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Dual Responsive Pectin Hydrogels and Their Silver Nanocomposites: Swelling Studies, Controlled Drug Delivery and Antimicrobial Applications

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Novel dual responsive pectin hydrogels composed from poly(acrylamidoglycolic acid-*co*-vinylcaprolactam)/ Pectin (PAV-PC) and also PAV-PC hydrogels are used as templates for the production of silver nanoparticles. 5-Fluorouracil is an anticancer drug and has been loaded *in situ* into PAV-PC hydrogels. Structure and morphology characterization of PAV-PC hydrogels were investigated by fourier transform infrared spectroscopy, differential scanning calorimetry, thermo gravimetric analysis, X-ray diffraction studies, scanning electron microscopy and transmission electron microscopy. The results revealed a molecular level dispersion of the drug in PAV-PC hydrogels. *In vitro* release of 5-fluorouracil from the PAV-PC hydrogels has been carried out in GIT fluids as well as in various temperatures. 5-Fluorouracil released from PAV-PC hydrogels was 50% at pH 1.2, and 85% at pH 7.4 within 24 h. The release profile was characterized with PAV-PC hydrogels and initial burst effect was significantly reduced in two buffer media (1.2 and 7.4), followed by a continuous and controlled release phase, the drug release mechanism from polymer was due to Fickian diffusion. *In situ* fabrication of silver nanoparticles inside the hydrogel network *via* the reduction of sodium borohydrate by PAV-PC chains led to hydrogel nanocomposites. The diameter of the nanocomposites was about 50-100 nm, suitable for uptake within the gastrointestinal tract due to their nanosize range and mucoadhesive properties. These nanocomposite PAV-PC hydrogels showed strong antimicrobial activity towards *Bacillus subtilis* (G+ve) and *Escherichia coli* (G-ve).

Key Words : Pectin, Nanocomposite hydrogels, Controlled release, 5-Fluorouracil, Antimicrobial activity

Introduction

Hydrogels are three-dimensional high-molecular weight networks composed of a polymer backbone, water and a crosslinking agent. They are gaining tremendous importance in a wide variety of applications in medical, pharmaceutical and related fields, *e.g.* wound dressings,[1] contact lenses,[2] artificial organs and drug deliverysystems.[3] Bio-hydrogels from plants or animal derived macromolecules, in general, are biodegradable, because they are susceptible to human enzymes of the many carbohydrate-based biopolymers, pectin, chitosan, hyaluronate, heparin sulfate, and sodium alginate have a long history of safe use, and are well documented for biocompatibility, biodegradability, and low toxicity. These bulk (macro) and nano-sized hydrogels, which are known to be smart materials with tunable properties, morphology, size, and hydrophilic/hydrophobic balance, are indispensable materials for numerous applications in biomedical fields such as drug delivery, catalysis, and the development of antimicrobial materials.[4-7] Due to their hydrophilic surface has a low interfacial free energy in contact with body fluids, which results in a low tendency for protein and cells to adhere to these surfaces. Moreover, the soft and rubbery nature of hydrogels minimizes irritation to surrounding tissues. As a result, in comparison to other synthetic materials, hydrogels resemble nature living tissues closely in their physical properties due to their high water contents and softness, which also contributes to biodegradability and biocompatibility. Therefore, it can find applications in different technological areas such as materials for contact lenses and protein separation, matrices for cell encapsulation and devices for controlled release of drugs and proteins.[8-12]

Pectin is a naturally occurring biopolymer, which constitutes a(1-4)-linked α-D-galacturonic acids, with varying degree of methylation of carboxylic acid residues.[13] It has been used widely in the food and beverage industry as a gelling agent, a thickening agent and widely used in the production of jams and jellies, confectionary products and for stabilization of yogurts[14] and drug production such as for antidiarrheic, detoxicants and as protectors of gastrointestinal tract.[15,16] However there has been recent interest in the use of pectin gels in controlled drug delivery.[17,18] Its gelling ability and solubility strongly depends upon the pH of the surrounding media. This is in part due to their long standing reputation of being non-toxic and the low production cost of pectin make them of great interest for the formulation of controlled release dosage forms.[19] Pectin could be used to deliver drugs orally, nasally and vaginally[20-26] which are generally well accepted by patients.[2,7,27] Pectin has the mucoadhesive property and their films have low thermal stability and poor mechanical properties; hence it was blended with different polymers to improve its thermal and mechanical stability.[28-31] The biodegradable polymers are important by colonic bacteria holds great promise. Pectin is a polysaccharide having

bacterial enzymes that are available in sufficient quantity to be exploited in colon targeting of drugs.[32] 5-FU is a most commonly used chemotherapeutic drug, it is a pyramidine analog which is widely used in the treatment of cancer due to its potent curative effect. 5-FU having short half life due to rapid metabolism, incomplete and uniform oral absorption due to rapid metabolism by dihydropyramidine dehydrogenage and non selective action against healthy cells. Hydrogels have been used as a drug carriers, the 5-FU can be loaded into the hydrogel network and release a 5-FU effectively by swelling and shrinking effects simultaneously. The drug release from the dual responsive (pH/temperature) hydrogels much faster than chemical bond linking polymer drug compounds and can controlled by changing the external environment.[33] 5-FU having short half life, serious toxicity and low bio-availability to overcome this 5-FU incorporated into pectin hydrogel network, to increase the release time of 5-FU and its efficacy, to reduce the 5-FU associated side effects and there by improve its therapeutic index.

