ax-1m	0.2	2.0	
	Ax-1n	0.2	1.0	
	Ax-1o	0.2	0.5	
Semi IPNs→				
AxG	SAx-1	0.5	-	1:1
	SAx-1a	0.4	-	
	SAx-1b	0.3	-	
	SAx-1c	0.2	-	
AGx	SGx-1	-	4.0	1:1
	SGx-1a	-	2.0	
	SGx-1b	-	1.0	
	SGx-1c	-	0.5	

This indicates that, there is an interaction between carboxylic group of poly(acrylic acid) chain and amino group of gelatin chain of the hydrogel, which hinder the ionization of respective groups, as a result ion- ion repulsion decreases and reduces the rate of the swelling of full (AxGx) and semi IPNs (AxG) in comparison to simple hydrogel Ax [36]. No noticeable change in pH during the degradation was observed. As it is evident that, reducing the concentration of cross-linker, leads to loosen meshed network, which enhanced the rate of diffusion of solvent and subsequently increases the rate of degradation [37-40]. The aim of the current work was to have a controlled release of drug for at least 4 weeks, so that these devices can be used for the treatment of bone infection, hence we did not further reduce the cross-linker concentration, which would have affected the mechanical strength as well as drug release.

Table 2: Time taken by the hydrogels to degrade completely in water (pH ~5.8) and phosphate buffer (pH 7.4) at 37°C.

AA: Ge ratio (w/w)	Sample Designation	BAm Concentration (mole %)	Glutaraldehyde Concentration (w %)	Degradation Time (days) in Water	Degradation Time (days) in Phosphate buffer
1:0	Axa	0.3	-	24	21
1:0	Axb	0.2	-	24	20
0:1	Gxa	-	2.0	10	9
0:1	Gxb	-	1.0	8	7
1:1	Ax-1a	0.5	2.0	37	35
1:1	Ax-1b	0.5	1.0	32	32
1:1	Ax-1c	0.5	0.5	30	29
1:1	Ax-1d	0.4	4.0	38	36
1:1	Ax-1e	0.4	2.0	38	36
1:1	Ax-1f	0.4	1.0	32	30
1:1	Ax-1g	0.4	0.5	30	25
1:1	Ax-1h	0.3	4.0	42	35
1:1	Ax-1i	0.3	2.0	18	15
1:1	Ax-1j	0.3	1.0	14	13
1:1	Ax-1k	0.3	0.5	9	7
1:1	Ax-1l	0.2	4.0	35	30
1:1	Ax-1m	0.2	2.0	17	14
1:1	Ax-1n	0.2	1.0	12	11
1:1	Ax-10	0.2	0.5	8	6
1:1	SAx-1a	0.4	-	40	35
1:1	SAx-1b	0.3	-	37	33
1:1	SAx-1c	0.2	-	15	17
1:1	SGx-1a	-	2.0	17	15
1:1	SGx-1b	-	1.0	14	12
1:1	SGx-1c	-	0.5	10	9

The data represents the mean of three experiments; AA: Acrylic acid, Ge: gelatin, BAm: N,N'-methylenebisacrylamide.

In-vitroDrug Release

The effect of simple geometry on drug release is described by power law model presented in equation 1.

$M_t = M_\infty t^n$ (1)

Where M_t and M_∞ are the respective mass of drug release at time **t,** infinity and **n** is the diffusion exponent. Information about the release mechanism, can be gained by fitting the drug release data and comparing the value of **n** to the semi empirical value of various geometry's [41,42]. For a cylindrical geometry, the value of **n**≤0.45 or less correspond to purely Fickian diffusion mechanism, the value between 0.45 to 0.89 indicates anomalous release mechanism, and a value of **n** greater than 0.89 indicates a relaxation-controlled release mechanism.Hydrogels at AA:Ge : 1:1 (w/w) (polymer: drug : 4:3.4 w/w)

