Saudi Pharmaceutical Journal 31 (2023) 101671

HOSTED BY

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Novel Natrosol/Pectin-co-poly (acrylate) based pH-responsive polymeric carrier system for controlled delivery of Tapentadol Hydrochloride

Nadiah Zafar [a,*], Asif Mahmood [b], Sehar Ilyas [a], Hira Ijaz [c], Rai Muhammad Sarfraz [d], Wael A. Mahdi [e], Mounir M. Salem-Bekhit [e,*], Mohamed A. Ibrahim [e], Yacine Benguerba [f], Barbara Ernst [g]

[a] Department of Pharmaceutics, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia
[b] Department of Pharmacy, University of Chakwal, Chakwal, Pakistan
[c] Department of Pharmaceutical Sciences, Pak-Austria Fachhochschule Institute of Applied Sciences and Technology, Mang, Khanpur Road, Haripur 22620, Pakistan
[d] College of Pharmacy, University of Sargodha, Sargodha, Pakistan
[e] Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia
[f] Laboratoire de Biopharmacie Et Pharmacotechnie (LPBT), Ferhat Abbas Setif 1 University, Setif, Algeria
[g] Université de Strasbourg, CNRS, IPHC UMR 7178, Laboratoire de Reconnaissance et Procédés de Séparation Moléculaire (RePSeM), ECPM 25 rue Becquerel, F-67000, Strasbourg, France

ARTICLE INFO

Article history:
Received 7 April 2023
Accepted 6 June 2023
Available online 12 June 2023

Keywords:
Natrosol
Pectin
Interpenetrating network
Controlled release
Tapentadol Hydrochloride

ABSTRACT

Background & Objectives: This study aimed to create a controlled delivery system for Tapentadol Hydrochloride by developing interpenetrating networks (IPNs) of Natrosol-Pectin copolymerized with Acrylic Acid and Methylene bisacrylamide, and to analyze the effects of various ingredients on the physical and chemical characteristics of the IPNs.

Methods: Novel Tapentadol Hydrochloride-loaded Natrosol-Pectin based IPNs were formulated by using the free radical polymerization technique. Co-polymerization of Acrylic Acid (AA) with Natrosol and Pectin was performed by using Methylene bisacrylamide (MBA). Ammonium persulfate (APS) was used as the initiator of crosslinking process. The impact of ingredients i.e. Natrosol, Pectin, MBA, and Acrylic Acid on the gel fraction, porosity, swelling (%), drug loading, and drug release was investigated. FTIR, DSC, TGA, SEM and EDX studies were conducted to confirm the grafting of polymers and to evaluate the thermal stability and surface morphology of the developed IPNs.

Results: Swelling studies exhibited an increase in swelling percentage from 84.27 to 91.17% upon increasing polymer (Natrosol and Pectin) contents. An increase in MBA contents resulted in a decrease in swelling from 85 to 67.63%. Moreover, the swelling was also observed to increase with higher AA contents. Significant drug release was noted at higher pH instead of gastric pH value. Oral toxicological studies revealed the nontoxic and biocompatible nature of Natrosol-Pectin IPNs.

Interpretation & Conclusion: The developed IPNs were found to be an excellent system for the controlled delivery of Tapentadol Hydrochloride.

1. Introduction

Crosslinked nexus exhibits fascinating biocompatibility due to which they find applications in pharmaceutical and biomedical fields (Ijaz et al., 2022). These nexuses are three-dimensional architectures that can imbibe a remarkable amount of water while retaining their mechanical attributes. These nexuses are known as intelligent material/ smart materials as these nexuses respond to external stimuli like ionic strength, pH and temperature which in turn promote phase transition, augmented volume and pore size of the matrix (Danish et al., 2021). Hydrophilic pendent moieties (-SO_3H, –OH, $CONH_2$ and –CONH) aid in retaining media thereby improving swelling which results in the formation of elastic, soft

* Corresponding authors at: Department of Pharmaceutics, College of Pharmacy, King Saud University, Saudi Arabia (Nadiah Zafar), Department of Pharmaceutics, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia (Mounir M.Salem-Bekhit).
E-mail addresses: nadiahzafar@uitm.edu.my (N. Zafar), mbekhet@ksu.edu.sa (M.M. Salem-Bekhit), Barbara.ernst@unistra.fr (B. Ernst).

Peer review under responsibility of King Saud University. Production and hosting by Elsevier.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2023.06.004
1319-0164/

architecture whose biological, chemical and physical properties can be manipulated as per desired response. A suitable section of polymeric microstructure is crucial as per desired applications. However, natural and semisynthetic polymers are more crucial for biomedical applications due to their excellent biocompatibility and biodegradability (Azam et al., 2021).

Pectin is a complex polysaccharide comprising of galacturonic acid units which are joined together through 1, 4-α-glucoside bonds (Padmasri et al., 2020; Danish et al., 2021; Azam et al., 2021). The literature survey supports the fact that Pectin has controlled release attributes as well as gelation capability which is attributed to the degree of esterification. Moreover, Pectin depicts pH-responsive behavior which aids in colon targeting (Gupta et al., 2002; Liu et al., 2007; Sundar et al., 2012; Grande et al., 2019; Naik et al., 2020). Natrosol (hydroxyethyl cellulose) (HEC) is a synthetic polymer; a polysaccharide derivative having thickening, gelling, stabilizing, and water-retaining properties (El Fawal et al., 2018). As Natrosol has 100 times the capacity to swell from its original weight, it is abundantly employed in pharmaceuticals to enhance the absorption of hydrophobic drugs (Torrest 1982). It has the unique property of displaying higher viscosity at lower temperatures and showing negligible gelation at higher temperatures (Harsoor 2011; Singh et al., 2013).

Acrylic Acid (AA) was employed as a monomer. It has unsaturated carboxylic groups with vinyl groups at each end. These carboxylic groups are responsible for their pH-responsive behavior. It promotes swelling at a pH of 6.0–––8.5. Inter-molecular interactions (H-bonding and electrostatic bonds) in IPNs are due to AA (Vera et al., 2003; Zhang et al., 2014).