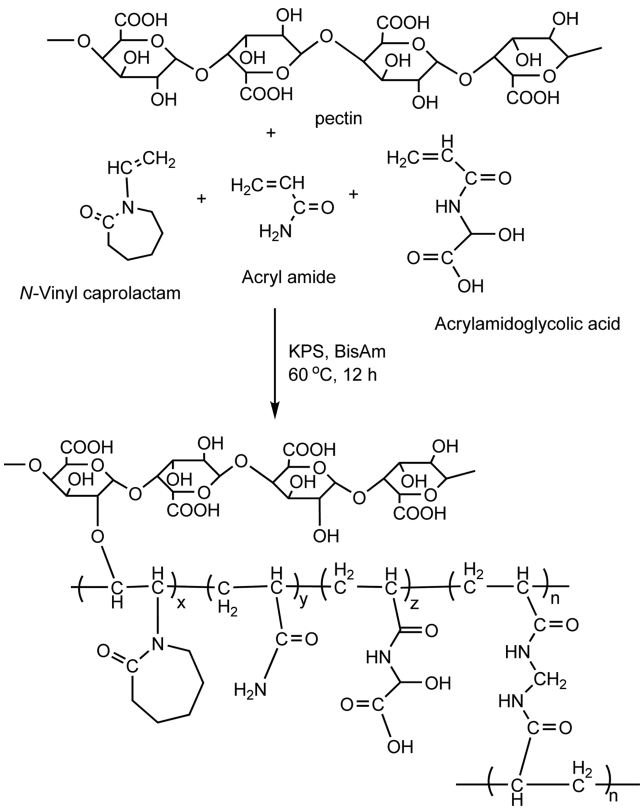
The design and synthesis of metal nanoparticles or polymer composites have been very interesting in the field of nanoscience and nanotechnology[34] due to their size effects compared to those of bulk metal and molecular compounds. The noble metals, especially silver and gold, have attracted great attention due to their innumerable applications in various fields of sciences. For better stabilization or dispersion of metal nanoparticles or polymer composites in aqueous media, various polymers (such as natural or synthetic)/ protective agents were used as a supporting materials and play a wonder role in controlling the nanostructures of the particles. These nanocomposites are used as catalysts and in the biomedical applications.[35] Nowadays the macroscopic gels are becoming most promising as templates/nano reactors for *in situ* synthesis of smaller size metal nanoparticles and this strategy has brought up a new concept in hybrid or composite systems in chemistry and engineering science.[36,37] Silver nanoparticles are considered as nontoxic and environmentally friendly antibacterial materials, but due to their poor binding characteristic with surfaces, their utility has been restricted. Therefore, polymer-stabilized nanoparticles and nanoparticles embedded in hydrogel networks are outstanding approaches for antibacterial applications.[37-39] The hydrogel, microgel, and nanogel systems were used as templates to produce metal nanoparticles, the silver nano composite hydrogel architectures as promising antibacterial materials, in which silver nanoparticles incorporated into the PAV-PC hydrogels. These PAV-PC silver nano composite hydrogels were used as biomedical applications.

The present study describes the development of novel specific drug delivery systems for 5-FU using PAV-PC hydrogel carrier. The PAV-PC hydrogels were evaluated for physicochemical properties, drug content, dissolution, swelling studies and *in vitro* drug release studies. The dissolution profile and *in vitro* release kinetics showed that pectin hydrogels were promising for controlled delivery of the 5-FU. PAV-PC hydrogels are used as templates for production of silver nanoparticles and used for antimicrobial study against the *Bacillus* (G+ve) and *E. coli* (G-ve). The results demonstrate that pectin hydrogels are promising for colon targeting of 5-FU to synchronize the chronobiological symptoms for effective treatment of anticancer.

Experimental

Materials. Pectin (PC), Himedia (Degree of esterification is 65-70%), *N*-vinylcaprolactam (NVC), acrylamidoglycolic acid (AGA), *N,N'*-methylenebisacrylamide (99%) (MBA), silver nitrate (99.0%), 5-fluorouracil (5-FU) and sodium borohydrate were obtained from Aldrich and used without further purification. Ammonium per sulphate (APS) was obtained from s.d. fine chemicals and all other chemicals were analytical grade and were used as received. All experiments were carried in double distilled water (DDW).

Synthesis of PAV-PC semi-IPN Hydrogel. PAV-PC *co*-polymeric semi-IPN hydrogels were prepared by simple redox polymerization method. Monomers were dissolved in 3 mL of distilled water, to this solution 1 mL of crosslinking agent (1 wt % aqueous solution), 1 mL of potassium persulfate solution (10 wt %), and 2 mL of 2 wt % PC solution. The free-radical crosslinking polymerization was carried out in a 50 mL beaker maintained at 55 °C temperature for 10 h in order to complete network formation. These gels were cut into disks 10 mm in diameter and 2 mm in thickness, and then immersed in an excess of DDW for one day to remove the residual unreacted monomer.



Scheme 1. The schematic representation of the synthesis of the PAV-PC hydrogels.

Table 1. Preparation of PAV-PC hydrogels and release kinetics parameters of different formulations

Code	AGA/NVC (gm)	Pectin (2%) mL	MBA (1%) mL	%EE	n	r^2	$k \times 10^{-3}$
A	0.125/0.25	2	1	8.5	0.7209	0.9799	1.349
B	0.25/0.25	2	1	11.3	0.4271	0.9867	1.349
C	0.50/0.25	2	1	15.6	1.0655	0.9734	0.192
D	0.25/0.25	2	2	10.9	0.9978	0.9902	0.309
E	0.25/0.25	2	0.5	11.1	0.8552	0.9804	1.052
F	0.25/0.00	2	1	17.4	0.7126	0.9865	0.468
G	0.00/0.25	2	1	20.0	1.0687	0.9791	0.217
H	0.25/0.50	2	1	22.1	1.0078	0.9652	0.099
I	0.25/0.25	0	1	5.7	0.5238	0.965	13.797
J	0.25/0.25	1	1	6.5	0.5633	0.9789	11.58
K	0.25/0.25	3	1	8.1	0.832	0.9846	1.988
L	0.25/0.25	4	1	10.4	0.414	0.98	19.09

For FT-IR analysis, the swollen hydrogels were dried at room temperature for 2 days and vacuum dried until attainment of constant weight. The scheme is presented in Scheme 1. The details of monomer and polymer weights are presented in Table 1.