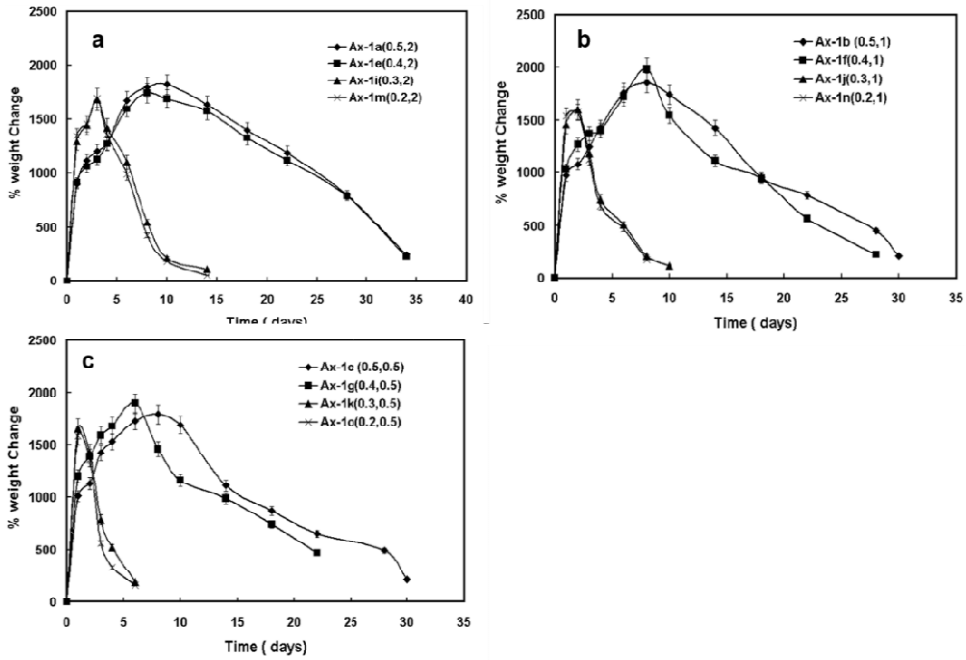


Figure 1: Effect of N,N'-methylenebisacrylamide concentration at (a) 2 wt. % (b) 1 wt %, and (c) 0.5 wt % glutaraldehyde concentration on *in-vitro* degradation of full IPNs hydrogel in phosphate buffer (pH 7.4) at 37^0C.