Pectin/HEC/AA react with cross-linker forming 3-D nexus which serves as a matrix for controlled drug delivery. Some studies based on HEC and Pectin depicted both polymers as excellent biomaterials for cartilage regeneration. Careful control of synthesis parameters results in desired polymeric nexus with optimized attributes. Crosslinking agents (MBA) in such applications are vital as they allow bond formation between pendent groups of polymers (Pectin/HEC) and monomer (AA). The most commonly employed crosslinking agents are genipin, glutaraldehyde (GTA), and dextran. However, the use of the above-mentioned crosslinking agents is restricted due to cost and reported toxicity. In this work, we offered an alternative route by using MBA to formulate a less costly 3-D polymeric nexus with tunable attributes. MBA aids in crosslinking of one polymeric chain to another via covalent bonds. It is a water-soluble, white crystalline powder. It increases the density of the gel and stabilizes it by forming inter-molecular linkages between linear-polymeric chains (Pizzorusso et al., 2012).

Pectin/HEC/AA based nexus serves as a vehicle for controlled delivery of Tapentadol Hydrochloride. Tapentadol hydrochloride is a centrally-acting analgesic, having double action: noradrenaline reuptake inhibitor and μ-opioid receptor agonist. It has rapid oral absorption after administration. It has a short half-life of 4 h. It possesses poor protein binding of 20% and steady-state concentration is attained within 25 to 30 h following oral dosing after every 6 h. The recommended dose is 500–600 mg in divided doses after every 4 to 6 h (Harsoor, 2011). Bioavailability is very low (32%) due to extensive first-pass metabolic effects. It is metabolized by way of conjugation into glucuronides and hydroxyl derivatives (Singh et al., 2013). Low oral bioavailability and frequent dosing make it an ideal candidate to be incorporated into a controlled-release drug delivery system. The main advantages of employing Pectin/HEC/AA scaffolds are the controlled release drug delivery, good biodegradability, non-immunogenicity and excellent biocompatibility with negligible chances of dose dumping. Hence, the main idea of this work was to contribute to the development of a low-cost, biocompatible, and biodegradable carrier system that would allow a reduction in the dosing frequency of Tapentadol Hydrochloride while maintaining its excellent analgesic effect.

2. Material and Methods

2.1. Materials

Natrosol, Pectin, Acrylic Acid (AA) and Ethanol were procured from Sigma Aldrich, Germany. Ammonium persulfate (APS), methylene bis-acrylamide (MBA), hydrochloric acid (HCl) and potassium phosphate were purchased from Merck, Germany. Tapentadol Hydrochloride was graciously gifted by Jenner Pharmaceuticals, Lahore, Pakistan.

3. Methods

3.1. Development of Natrosol/Pectin-co-poly acrylate interpenetrating network

Free radical polymerization technique was used. A pre-weighed quantity of Natrosol was added slowly to already warmed (40 °C) 5 ml of distilled water. It was agitated until a transparent and slightly viscous solution was formed. It was labeled as solution A. A pre-weighed quantity of Pectin was added in already warmed (40 °C) 5 ml distilled water in divided portions to obtain a clear and slightly yellowish solution B. After that, the two solutions (A and B) were agitated together under constant stirring at 40 °C, in order to make a mixed polymeric solution, named solution C. Precisely weighed APS was dissolved in distilled water (5 ml) and named solution D. Specific amount of cross-linker MBA was added into a known quantity of AA under continuous stirring for 10–15 min to get clear solution E and the temperature was maintained at 40 °C. Next, solution D was carefully poured into polymeric solution C, and solution E was then added to this solution dropwise continuously for 5–10 min. Then, this solution was sonicated for 3–5 min at about 5000 rpm. The solution was sonicated to enhance mixing and remove any trapped or dissolved oxygen. The final solution was placed in test tubes and sealed with aluminum foil. A test tube stand was used to hold these test tubes in a water bath. Primarily, the temperature of the water bath was kept at 45 °C for about an hour. After an hour, the temperature was increased to 50 °C for two hours. Free-radical polymerization was completed during this time interval, leading to the development of IPNs. Test tubes were cooled at room temperature. IPNs were sliced into discs of specific dimensions with the help of a sharp blade and washed with a solution containing ethanol and water (30:70) respectively. Discs were then transferred into Petri dishes. First, the drying discs were done at room temperature and then dried in an oven at 45 °C to remove all traces of water in them. Twelve different IPN formulations (IPN1-IPN12) were developed by utilizing varying quantities of Pectin, MBA, Natrosol, and Acrylic Acid as mentioned in Table 1. Dried IPNs discs were stored for further analysis.

3.2. Optimization of formulation

In optimization process Central Composite Statistical Design was employed to optimize the nexus by using Design Expert® software (version 13.0.5.0. Stat-Ease., Minneapolis, MN, USA). In this design 4 factors and 2 levels of each variable (-1, 0 and + 1) was employed to studied the impact of independent variable (ratio of polymer, monomer and crosslinker) on dependent variable like swelling and drug release. Observed response was recorded. All experiment were conducted in triplicate and results were presented as ± SD.

Table 1
Composition of Natrosol/Pectin co-poly acrylate IPNs (IPN1-IPN12).

Formulation Code	Natrosol (g)	Pectin (g)	MBA (g)	Acrylic Acid (g)	APS (g)
IPN-1	0.1	0.1	0.1	6	0.2
IPN −2	0.1	0.1	0.2	6	0.2
IPN −3	0.1	0.1	0.3	6	0.2
IPN −4	0.1	0.15	0.2	6	0.2
IPN −5	0.1	0.2	0.2	6	0.2
IPN −6	0.1	0.25	0.2	6	0.2
IPN −7	0.15	0.1	0.2	6	0.2
IPN −8	0.2	0.1	0.2	6	0.2
IPN −9	0.25	0.1	0.2	6	0.2
IPN −10	0.1	0.1	0.2	8	0.2
IPN −11	0.1	0.1	0.2	10	0.2
IPN −12	0.1	0.1	0.2	12	0.2