Fabrication of PAV-PC Silver Nanocomposite Hydrogels. A solution of silver nitrate in water (5 mM) was added into the swollen PAV-PC hydrogel and allowed to equilibrate. The equilibrated hydrogels are immersed to the freshly prepared $NaBH_4$ solution. The reaction was left to proceed overnight in the dark, the colour of the hydrogel turned from yellow to brown. The silver nanocomposite hydrogels were immersed in distilled water which was replaced at regular intervals over a period of one day, to remove unreacted silver salts. The silver nanocomposite PAV-PC hydrogels are named as PPHNCS.

The dried PAV-PC semi-IPN hydrogels of the same weight were equilibrated in DDW at 30 °C for 2 days. These PAV-PC hydrogels were treated first with Ag salt and subsequently with $NaBH_4$ solutions as explained in the experimental section.

Swelling Studies. Swelling ratio of the PAV-PC hydrogels was measured as a function of temperature and pH of swelling medium. For the pH and temperature measurement, the PAV-PC hydrogels was soaked in solutions of different pH values ranging from 1.2 and 7.4 and temperatures ranging from 25 °C and 37 °C respectively. The dried samples were immersed in various solutions with certain pH and temperature for 24 h. The solutions was replaced frequently through the swelling process to ensure complete equilibration at the desired pH and temperature, the weight of the swollen semi-IPNs was measured against time, after the surplus surface adhered water of swollen hydrogel removed by filter paper. The percentage of water absorption/water retention and equilibrium swelling ratio of PAV-PC hydrogels can be determined by the following equations and observed in Figure 3.

$$\text{Swelling ratio (\%)} = \left(\frac{W_t - W_o}{W_o}\right) \times 100 \quad (1)$$

$$\text{Equilibrium swelling ratio (\%)} = \left(\frac{W_\infty - W_o}{W_o}\right) \times 100 \quad (2)$$

Where W_o is the initial dry weight and W_t is the swollen weight of the hydrogel at time t. This experiment was performed in triplicate. Here, W_∞ is the weight of equilibrium swollen hydrogels. This study of PAV-PC hydrogels have been performed using an electronically controlled oven, initial mass of the hydrogel was taken over a single-pan digital microbalance (Sartorius, Model BSA224S-CW, Singapore) sensitive to ± 0.01 mg.

Encapsulation Efficiency of 5-Fluorouracil. PAV-PC hydrogels were loaded with 5-fluorouracil (5-FU), an anticancer drug by swelling equilibrium method. The hydrogels were allowed to swell in the 5-FU solution of known concentration for 24 h at 37 °C. In general solubility of 5-FU in water is very low (13 mg/mL), but the solubility of the sodium salt increases up to 65 mg/mL.[32] In order to load the maximum 5-FU into the hydrogel matrix, hydrogel disks were immersed in NaOH neutralized 5-FU aqueous solution; 5-FU in the solvent was adsorbed onto the PAV-PC hydrogels. The loading efficiency of 5-FU in the hydrogel was determined spectrophotometrically. About 0.53 gm of the drug loaded hydrogel were placed in 50 mL of buffer solution and stirred vigorously for 48 h to extract drug from the hydrogels the solution was filtered and assayed by UV spectrophotometer (LAB INDIA, UV-3092) at fixed λ_{max} value of 271 nm. The results of % 5-FU loading and encapsulation efficiency were calculated, respectively using Eqs. (3) and (4). these data of %EE is presented in Table 1.

$$\%\text{5-FU loading} = \left(\frac{\text{Amount of 5-FU in the PAV–PN hydrogel}}{\text{Amount of PAV–PN hydrogel}}\right) \times 100 \quad (3)$$

$$\%\text{ Encapsulation efficiency of 5-FU} = \left(\frac{\text{Actual loading of 5-FU}}{\text{Theoretical Loading 5-FU}}\right) \times 100 \quad (4)$$

***In Vitro* Release Studies of 5-FU.** PAV-PC hydrogels were evaluated for the *in vitro* drug release in simulated gastro-

intestinal fluids (SGF). The 5-FU dissolution test of PAV-PC hydrogels was performed dissolution system (LABINDIA DS8000) by the paddle method specified in USP XXIII. PAV-PC hydrogels were immersed over the surface of 500 mL of dissolution medium (SGF). The content was rotated at 100 rpm at 37 °C ± 0.5 °C. Perfect sink conditions prevailed during the 5-FU dissolution study period. The simulation of gastrointestinal transit condition was achieved by altering the pH of dissolution medium at different time intervals. 5-FU release kinetics was analyzed by the following eqs. plotting the cumulative release data *versus* time.

$$\frac{M_t}{M_\infty} = kt^n \quad (5)$$

Where M_t and M_∞ are the amount of drug released by the hydrogel at time t and at equilibrium, k is a specific constant of the hydrogel, and n is a specific exponent of the mode of transport of the penetrate. The PAV-PC semi-IPN hydrogels was studied in buffer medium at pH 7.4 and pH 1.2. The samples were withdrawn from the dissolution medium at various time intervals using a pipette fitted with a micro filter. The receptor volume was maintained constant by replacing equivalent amount of SGF. The concentration of 5-FU in the samples was calculated based on average calibration curves. All dissolution studies were performed in triplicate.