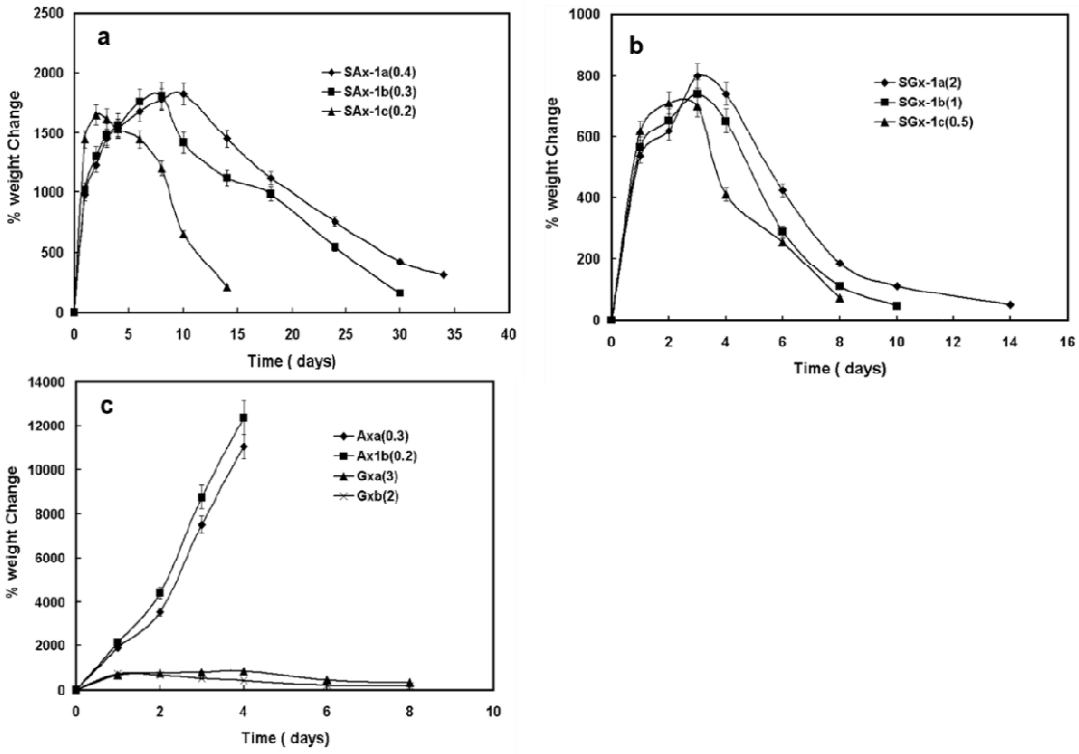


Figure 2: Effect of cross-linker concentration on degradation of hydrogels (a) N,N'-methylenebisacrylamide (BAm) concentration on semi IPNS AxG (b) glutaraldehyde concentration on semi IPNs AGx, and (c) N,N'-methylenebisacrylamide (BAm) / glutaraldehyde on simple hydrogel on in-vitro degradation in phosphate buffer (pH 7.4) at 37 0C.

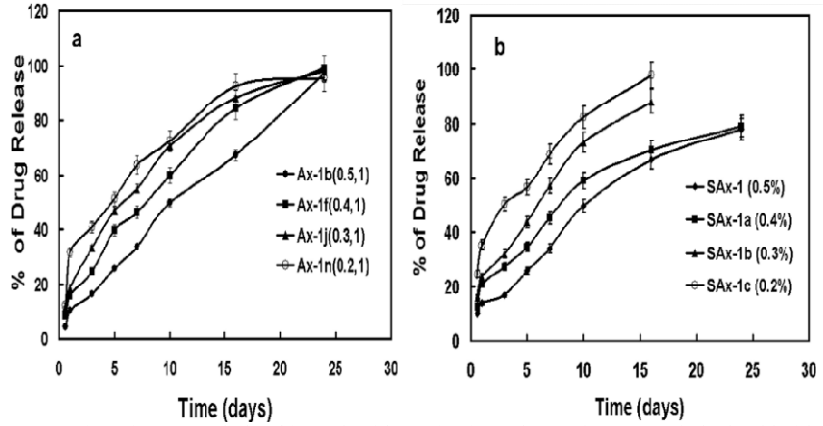


Figure 3: Effect of the concentration of N,N'-methylenebisacrylamide on in vitro release of vancomycin hydrochloride from (a) full IPNs (AA: Ge 1:1w/w) at 1 wt% glutaraldehyde and (b) semi IPNs AxG (AA:Ge: 1:1w/w) in phosphate buffer (pH 7.4) at 37 0C. Data represent the mean ±SD of four experiments.

Effect of Reduced Cross-linker Concentration on Release of Vancomycin Hydrochloride from Full and Semi IPNs

Figures 3a and 3b, show the effect of N,N'-methylenebisacrylamide concentration on release of vancomycin hydrochloride at 1wt% cross-linking concentration of glutaraldehyde from full and semi IPNs (AA:Ge : 1:1w/w), respectively in phosphate buffer (pH 7.4) at 37±0.1°C. From the Figures 3a and 3b, it is clear that as the N,N'-methylenebisacrylamide concentration decreases from 0.5 to 0.2 mole % in the polymer network, release of vancomycin hydrochloride due to burst effect increases from 10 ±1 to 27±2 % of total loaded drug, in full IPNs and 14±1 to 34±2.5 % in semi IPNs. The diffusion exponent for the 60% drug release was found to be in the range of 0.63 ± 0.10 in case of Ax-1b, Ax-1f, Ax-1j and Ax-1n, and for semi IPNs SAx-1, SAx-1a, SAx-1b and SAx-1c was in order of 0.60 ± 0.08; thereby indicating anomalous release mechanism of vancomycin hydrochloride from these full and semi IPNs hydrogels in phosphate buffer (pH 7.4). From all the full IPNs devices, almost 90 % of loaded drug was recovered in drug release experiments for a period of 25 days.