Following are the equations for optimization;
Linear

$$116 + 13*A-8*C + 4*D-6*B*C-5*B*D-3*C2-1*D \quad 2$$

Curvilinear

$$112 + 8*A + 4*A*B + 1*A*D-1*B*C + 1*B*D-2*C*D-1*B \quad 2$$

3.3. Sol-Gel fraction (%)

Dried interpenetrating network discs were cut or crushed into small pieces and their weight (Wo) was noted by using a digital weight balance. Weighed interpenetrating networks disc pieces were placed into Soxhlet extractor at 100 °C with deionized water for a period of 24 h. Then the gel pieces were taken out of the extractor and were dried in an oven at 40oC. Dried IPN pieces were reweighed (Wi) (Ranjha and Qureshi, 2014). Sol-Gel fraction (%) was determined by using Eqs. (1) and (2):

$$Sol\,fraction\,(\%) = \frac{Wo - Wi}{Wo} x\,100 \quad (1)$$

$$\text{Gel fraction} = 100 - \text{Sol fraction} \quad (2)$$

3.4. Porosity testing

Dried and weighed IPN discs of all 12 formulations (IPN 1-IPN 12) were dipped in absolute ethanol for a period of 12 h separately. Excess ethanol was removed from the discs by placing them in a desiccator and weight of discs was noted again (Shin et al., 2002) [20]. Porosity of IPN discs was calculated through Eq. (3):

$$Porosity = \frac{(M2 - M1)}{\rho V} \times 100 \quad (3)$$

Where, M1 was the mass of IPN before dipping in ethanol, M2 was the mass of IPN after dipping into ethanol. ρ was density of ethanol and V was volume of IPN disc.

3.5. Drug loading (%)

Drug can be loaded in IPNs by two approaches either by pre-loading or by post-loading method. In current study, pre-loading approach was used. Typically, a specified amount of drug (~1.5 g) was dissolved in water and this mixture was poured into polymerization solution and stirred for about 5 min. Then the mixture was sonicated and poured in test tubes before its polymerization in water bath.

3.6. Fourier transform infrared (FTIR)

FTIR analysis was performed to confirm, identify and to check compatibility of formulation ingredients. Crushed sample was mounted on a crystal-mark made of potassium bromide with the aid of a spatula and some force was put in on sample for formation of compact disc for FTIR testing. Infrared spectrums of formulated Natrosol-Pectin interpenetrating network, Tapentadol Hydrochloride, Natrosol, Pectin, AA and MBA were recorded at 500–4000 cm^{-1} by FTIR spectrophotometer [Bruker Tensor 27 series, Germany] (Khanum et al., 2018).

3.7. Thermal analysis (DSC/TGA)

DSC was used to measure variation in heat capacity of the materials, to evaluate glass transition temperature (Tg) as well as to know the crystalline or non-crystalline nature of samples at a specific temperature range. DSC was done by utilizing TA instrument SDT Q600 (West, Sussex, UK). The samples were grounded into powder form to perform DSC. 10 mg of sample was sealed in an aluminum pan. The samples were heated over 0-500oC at a heating rate of 20 °C/minute having constant nitrogen flow (20 ml/min) (Minhas et al., 2020).

TGA was used to determine the thermal stability of pure ingredients as well as developed IPN. Thermogravimetric analysis was performed by using TA instrument SDT Q600 (West, UK). The samples were grounded into powder form to perform TGA. 10 mg of the sample was sealed in an aluminum pan. The samples were heated over 0-700oC at a heating rate of 10 °C/minute by utilizing nitrogen flow (20 ml/min) continuously (Minhas et al., 2020).

3.8. Powered X-ray diffraction (PXRD)

XRD analysis is used to confirm the crystalline or amorphous nature of samples. XRD analysis was done by utilizing an X-ray diffractometer (Siemens model D500, Cu K α radiation). PXRD was performed at room temperature under a voltage of 40 kV. Samples were scanned at 2θ angle ranging between 6o-80o at the speed of 20/min (Wang et al., 2016).

3.9. Scanning electron microscopy (SEM)

SEM was used to determine the surface morphology of Natrosol-Pectin IPN by using SEM (Hitachi, Tokyo, Japan, Quanta) analysis. IPN disc was crushed into small pieces and then coated with gold before scanning (Wong and Dodou, 2017).

3.10. Energy dispersive X-ray (EDX) spectroscopy

The elemental composition of samples was determined with Energy Dispersive X-ray (EDX) studies. Atoms present in elements emit X-rays with specific energy amounts. This energy was used to determine and identify elements within each sample. EDX peaks were recorded by utilizing EDX analyzer (Model EX-400; Japan) (Ichibouji et al., 2009).

3.11. Swelling studies

The swelling behavior of IPNs was studied at both acidic pH 1.2 and basic pH 7.4. The weight of dried IPN discs was recorded and then soaked in 200 ml of respective buffer solutions. Discs were taken out from the buffer solution after the predefined time intervals i.e. 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, 8, 9, 10, 12, 24, 36, 48, 60 and 72hr. Excessive buffer was absorbed by placing the discs on filter paper and weight was measured until the weight became constant. Equilibrium swelling (%) was determined by using Eq. (4) as given below:

$$Equilibrium\,swelling\,(\%) = \frac{Ws - Wi}{Ws} x\,100 \tag{4}$$

Ws = Weight of swollen of disc at specific time interval
Wi = Initial dry weight of disc

3.12. Tapentadol Hydrochloride release studies

To study the release of Tapentadol Hydrochloride from Natrosol-Pectin IPNs, dissolution studies were performed at different pH (acidic 1.2, basic 7.4). Dissolution apparatus Type II (USP) was operated at a speed of 50 rpm and temperature was maintained at 37oC. Pre-weighed IPN discs containing drug were placed in 900 ml of the buffer solution. After fixed time intervals, samples were removed (0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 18, 24, 30, 36 and 48 h) and analyzed. The drawn samples were scanned at 214 nm by using UV-spectrophotometer (UV-1800, Japan) (Patil et al., 2012).

3.13. Release kinetic studies

The data obtained after dissolution process was used to determine the best fit kinetic model and mechanism of release of drug from Natrosol-Pectin IPNs.

Zero order model indicates slow release of active pharmaceutical ingredient (drug) from drug-delivery systems and it can be expressed by Eq. (5) (Dash et al., 2010):

$$F_t = F_o - K_o\,t \tag{5}$$

1st order models are used not only to study the drug release pattern but they also represent absorption & excretion profile of drugs. It can be expressed by Eq. 6 (Dash et al., 2010):

$$\ln(1 - F) = K_1 t \tag{6}$$

Higuchi was first scientist to describe drug release from a matrix system with the help of a mathematical model. It can be expressed by Eq. (7):

$$F_t = K_H t^{1/2} \tag{7}$$

Where, *Ft* = fraction of drug release in time *t*, Fo = total amount of drug in hydrogel, Ko, K1 and KH are zero order, first order and Higuchi models rate constants, respectively.