Antimicrobial Activity of PAV-PC Hydrogel Silver Nano Composites (PPSNC). *In vitro* antimicrobial activity of PAV-PC silver nanocomposites (PPSNC) was assessed against two strains of bacteria namely *Bacillus subtilis* and *Escherichia coli* by zone of inhibition. Nutrient agar medium was prepared by mixing peptone (5 g), beef extract (3 g), and sodium chloride (NaCl) (5 g) in 1000 mL DDW and the pH are adjusted to 7. Finally, agar (15 g) was added to this solution and this medium was sterilized in an autoclave at a pressure of 15 lbs for 30 min at 121 °C. This medium was transferred into sterilized petri dishes in a laminar air flow chamber. After solidification of the media, the strains were kept at 4 °C for the antimicrobial tests. Petri dishes were inoculated with test bacteria solutions *Bacillus*, and *E. coli.* culture (20 μL, 300 cells/μL) were uniformly spread on the solid surface of the media activated at 37 °C for 24 h. The filter paper discs disinfected of 6 mm in diameter was impregnated with sterile DDW and saturated with PPSNC, followed by lying on the agar plates. The agar plates of bacteria were incubated at 37 °C for 24 h. Tetracycline were used as control in this study, in order to form a visible zone.

Characterization. UV-vis spectra of PAV-PC semi-IPN silver nanocomposite hydrogels (10 mg in 1 mL of DDW) were carried out on a Lab India, UV-3092 spectrophotometer. PAV-PC semi-IPN hydrogels were characterized by Fourier transform infrared spectroscopy, (Perkin Elmer Spectrum Two, UK) performed in the range of 4000-500 cm^{-1}. Furthermore, to investigate the drug nature in the hydrogel matrix, DSC (Model-DSC SP, UK) analysis was preformed for pure 5-FU, 5-FU loaded hydrogel, and pristine hydrogel. TGA (Model-SDTQ600, USA) analysis was performed for pristine PAV-PC and PPSNC. Analysis of the samples was performed at heating rate of 10 °C/min under N_2 atmosphere at a purging rate of 100 mL/min. X-ray diffraction measurements were recorded using a Rigaku diffractometer (Cu radiation, λ = 0.1546 nm) running at 40 kV and 40 mA and were recorded in an angle of 5-60 °C at a speed rate of 5°/min to estimate the crystallinity of the sample. Morphology of the PAV-PC hydrogel was recorded using a scanning electron microscope (MERA\\TESCAN). The TEM measurements were performed on FEI Tecnai G2 S-Twin instrument operated at an accelerating voltage at 200 kV.

Results and Discussion

Synthesis of the PAV-PC Hydrogels. A simple free radical redox polymerization was used for formation of hydrogels. First the persulphate anion radicals formed then radical abstracts hydrogen from OH of the polymer to form alkoxy radicals on the substrate (pectin back bone). So, the resulted active substrate is radically initiate the polymerization of monomers (AGA, NVC and NVC) and MBA crosslinker led to a three dimensional network structure. In general carboxylate do not involve in the formation of radical in presence of persulphate anion radicals.

FTIR Analysis. According to the characteristic spectra of the pectin based hydrogel and drug loaded hydrogels in Figure 1, an attempt was made to determine the eventual presence of interactions between them. The two peaks around 1663 and 1608 cm^{-1} are due to amide-I and amide-II of acrylamide units. -CN and -CH stretching bands appear at 1440 and 2922 cm^{-1} respectively, further confirming the presence of amide groups. A broad band observed between 3600 and 3000 cm^{-1} is due to the overlapping of the -OH stretching bands of carboxylic acid and OH group of alcohol of AGA, PC and-NH of the amide groups. Moreover, a band is observed at 1694 cm^{-1} due to the presence of COO- of AGA and pectin in the spectrum of PAV-PC semi-IPN hydrogel in addition to a band at 1641 cm^{-1}. After drug

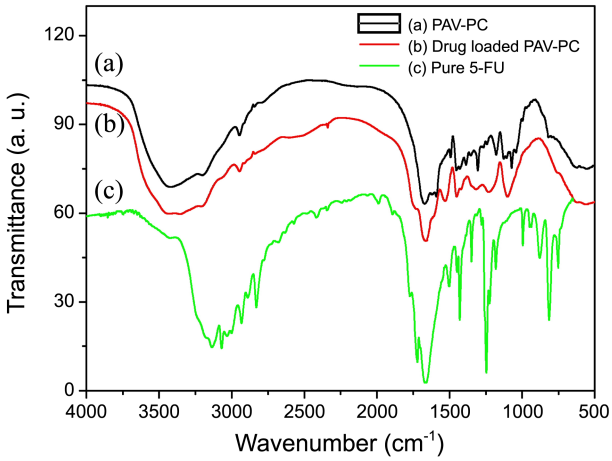


Figure 1. FTIR spectra of PAV-PC hydrogels composition C of (a) pure PAV-PC, (b) 5-FU loaded PAV-PC hydrogels and (c) pure 5-FU.

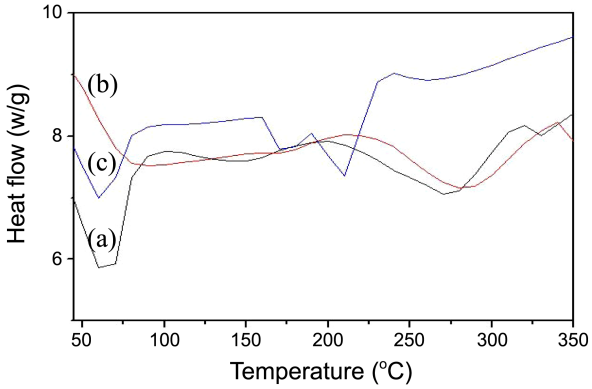


Figure 2. DSC thermo grams of (a) PAV-PC hydrogels and (b) PAV-PC drug loaded hydrogels and (c) pure 5-FU.

loading a peak at 2254 cm^{-1} and also at 1500 cm^{-1} is observed this indicates that the drug is molecularly dispersed in hydrogel network.