Effect of Reduced Concentration of Glutaraldehyde on Release of Vancomycin Hydrochloride from Full and Semi IPNs Hydrogels

Figures 4a and 4b, depict the effect of glutaraldehyde concentration on release of vancomycin hydrochloride at 0.3 percent crosslinking concentration of N,N'-methylenebisacrylamide from full and semi IPNs (AA:Ge: 1:1w/w) in phosphate buffer (pH 7.4) at 37±0.1°C. For full IPNs samples, Ax-1h (4 % glutaraldehyde) and Ax-1i (2 % glutaraldehyde), release of vancomycin hydrochloride was almost identical for the first 7 days of the experiments (Figure 6a) and about 50 % of loaded drug was released from both devices. Whereas, Ax-1k device, degraded within 7 days with 92±3 % release of the drug.

Furthermore, semi IPNs SGx-1 (4%), SGx-1a (2%) and SGx-1b (1%), vancomycin hydrochloride release pattern was statistically identical.Whereas, at 0.5 percent glutaraldehyde concentration, release of drug from full and semi IPNs (Ax-1k and SGx-1c) was higher than full and semi IPNs at higher cross-linking concentration of glutaraldehyde. This indicates that swelling characteristics of the IPNs also governs the release of vancomycin hydrochloride from these polymers. All the full and semi IPNs, show anomalous drug release mechanism in phosphate buffer (pH 7.4). Semi IPNs SGx-1b and SGx-1c became viscous within 10 days of release study and we wereunable to study their release profile(Figure 4b). Decreasing the cross-linker concentration usually loosen the network of the polymer matrix and provides less resistance for the permeation of water into the system and increase the diffusivity of loaded drug, as a result, the rate of drug release increases with decreasing the cross-linker concentration in full IPNs as well as in semi IPNs.

Evaluation of In-vitro Degradation by Matrix Assisted Laser Desorption Ionisation (MALDI) and Scanning Electron Microscope (SEM)

The table 3 represents molecular weight of degraded product of hydrogel which were calculated by MALDI. The m/z value of the degraded products were very low ich can easily pass-through renal system. The result obtained by

SEM enables the observation of the physical characterization of external morphology of *in vitro* and *in vivo* degradation of devices. Scanning electron micrographs of control samples of full Ax-1n (0.2,1%), cross section of vancomycin hydrochloride loaded full IPNs Ax-1n (0.2,1%), and 10 days *in vitro*degraded Axn-1n, are given in Figures 5a, b, and c, respectively. External surface of the control sample is relatively smooth except for debris (Figure 5a) and uniform distribution of drug was observed in full IPNs (Figure 5b). Emergence of large grooves and extensive porous morphology in degraded samples indicate the progress of degradation in the hydrogel (Figure 5c).

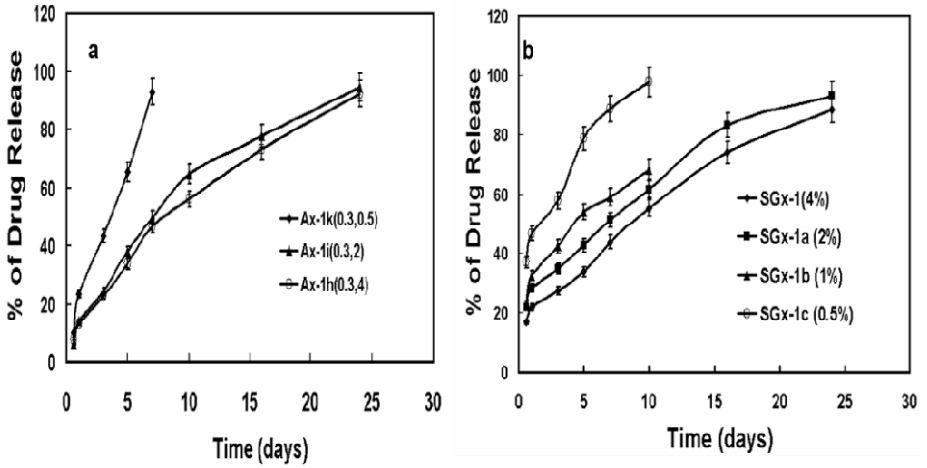


Figure 4: Effect of the concentration of glutaraldehyde on in vitro release of vancomycin hydrochloride from (a) full IPNs (AA: Ge 1:1w/w) at 0.3 mole % N,N'-methylenebisacrylamide and (b) semi IPNs AGx (AA:Ge: 1:1w/w) in phosphate buffer (pH 7.4) at 37 0C. Data represents the mean ±SD of four experiments.