Hixson and Crowell proposed a mathematical model expressed as Eq. (8):

$$\frac{Wo1}{3} - \frac{Wt1}{3} = k\,t \tag{8}$$

This model is used to study the drug release mechanism from a polymeric drug delivery system. Eq. (9) can be used for calculation of value of "n".

$$\frac{Mt}{M^{\infty}} = k_{kp} t^n \tag{9}$$

Mt/M∞ is the portion of Tapentadol Hydrochloride released at time t, Kkp and n are the rate constant and release exponent, respectively.

3.14. Oral toxicity experiment

According to OECD guidelines, oral toxicity testing was conducted on healthy rabbits in order to examine the safety level of prepared Natrosol-Pectin IPN carrier system. 12 male rabbits were selected for this study having weight of 1.25–1.75 kg out of which, 6 rabbits were grouped together as control group while other 6 rabbits were grouped together as a test group. Healthy food as well as water was provided to both animal groups on regular basis. A specific dose of crushed IPN system was given to the test group (2 g/kg). Physical activities, weight and appearance of rabbits were checked on regular basis for consecutive 2 weeks. At 14th day, blood samples were drawn from each group to perform biochemical test, LFT, RFT and lipid profile. After that, the rabbits were scarified on 14th day for histopathological examination to remove vital organs including liver, heart, brain, spleen, lungs, stomach and small intestine (Mahmood et al., 2019).

4. Results

4.1. Sol-gel fraction (%)

The influence of variable concentrations of Pectin, MBA, AA and Natrosol on gel fraction was evaluated as shown in Fig. 1. The results depicted that as the concentration of MBA, Pectin, Natrosol and AA was increased, the gel fraction (%) was also increased and sol fraction was decreased. Gel fraction was increased in the range of 72–97% for all 12 developed IPN formulations. Fig. 1 showed that as a concentration of MBA was increased (IPN1-IPN3) from 100 mg to 300 mg, gel fraction also increased correspondingly from 72.86% to 94.78% which is attributed to enhanced physical entanglements and crosslinking in-between polymeric chains eventually enhancing gel formation. When the amount of Pectin was increased (IPN4-IPN6) from 150 mg to 250 mg, the gel fraction also increased from 92.86% to 96.75%. Moreover, by increasing the Acrylic Acid contents (IPN10-IPN12), the gel fraction was also increased from 92.86% to 95.29% while sol fraction was reduced from 7.14 to 4.71%. Finally, when the amount of Natrosol was increased (IPN7-IPN9.) from 150 to 250 mg, gel fraction (%) also increased from 92.16% to 97.73% while sol fraction was reduced from 7.84% to 2.27%. Gelling time was also considerably reduced from 2 h to almost 1 h owing to the high gelling capacity of Natrosol in presence of a cross-linker.

4.2. Porosity testing

The results of porosity studies are presented in Fig. 2. It was observed that increasing cross-linker (MBA) concentration (IPN1-IPN3), decreased the percentage porosity from 64.23% to 49.56%, due to the increased cross-linked density and resulting dense cross-link nexus. On the other hand, increasing the amount of Natrosol in IPN7-IPN9 increased the porosity percentage from 49.63% to 64.25%, owing to increased ethanol absorption. Similarly, increasing the ratio of Pectin in the reaction mixture (IPN4-IPN6) increased the porosity from 40.24% to 60.25%, due to an increase

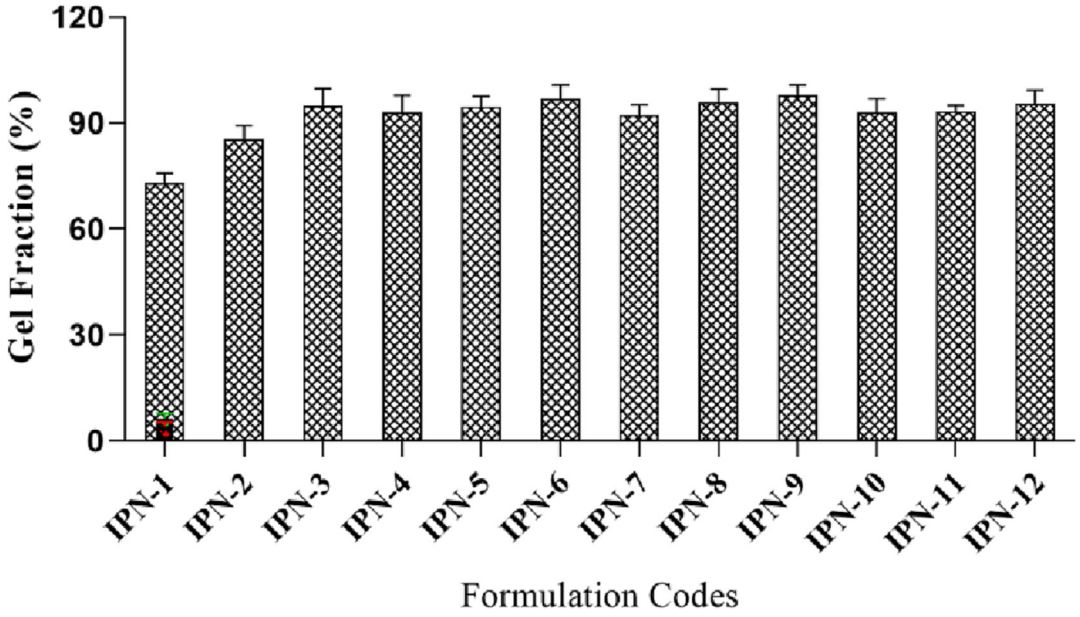

Fig. 1. Results of Gel fraction (%) (IPN1 – IPN-12).