Differential Scanning Calorimetry (DSC) Studies. DSC Thermo grams of pristine hydrogel, 5-FU loaded hydrogels, and pure 5-FU are displayed in Figure 2. 5-FU shows a sharp peak at 285.16 °C due to polymorphism and melting, but in case of 5-FU loaded hydrogels no characteristic peak was observed at 285.16 °C, suggesting that 5-FU is molecularly dispersed in the hydrogel network.

X-ray Diffraction (XRD) Studies. The crystallinity of the pectin hydrogels has been demonstrated by XRD studies, and also to determine the maximum amorphous drug loading in polymeric hydrogels at room temperature. XRD data indicate the crystallinity for well-defined characteristic patterns of the 5-FU, PAV-PC hydrogel and to identify the formation of 5-FU loaded PAV-PC polymorphs in the hydrogel network. The 5-FU exhibit a characteristic crystalline peak at 2θ of 2.9° (Fig. 3(a)). The crystalline 5-FU, pristine PAV-PC hydrogel, and 5-FU loaded PAV-PC hydrogel were shown in Figure 3. It indicates that the PAV-PC hydrogel was an amorphous material in nature. For 5-FU loaded PAV-PC hydrogel, the characteristic peaks of crystalline 5-FU were not detected in the X-ray diffractograms, this indicates that 5-FU molecularly dispersed in the polymer matrix.

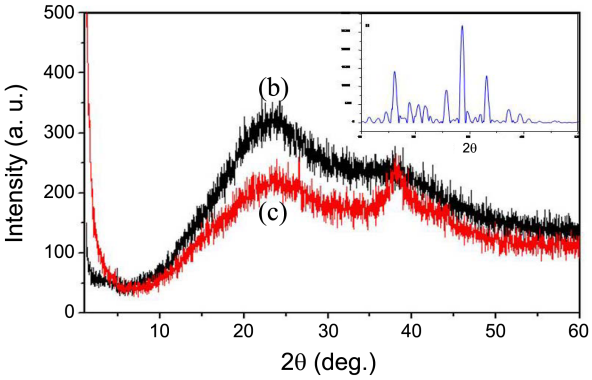


Figure 3. XRD pattern of the (a) pure 5-FU, (b) PAV-PC and (c) drug loaded PAV-PC hydrogels sample code is C.

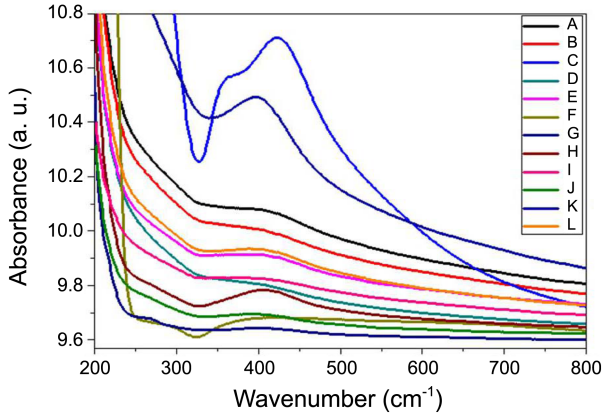


Figure 4. UV-absorption of PAV-PC silver nanocomposite samples from A-L.

UV-visible Spectroscopic Studies. The presence of embedded silver nanoparticles within the hydrogel networks is confirmed by using UV-vis spectrophotometer. As presented in Figure 4, all the UV-vis spectra of silver nanoparticles embedded in semi-IPN hydrogel networks have shown a distinct characteristic absorption peak around 420 nm. This is due to surface plasmon resonance effect of silver nanoparticles present in the hydrogel networks. With increase of pectin and AGA content in the hydrogel composition, the silver salt loading probably increases, resulting in more silver nanoparticle formation in the hydrogel structure which in turn progressively increases the absorption in the UV-vis spectra (Fig. 4).

Thermo Gravimetric Analysis (TGA). Thermogravimetric analysis provides information on the thermal stability of the nanocomposites relative to the polymers. In Figure 5, the PAV-PC and PAV-PC-Ag nanocomposite hydrogel has followed two decomposition steps and 95% degradation of the hydrogel chains occurred below 600 °C. However, it is noted as two degradation steps, the first step starts from around 110 °C indicates that weight loss of water molecules and the second step starts from 340 °C with 90% weight

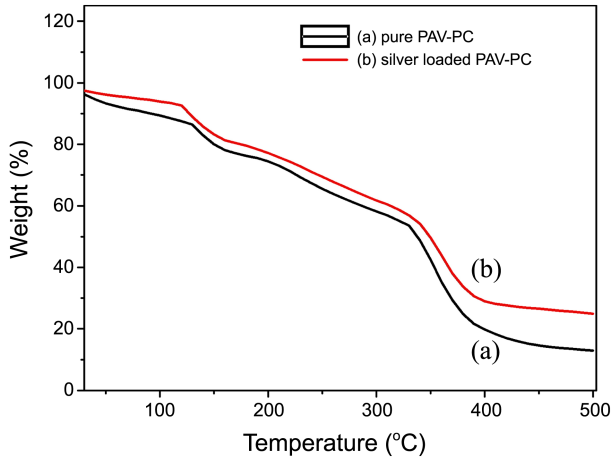


Figure 5. TGA curves of (a) PAV-PC hydrogel (b) PAV-PC silver nanocomposite hydrogel.