Table 3: Matrix assisted laser desorption ionization mass spectral peaks of the degradation products of hydrogels in phosphate buffer (pH 7.4) at 37±0.1 °C.

Systems	Peaks (m/z)
Gxb	443, 478, 487, 492, 643, 671, 790
Axa	364, 393, 407, 439, 449, 490
Ax-1J (Ax:Gx)	354, 355, 372, 386, 655, 673
SAx-1b (AxG)	336, 358, 392, 384, 444, 470
SGx-1b (AGx)	322, 340, 460, 455

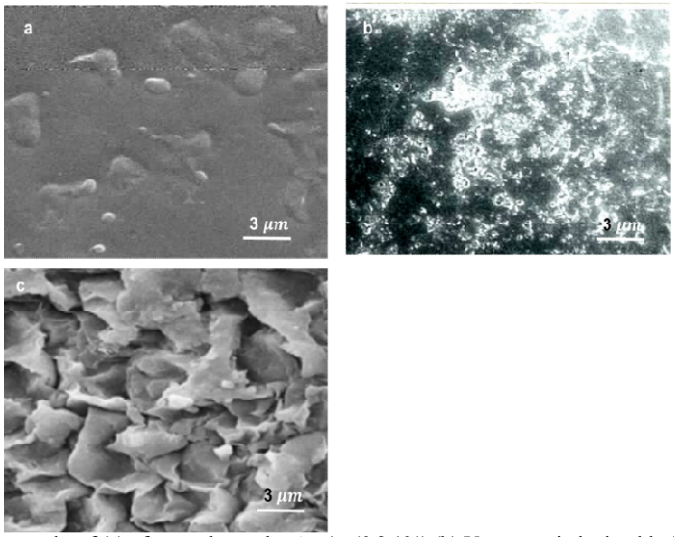


Figure 5: Scanning electron micrographs of (a) of control samples Ax-1n (0.2,1%) (b) Vancomycin hydrochloride loaded Ax-1n (0.2,1%), (c)15 days in- vitro degraded samples of Ax-1n (0.2,1) in phosphate buffer (pH 7.4).

IV. CONCULSION

The results of the current study revealed that degradation and drug release from IPNs aredependent upon the cross-linking concentration of respective chains. Time taken for *in-vitro*degradation reduced significantly with reduction in cross-linker concentration of acrylic acid and gelatin chain. Presence of vancomycin hydrochloride does not affect the rate of degradation loaded hydrogel. MALDI data depicted that degraded product have low molecular weight with no systemic toxicity. *In-vitro* release profile of drug shows burst effect followed by controlled release. Diffusion and degradation of hydrogel govern the drug release.

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CONFLICT OF INTREST

There is no conflict of interest.