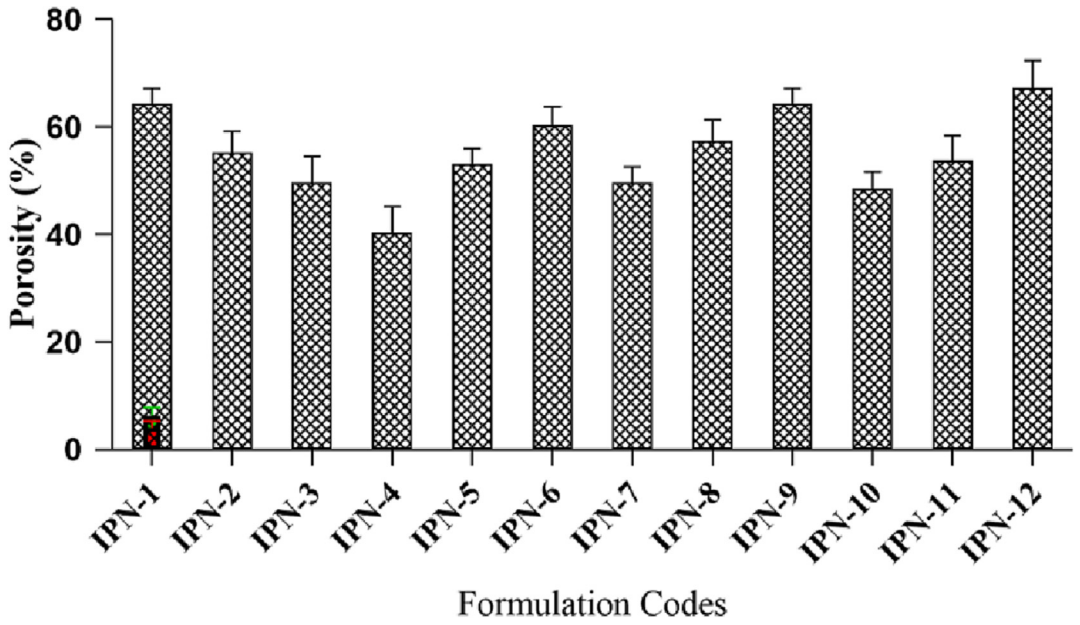

Fig. 2. Effect of formulation contents on Porosity (IPN1 – IPN-12).

in viscosity. Finally, increasing the amount of Acrylic Acid in IPN10-IPN12 caused a corresponding increase in porosity ranging between 48.45% and 67.15%.

4.3. FTIR analysis

IR Spectrum of Tapentadol Hydrochloride (Fig. 3A) depicted a prominent peak at 3157 cm-1 which was due to –OH stretching of the enol group (C = C-O–H) in phenols. One more characteristic peak was observed at 1594 cm-1 indicating N–H stretching due to hydrogen bonding with Cl. Sharp peaks were observed at 796 cm-1, 2672 cm-1, 1252 cm-1, 1216 cm-1, and 947 cm-1 due to C-Cl bonding, N–H, C-O, and C–C stretching respectively (Arjunan et al., 2015). The IR spectrum of Natrosol (Fig. 3B) showed strong peaks at 3306 cm-1, 1552 cm-1, 1409 cm-1, and 1017 cm-1 because of O–H, C–H stretching, and asymmetric stretching of C-O-C (El-Sayed et al., 2020). The IR spectrum of Pectin (Fig. 3C) displayed prominent peaks at 3308 cm-1, 1657 cm-1,1626 cm-1 and at 1230 cm-1 indicating –OH, >C = O, C = C, and C-O stretching, respectively (Rehmani et al., 2017). The IR spectrum of Acrylic Acid (Fig. 3D) displayed peaks at 1409 cm-1 and 1702 cm-1 due to C = C and C-O vibrational movements, respectively (Feng et al., 2018). IR of MBA (Fig. 3E) showed characteristics peaks at 3302 cm-1, 1656 cm-1,1625c, 1538 cm-1, 1409 cm-1, 1383 cm-1, 1304 cm, and 1224 cm-1 because of the presence of N–H groups and C = O bond (Ibrahim et al., 2019). FTIR spectrum of unloaded preparation (Fig. 3F) of Natrosol-Pectin IPN represented characteristics peaks at 1154 cm-1, 1704 cm-1, and 2929 cm-1. All characteristic peaks of

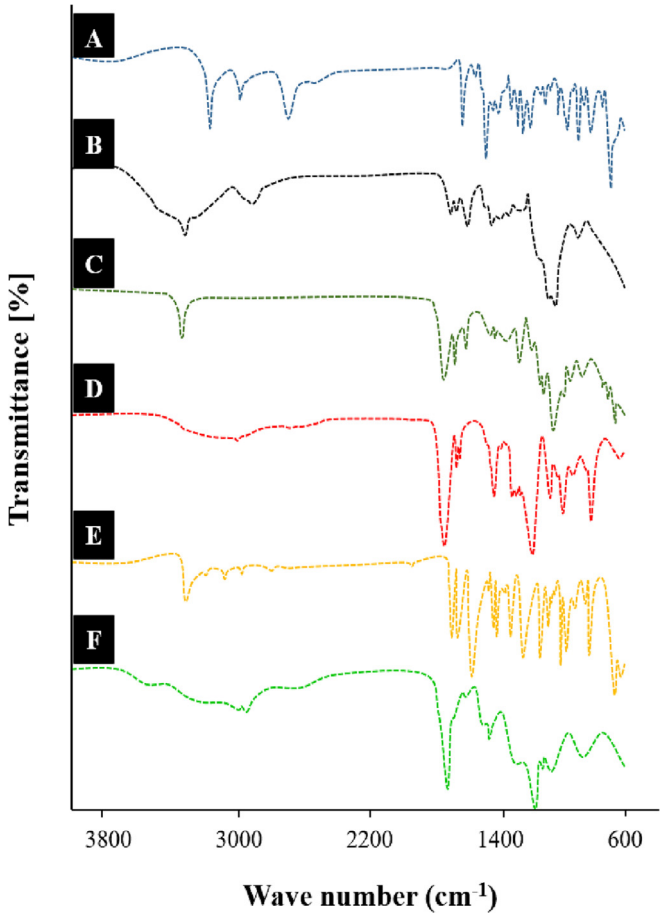

Fig. 3. FTIR spectra of A) Tapentadol Hydrochloride B) Natrosol C) Pectin, D) Acrylic Acid, E) MBA and F) Developed IPN.

individual ingredients were found to be absent from formulated IPNs which showed the formation of a new structure of interpenetrating network.

4.4. Thermal analysis (DSC/TGA)

DSC/TGA studies were used to test the thermal stability of pure ingredients, drug and developed interpenetrating networks. It was performed at a range of 0–700 °C. Fig. 4A showed DSC/TGA thermograms of Tapentadol Hydrochloride. Two sharp endothermic peaks were observed at 210 °C and 300 °C corresponding to the phase transition temperature of Tapentadol Hydrochloride. A small exothermic peak was observed at 440 °C due to the complete combustion of the pure drug. TGA thermogram depicted that weight loss event started at above 250 °C and more than 90% weight loss occurred at 305 °C.