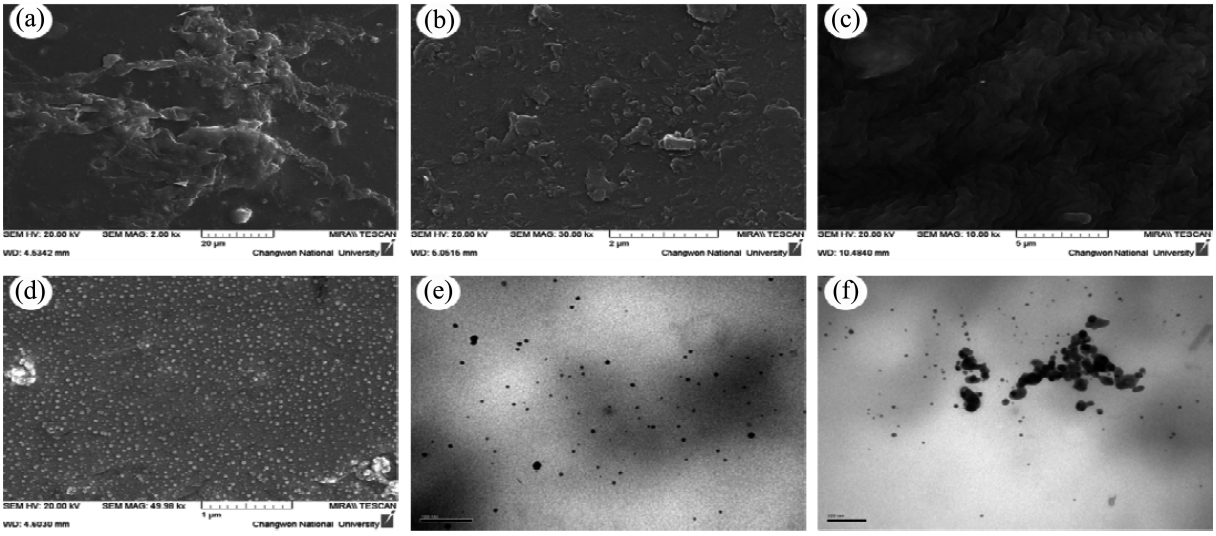


Figure 6. SEM images of (a) poly(Am-*co*-AGA)/pectin sample code is F, (b) poly(Am-*co*-NVC)/pectin sample code is G, (c) poly(Am-*co*-NVC-*co*-AGA)/Pectin sample code is C, (d) PAV-PC silver composites and (e), (f) TEM image of PAV-PC silver composite hydrogels sample code is C.

losses were occurred even at 500 °C in the case of PAV-PC-Ag composite hydrogel. The difference in decomposition between the hydrogel and silver nanocomposite hydrogel is found to be 20% and it confirms that the presence of silver nanoparticles (weight loss) in the hydrogel. As the percentage of metallic nanoparticles increases, difference in weight will be more. In present invention, silver nanoparticles play a role in degradation of hydrogel, which restricts the percentage of weight loss.

SEM and TEM Analysis. Scanning electron microscope images of poly(Am-*co*-AGA)/pectin, poly(Am-*co*-NVC)/pectin, poly(Am-*co*-NVC-*co*-AGA)/Pectin and PAV-PC silver nanocomposite hydrogels are shown in Figure 6, and also the TEM images of PAV-PC silver nanocomposite hydrogels. In Figure 6(a), PAV-PC hydrogels having the rough surface area, while in the case of Figure 6(b), PAV-PC also same but in case of Figure 6(c), the surface area of PAV-PC hydrogel is smooth. The SEM and TEM images of PAV-PC silver nanocomposites are observed in the Figure 6(d) and 6(e), 6(f). It was observed that pectin silver nanoparticles were circular in spherical shape with uniformly distributed throughout the hydrogel networks and maximum particles range within 50-100 nm in size.

Effect of Temperature at (25 and 40 °C). The temperature responsive swelling behavior of PAV-PC hydrogels was examined at different temperatures (25 and 40 °C) in neutral medium at preoptimized time (24 h) (Fig. 7(a)). Initially swelling ratio was found to decrease with increase in temperature. At higher temperature the non-elasticity of the polymeric matrix increased and also the effect of thermo sensitive monomer *N*-vinylcaprolactam (PNVC). PNVC containing hydrogels have LCST behavior, at below the LCST the PNVC hydrogels having good solubility due to interactions between water molecules and hydrophilic moieties. So that it can accommodate lesser water molecules. Also, at higher temperatures, the secondary bonding forces such as hydrogen bonds between water molecules and hydrophilic groups of the hydrogel network plays an important role. These hydrogen bonds formed a cage like structure and decreased the

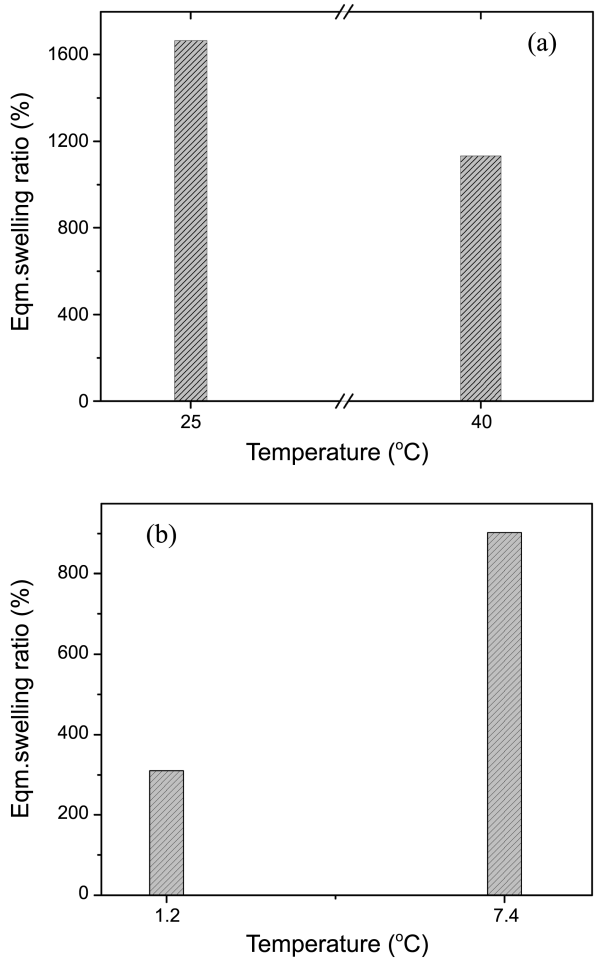


Figure 7. Equilibrium swelling ratio of (a) PAV-PC and (b) PAV-PC-Ag nanocomposite hydrogels.

water holding capacity of PAV-PC hydrogels which ultimately resulted in decreased swelling ratio. However, at higher temperatures, the polymer matrix collapsed and the hydrogen bonds broke leading to desorption of water molecules and also the shrinking effect of thermo sensitive monomer due to this decreased the swelling ratio of PAV-PC hydrogels.