REFERENCES

[1] Adams K, Couch L, Cierny G, Mader JT. In vitro and in vivo evaluation of antibiotic diffusion from antibiotic-impregnated polymethylmethacrylate beads. ClinOrthopRelatRes. 1992,278:244–252.
[2] Chidambaram S,DebabrataB,Biswanath S, SomeswarD.Local drug delivery system for the treatment of osteomyelitis: In vitro evaluation.
[3] Calhoun JH, Mader JT. Antibiotic beads in the management of surgical infections. Am J Surg. 1989,157:443–449.Drug Development and Industrial Pharmacy, 2011 37:538-546.
[4] Calhoun JH, Mader JT. Treatment of osteomyelitis with a biodegradable antibiotic implant. Clin OrthopRelat Res.1997,341:206–214.
[5] Carek PJ, Dickerson LM. Sack JL. (2001) Diagnosis and management of osteomyelitis. Am. Fam. Physician. 2001, 63:2413–2420.
[6] Ji-Hyun L, Jong-Min B, Young-Soo Y, Joo Hyun K, Chi Bum A, Kuk HS, Joo-Hyung K, Eun SC, Jin WL (2020) Development of a heat labile antibiotic eluting 3D printed scaffold for the treatment of osteomyelitis. Sci Rep. 2020, 10: 7554.
[7] Amit GK, Raja B, DeepthyM,enonandManithaBN.Biodegradable nanocomposite fibrous scaffold mediated local delivery of vancomycin for the treatment of MRSA infected experimental osteomyelitis. Biomater. Sci. 2020, 8: 2653-2665.
[8] Leah HC, Emily MM, Lauren Apitherapeutic and delivery vehicles for local treatment of osteomyelitis. J OrthopRes.2020,38(10): 2091-2103.
[9] Kavanagh N, Ryan EJ, Widaa A, Sexton G, Fennell J, O'Rourke S, Cahill KC, Kearney CJ, O'Brien FJ, Kerrigan SW. Staphylococcal osteomyelitis: disease progression, treatment challenges, and future directions. Clin Microbiol Rev. 2018, 31: e00084-17.
[10] Gao P, Nie X, Zou M, Shi Y, Cheng G. Recent advances in materials for extended-release antibiotic delivery system. J Antibiot. 2011,64:625– 634.
[11] Jason AI.Edward MS, Stephen LK, Hani AA. Biomaterials approaches to treating implant-associated osteomyelitis. Biomaterials.2016,81: 58-71.
[12] Murugesan G, Nachimuthu L, Kannan S, MarudhamuthuM,MariappanR.Calcium alginate nanoparticle crosslinked phosphorylated polyallylamine to the controlled release of clindamycin for osteomyelitis treatment.Drug Development and Industrial Pharmacy .2021,47:280-291.
[13] Mohammad FA, Deepak Y,Sumeet K, Jagdish C, SamimM.Comparison of antibacterial activity of Ag nanoparticles synthesized from leaf extract of Parthenium hystrophorus L in aqueous media and Gentamicin sulphate: in-vitro.Drug Development and Industrial Pharmacy .2015,41:43-50.
[14] Hesaraki S, Moztarzadeh F, Nemati R, Nezafati N. Preparation and characterization of calcium sulfate-biomimetic apatite nanocomposites for controlled release of antibiotics. J. Biomed. Mater. Res. B Appl. Biomater.2009, 91: 651–661.
[15] Sripriya R, Kumar MS, Ahmed MR, Sehgal PK. Collagen bilayer dressing with ciprofloxacin, an effective system for infected wound healing. JBiomaterSciPolym Ed2007, 18:335–351.
[16] Zhang W, Guangyu L, Yang L, Wei W, Tao S, JinzhuF. Approach to osteomyelitis treatment with antibiotic loaded PMMA. MicrobPathog. 2017,102: 42-44.
[17] Hancock REW, Sahl H-G. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol. 2006, 24:1551–1557.
[18] Tatiana P, Catarina CC, Paulo C, NunoA, Susana RS, Fernando JM. Combining local antibiotic delivery with heparinized nano hydroxyapatite/collagen bone substitute: a novel strategy for osteomyelitis treatment. Mater Sci Eng C, 2021, 119:111329.
[19] Caplin JD, García AJ. Implantable antimicrobial biomaterials for local drug delivery in bone infection models. Acta Biomater. 2019,93: 2-11.
[20] .Majeti NV, Ravi K. Polymeric Controlled Drug-Delivery Systems: Perspective Issues and Opportunities. Drug Development and Industrial Pharmacy. 2001, 27: 1-30.
[21] Kapoor S, Kundu SC. Silk protein-based hydrogels: promising advanced materials for biomedical applications. Acta Biomater. 2016, 31:17–32.
[22] Thompson K, Petkov S, Zeiter S, Sprecher CM, Richards RG, Moriarty TF, Eijer H. Intraoperative loading of calcium phosphatecoated implants with gentamicin prevents experimental Staphylococcus aureus infection in vivo. PloS One. 2019,14: e0210402.
[23] Release behavior, mechanical properties, and antibacterial activity of ciprofloxacin-loaded acrylic bone cement: a mechanistic study.Marzieh G, Arash M, Hamid RM. Drug Development and Industrial Pharmacy .2020,46:1209-1218.
[24] Alghamdi AA, Saeed WS, Al-Odayni AB, Alharthi FA, Semlali A, Aouak T. Poly(ethylene-co-vinylalcohol)/poly(δ-valerolactone)/aspirin composite: model for a new drug-carrier system. Polymers,2019, 11:439.