DSC/TGA thermograms of Pectin were recorded as shown in Fig. 4B. DSC thermogram presented an initial endothermic peak near 100 °C due to loss of moisture contents. It was followed by another endothermic peak at 190 °C. The exothermic event started at 250 °C and continued up to 570 °C leading to the complete combustion of the polymer. In TGA analysis weight loss started at 100 °C due to loss of moisture contents. 15% weight loss occurred at 235 °C owing to the breakage of polymeric chains. 40 % weight loss occurred at 275 °C. Above 280 °C a major weight loss phase occurred leading to complete degradation at 570 °C.

DSC thermogram of Natrosol (Fig. 4 °C) exhibited exothermic peaks between 275 °C and 330 °C representing thermal degradation of polymeric linkages. TGA thermogram of Natrosol displayed one step degradation phase starting from 250 °C. Above 300 °C the remaining intact mass was up to 30 %.

DSC thermogram of Natrosol-Pectin IPNs loaded with model drug Tapentadol Hydrochloride (Fig. 4D) showed a broad endothermic band between 200 and 300 °C followed by exothermic peaks between 375 and 575 °C. Two sharp exothermic peaks were seen at 450 °C and 525 °C which were due to the complete combustion of the developed network. TGA thermogram presented an initial major weight loss event at 220 °C and the remaining 40 % weight loss occurred at 350 °C which continued up to 500 °C where only 10 % of mass remained intact. According to the above results, it was concluded that formulated IPNs were much more stable when compared with individual ingredients.

4.5. Powdered X-ray diffraction (PXRD)

X-ray diffractograms of Tapentadol hydrochloride, Natrosol, Pectin, and Natrosol-Pectin-co-poly acrylate IPNs were recorded. It was performed to determine the crystalline or amorphous nature of test samples. Diffractogram of Tapentadol Hydrochloride displayed many evident and sharp peaks such as 17.08°, 18.32°, 21.72°, 25.48° and 34.4° and these peaks substantiated the crystallinity of the drug. The Diffractogram of Natrosol exhibited only two distinguished peaks at 8.04° and 20.84° (Tian et al., 2020). The absence of sharp peaks confirmed the amorphous nature of the polymer. Diffractogram of Pectin presented a number of sharp and prominent peaks at 8.08°, 12.28°, 13.16°, 16.76°, 19.60°, 22.04°, 25.28°, 31.08° and 39.04° thus confirming its crystalline nature (Tian et al., 2020). Diffractogram of Natrosol/Pectin-co-poly acrylate IPNs showed that all the recorded peaks in diffractograms of individual ingredients have been transformed into fused peaks thus confirming the conversion of crystalline nature of drug into amorphous form within the developed IPN. Results are shown in Fig. 5.

4.6. SEM analysis

SEM micrographs of Natrosol-Pectin IPNs are shown in Fig. 6. SEM micrographs displayed wavy, rough, irregular and highly porous structure of prepared network. Wavy structure was because of overlapping of cross-linked polymeric chains which would have happened during drying process. Porous structure depicted that IPN would possess high swelling capability which aids in drug loading and release.

4.7. Energy dispersive spectroscopy

EDX studies were performed in order to recognize the composition of Tapentadol Hydrochloride, IPN loaded with Tapentadol Hydrochloride and unloaded IPN. Fig. 7A shows EDX spectrum of Tapentadol Hydrochloride. Usually, EDX spectrum displays the presence of a specific element as well as % the weight of each specified element found in that particular compound. Tapentadol Hydrochloride contained 71.74 % carbon, 9.34 % oxygen and 18.93 % chlorine as shown in Table 2. EDX spectrum of unloaded IPN formulation (Fig. 7B) was having 42.86 % carbon, 50.1 % Oxygen and 7.04 % Sulphur. EDX spectrum of Tapentadol Hydrochloride loaded IPN is shown in Fig. 7C. It can be clearly seen that chlorine is absent in unloaded IPN while it is present in Tapentadol Hydrochloride loaded IPN. Moreover, it contained 68.18 % carbon, 9.19 % oxygen and 22.64 % chlorine (see Table 3).

4.8. Equilibrium swelling studies

Equilibrium swelling studies were carried out to investigate the effect of two different pH environments i.e. 1.2 and 7.4 on the