Effect of pH (1.2 and 7.4) on Swelling Ratio. The smart swelling behavior of PAV-PC hydrogels with respect to change in solution pH was observed in the aqueous solutions of different pHs (1.2 and 7.4), Figure 7(b) shows the equilibrium swelling ratio of pectin semi-IPN hydrogels in pH buffers at room temperature. Due to the effects of ionic strength, the equilibrium swelling ratio of the hydrogels in pH buffers were universally smaller than those in ultrapure water. While thermo responsive poly(NVC) does not respond to the changes in pH, but poly(AGA) is a typical pH sensitive polymeric material that can deprotonate its carboxyl moieties in alkaline solution and protonate them in acidic solution. The extent of ionization of the carboxylic groups of pectin, which produces greater number of carboxylate ions along the pectin molecules, grows with the increasing pH value of the swelling medium from 1.2 to 7.4. These anionically charged centers repel each other and produce a rapid relaxation in the network chains, thus resulting in a rise in the degree of liquid uptake. Figure 8 shows the influence of various formulations on the swelling ratio of PAV-PC hydrogels and PAV-PC silver nanocomposites hydrogels. Considerable variation in the swelling ratio of PAV-PC hydrogels was found when the hydrogels were modified or loaded with Ag nanoparticles. The swelling ratio of PAV-PC hydrogels greater than the PAV-PC silver nanocomposite hydrogels. The silver ions present in the hydrogels are responsible for a moderate increment of the swelling behavior of silver loaded PAV-PC hydrogels.

Drug Release Behavior of Pectin semi-IPN Hydrogels. The dual sensitive release behavior of pectin semi-IPN hydrogels was evaluated in detail based on both pH and temperature variations. The drug release behavior of the various semi-IPN hydrogels was investigated at 25 °C (below LCST) and 37 °C (above LCST), depending on the release time, as shown in Figure 9(a), 9(b) and 9(c). both the amount of released drug and the release rate decreased as the temperature increased from 25 °C to 37 °C due to the higher shrinkage of the hydrogels. For the pH sensitive characteristics of the semi-IPN hydrogels, shown in Figure 9(a) and 9(b) the amounts of drug release increased drastically as the pH increased from 1.2 to 7.4 due to the faster swelling of the hydrogels.

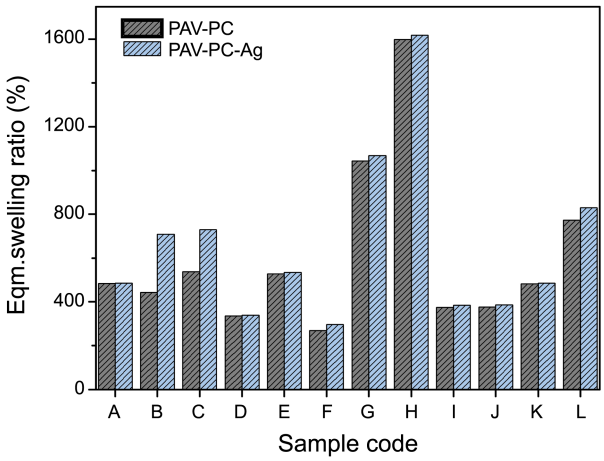


Figure 8. Influence of various formulations on the swelling ratio of PAV-PC hydrogels and PAV-PC silver nanocomposites hydrogels.

The content of crosslinker in the hydrogels also played some role in controlling the release behavior of the semi-IPN hydrogels, similar to the case of the swelling behavior. Therefore, the drug release behavior of the PAV-PC hydrogels can be controlled by varying the pH, temperature, and content of crosslinking agent. The *in vitro* drug-release studies were performed in pH 1.2 and 7.4 buffer media more than 45 h. hence, the present hydrogel developed is appropriate for the controlled release of 5-FU over an extended period of time up to 45 h.

Antimicrobial Activity. The synthesized PAV-PC silver nanocomposites were assayed for antibacterial activity against *Bacillussubtilis* (G+ ve) and *Escherichia coli* (G-ve) by taking tetracycline (30 mcg) as control by using disc diffusion methods. The silver nanoparticles showed zone of inhibition against both the bacteria. Maximum zone of inhibition was shown in *Escherichia coli*. The sample E was shown 75% efficiency in tetracycline (20.0 ± 1.0) in the same scenario A, B, D, G and H has shown 50% of effici-