[25] Xiaoli Y, Kunyan W, Lei Y, QiNing Y, Hongxia X, YanboL.Multi-stimuli-responsive poly(hydroxyethyl methacrylate-co-N-vinyl pyrrolidone-co-methacrylic acid-co-N-isopropylacryl amide) hydrogel: synthesis, characterization and application in drug release.IranPolym J. 2019, 11: 957-967.
[26] Hoffman AS. Hydrogels for biomedical applications, AdvDrug Deliv Rev. 2012,64:18–23.
[27] Hosseini H, Tenhu H., and Hietala S. Rheological properties of thermoresponsive nanocomposite hydrogels. JApplPolymSci. 2016, 133:43123.
[28] Rodkate N, Rutnakornpituk **B**, Wichai U, Ross G., and Rutnakornpituk M (2015) Smart carboxymethylchitosan hydrogels that have thermo-and pH-responsive properties.JApplPolym Sci. 2015,132:41505.
[29] WangK, Hao Y, Wang Y, Chen L, Mao L, Deng Y, Chen J, Yuan S, Zhang T, Ren J, and Liao W. Functional Hydrogels and Their Application in Drug Delivery, Biosensors, and Tissue Engineering. International Journal of Polymer Science. 2019, Article ID 3160732 (DOI: https://doi.org/10.1155/2019/3160732)
[30] Yang JA, Yeom J, Hwang BW, Hoffman AS, and Hahn SK. In situ-forming injectable hydrogels for regenerative medicine. Prog Polym Sci. 2014,39:1973–1986.
[31] Chang C, and Zhang L. Cellulose-based hydrogels: present status and application prospects. CarbohydrPolym2011, 84:40–53.
[32] Murakami T, Schmidt BVKJ, Brown HR, and Hawker. One-pot “click” fabrication of slide-ring gels. Macromolecules.2015,48:7774–7781.
[33] Burugapalli K, Bhatia D, Koul V, Choudhary V. Interpenetratingpolymer networks based on poly(acrylic acid) and gelatin I:swelling and thermal behavior. J. Appl. Polym. Sci.2001,82:217–227.
[34] Walker CA, Kopp B. Sensitive Bioassay for Vancomycin. Antimicrob Agents Chemother.1978,13: 30-33.
[35] Bauer AW, Kirby WM., Serris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol.1966,45:666YN493-496.
[36] Changez M, Burugapalli K, Veena K, Veena C. The effect of composition of poly(acrylic acid)–gelatin hydrogel ongentamicin sulphate release: in vitro, Biomaterials.2003, 24 : 527–536.
[37] Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC (1978) Enzymatic determination of total serum Urea. Clin Chem.1978, 20:470-475.
[38] Changez M, Veena K, Burugapalli K, Amit KD, Veena (2004) Studies on biodegradation and release of gentamicin sulphate from interpenetrating network hydrogels based on poly(acrylic acid) and gelatin: in vitro and in vivo, Biomaterials,2004, 25: 139–146.
[39] Wang-Xun W, Yen-Chuan H, Wen-Fu L (2020) Effect of poly(ethylene glycol)-derived cross-linkers on the properties of thermos sensitive hydrogels.IranPolym J,2 020, 29: 679–691.
[40] KabiriK, OmidianH, Hashemi SA,Zohuriaan-Mehr MJ. Synthesis of fast-swelling superabsorbent hydrogels: effect of crosslinker type and concentration on porosity and absorption rate. Eur PolymJ.2003,39: 1341-1348.
[41] Kinetics Peppas N, Ritger PL (1987) A simple equation for description of solute release I. Fickian and non-Fickian release from non-swellable device in the form of slabs, cylinder or disc. J ControlRelease.,1987, 5:23–36.
[42] Peppas N, Ritger PL (1987) A simple equation for description of solute release II. Fickian and anamolous release from swellable device. J ControlRelease.1987, 5:37–42.