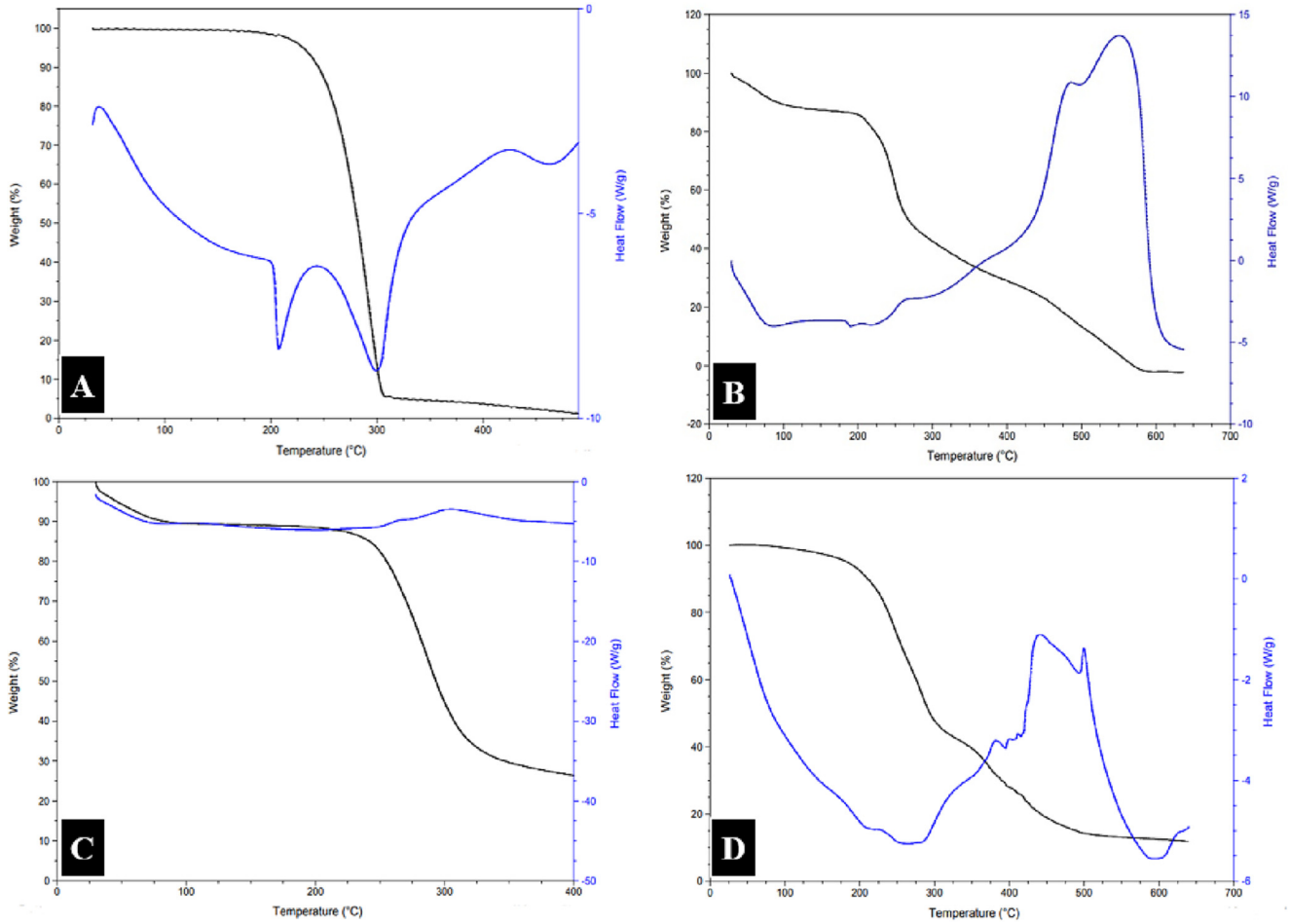

Fig. 4. DSC/TGA thermograms of A) Tapentadol Hydrochloride, B) Pectin, C) Natrosol and D) Developed IPN.

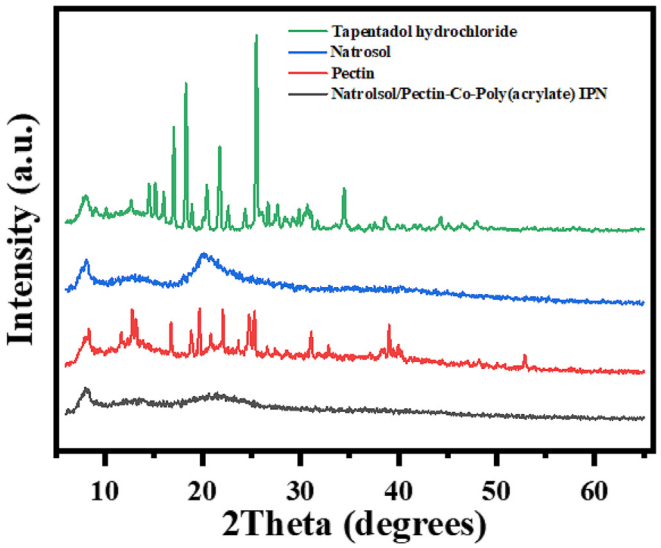

Fig. 5. X-ray diffractograms of Tapentadol Hydrochloride, Natrosol, Pectin and developed IPN.

developed network as shown in Fig. 8. At pH 1.2, swelling was minimal as carboxyl groups responsible for chain relaxation were unable to get ionized at pH 1.2. While at pH 7.4, pronounced swelling was noticed due to ionization of carboxylic groups into carboxylate ions as a result they repelled each other thereby promoting higher uptake of swelling media and subsequently displaying higher swelling.

4.9. Effect of cross-linker concentration on swelling

It was noted that by increasing the concentration of MBA, (IPN1-IPN3) swelling decreased from 85 – 67.63 % (pH 7.4) as shown in Fig. 9. Higher quantity of cross-linker resulted in the development of extremely firm network and masked the carboxylic groups due to which the electrostatic repulsion for chain elongation was decreased and thus, high crosslinking density justified low swelling behavior. No significant change in swelling was observed at pH 1.2.

4.10. Effect of pectin contents on swelling

By increasing the concentration of Pectin (IPN4-IPN6), no significant swelling change was seen at pH 1.2. However, at pH 7.4, we observed that when a greater concentration of Pectin was used, the swelling was increased simultaneously from 84.27 to 90.12 %. This was mainly contributed by two main reasons: primarily, Pectin comprises of carboxymethyl groups ($-COOCH_3$) which ionize greatly at basic pH 7.4 and remain non-ionized at acidic pH 1.2. When carboxymethyl ($-COOCH_3$) groups get ionized, electrostatic repulsions are enhanced. Such repulsions among polymeric chains of polymer result in the expansion of IPN. Secondly, increased porosity was observed by increasing the concentration of Pectin

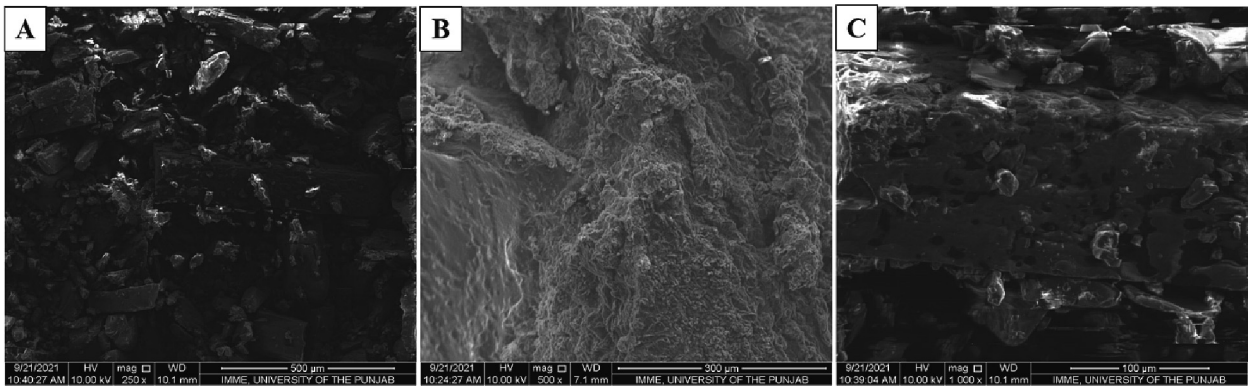

Fig. 6. SEM photomicrographs of IPN at different magnification powers A) 250x, B) 500x and C) 1000x.