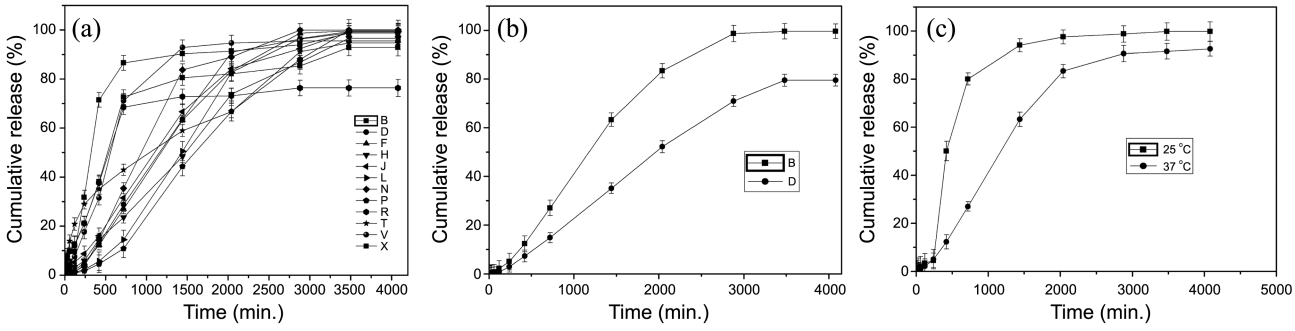


Figure 9. Cumulative release of PAV-PC hydrogels (A-L) at (a) pH 7.4, (b) sample C at pH 1.2 and pH 7.4 and (c) sample H at pH 7.4 with different temperatures.

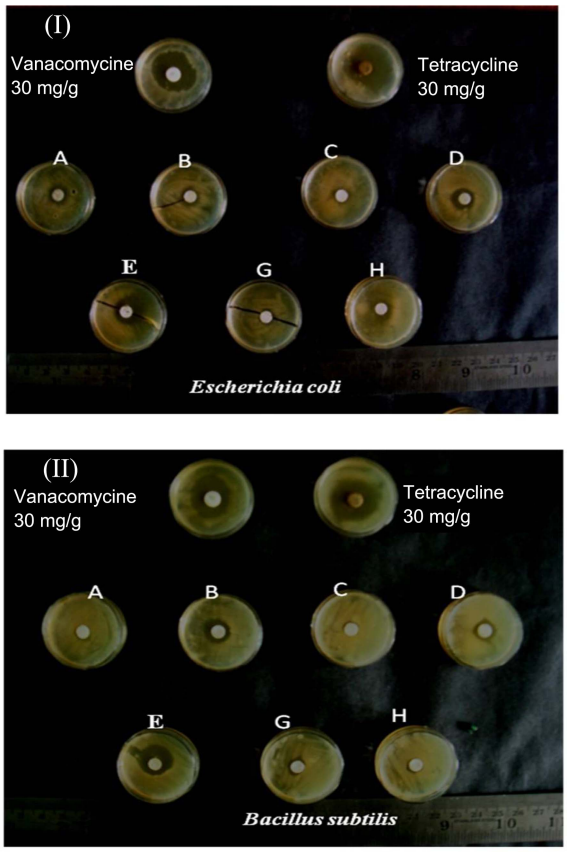


Figure 10. Anti-bactericidal activity of PAV-PC silver nanocomposites toward and *E. coli* (I) and *B. subtilis* (II).

ency and C doesn't shown minimum zone of inhibition. Whereas E also shown a good resistance against *Bacillus subtilis* B, D and G had shown 50% of moderate zone against tetracycline (21.3 ± 0.5) C samples doesn't shown zone of inhibition. Although pectin showed inhibitory effect against the two bacteria, the water-solubility and pH value that is far from the human physiological pH value limited its application. In the case of antibacterial mechanism against *Bacillus subtilis* and *Escherichia coli* it is generally believed that the positive charge of the *co*-polymeric group resulted in a polycationic structure absorbed onto the negatively charged cell surface of bacteria led to great alteration of the structure of outer membrane which caused release of major proportion of proteinaceous material from the cell. Cytoplasmic membrane is disrupted followed by the binding of PAV-PC nanocomposites, and then releasing cytoplasmic constituents, such as K^+ ions, DNA and RNA, ultimately leading to bacterial death.[40,41]

Table 2. Anti bacterial activities of the synthesized silver nanocomposite hydrogels on (I) gram negative (G−ve) and (II) gram-positive (G+ve) organisms

Sample	*E. coli* (G−ve)	*Bacillus subtilis* (G+ve)
Tetracycline-30 mcg/g	23 ± 1	21.33 ± 0.57
Vanacomycin-30 mcg/g	21 ± 1	18.66 ± 1.52
A	10.33 ± 1.52	6.66 ± 0.57
B	8 ± 1	11.33 ± 0.57
C	7 ± 1	6.66 ± 0.57
D	11 ± 1	10.33 ± 1.15
E	14 ± 1	15.33 ± 0.57
F	7 ± 1	8.66 ± 1.15
G	10.66 ± 1.52	6.66 ± 0.57

Conclusions

In conclusion, a series of novel dual responsive PAV-PC hydrogels and their silver nanocomposite hydrogels with no organic solvent were successfully prepared by a free radical redox polymerisation method. This hydrogel is biodegradable and has apparent temperature and pH responsiveness. The swelling ratio of PAV-PC hydrogels decreased accordingly when the temperature increases from 25 to 37 °C and increased when the pH value changed from 1.2 to 7.4. And also the dynamic swelling and deswelling behaviour has been investigated in this study. The silver nanoparticles are being formed not only on the surface of PAV-PC hydrogels but also throughout the hydrogel networks. In addition, the equilibrium swelling ratio of PAV-PC silver nano composite hydrogels are slightly higher than their PAV-PC hydrogels, due to dependent on their internal network structure. *In vitro* release behaviour of PAV-PC hydrogels have exhibited a prolonged release of 5-FU over an extended period of time up to 24 h. Release profiles varied depending upon the dual nature of the matrix. *In vitro* antimicrobial activity assessments exhibited PAV-PC silver nanocomposites showed pronounced inhibitory effect against the two bacteria (*B. subtilis* and *E. coli*). The PAV-PC hydrogels are promising derivative of pectin; it would be beneficial for some drug delivery systems and especially large potential applications in the oral administration of protein and peptide drugs and also in the pharmaceutical fields.

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