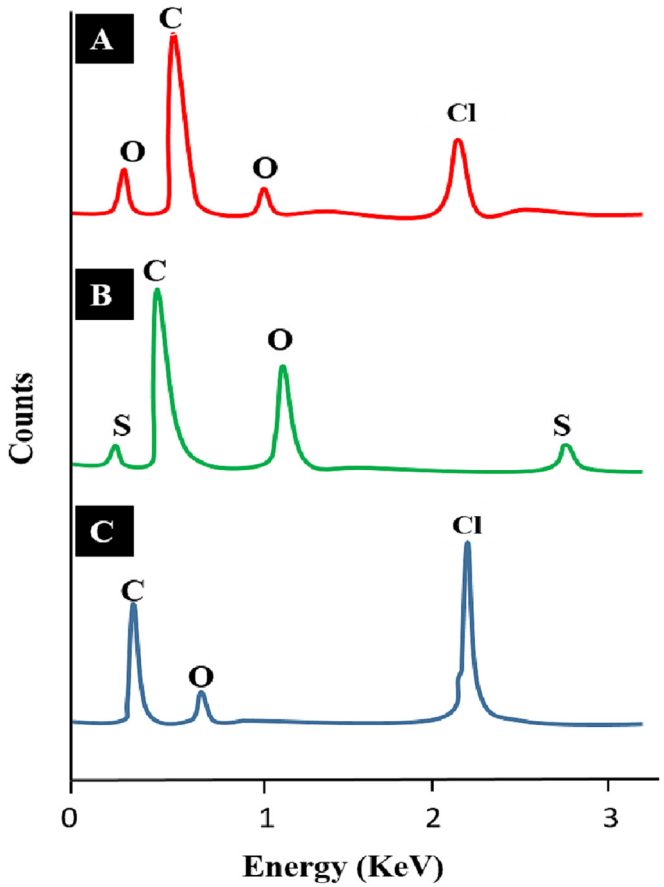

Fig. 7. EDX spectrum of A) Tapentadol Hydrochloride, B) Unloaded IPN, and C) Drug loaded IPN.

Table 2
Elemental composition of Tapentadol Hydrochloride, unloaded Natrosol-Pectin IPN and loaded Natrosol-Pectin IPN.

Types of material	Elements	% Weight	% Atomic
Tapentadol Hydrochloride	C	71.74	84.24
	O	9.34	8.23
	Cl	18.93	7.53
Unloaded Natrosol-Pectin IPN	C	42.86	51.58
	O	50.1	45.25
	S	7.04	3.17
Loaded Natrosol-Pectin IPN	C	68.18	82.4
	O	9.19	8.33
	Cl	22.64	9.27

in the simulated intestinal fluid (basic pH 7.4). Owing to this increase in porosity, an increase in solvent flux occurred into IPN formulations which in turn resulted in higher swelling. Sarioglu et al., (2019) developed Theophylline loaded Pectin base hydrogels. In their findings swelling was promoted parallel to Pectin contents at basic pH (Sarioglu et al., 2019).

4.11. Effect of natrosol contents on swelling

By increasing the concentration of Natrosol (IPN7- IPN9), no significant swelling was observed at pH 1.2 due to other components used for the formation of IPNs which retard its swelling at acidic pH. Furthermore, Natrosol contains numerous carboxylic acid groups (COOH) which are converted to carboxylate ions at basic pH. Results showed that when a higher concentration of Natrosol was used, the swelling was increased accordingly from 84.27 to 91.17 % at basic pH. Increased swelling was mainly due to the extremely hydrophilic nature of the polymer which justified sufficient imbibition of swelling media for better-swelling characteristics of IPNs.

4.12. Effect of acrylic acid contents on swelling

By increasing the concentration of Acrylic Acid (IPN10- IPN12), no significant swelling was shown at pH 1.2, however, swelling increased gradually at pH 7.4. The swelling percentage ranged between 78.24 and –92.5 %. The swelling was observed to be increased in simulated intestinal fluid (basic pH 7.4). Acrylic Acid comprises of carboxylic groups which ionize at alkaline pH and give rise to the expansion of IPNs. Therefore, by enhancing Acrylic Acid contents, the availability of carboxylic groups and their conversion into carboxylate ions was increased thereby leading to higher swelling.

Fig. 10 shows a three-dimensional RSM and contour plot of the response against the HEC/Pectin/AA and MBA concentration. Swelling with rising trend was observed with decrease in mole fraction of MBA. Additionally, maximum points on response curve were observed with increasing amounts of HEC/Pectin.

4.13. In-vitro release studies

Drug-loaded IPN discs were weighed and dipped into 900 ml buffer solutions of simulated gastric fluid (pH 1.2) as well as simulated intestinal fluid (pH 7.4) separately and samples were taken at predefined time intervals i.e. 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 18 and 24 h, and scanned at 214 nm wavelength for absorbance values. The impact of feed contents was evaluated. In general, drug

Table 3
Results of kinetic modeling (IPN1 – IPN12).

Formulation code	Zero order R^2	First order R^2	Higuchi model R^2	Korsemeyer-Peppas	
				R^2	*n*
IPN1	0.9989	0.9597	0.9015	0.9867	0.828
IPN2	0.9989	0.9643	0.8688	0.9949	0.948
IPN3	0.9989	0.9951	0.8230	0.9993	1.101
IPN4	0.9898	0.9399	0.8097	0.9999	1.205
IPN5	0.9957	0.9509	0.8477	0.9972	1.075
IPN6	0.9840	0.9460	0.8837	0.9857	0.928
IPN7	0.9974	0.957	0.8549	0.9952	1.047
IPN8	0.9976	0.9616	0.8920	0.9898	0.907
IPN9	0.9962	0.9535	0.9202	0.9828	0.916
IPN10	0.9981	0.9356	0.8129	0.9956	0.993
IPN11	0.9986	0.9426	0.8431	0.9943	0.913
IPN12	0.9983	0.9367	0.8661	0.9904	0.851

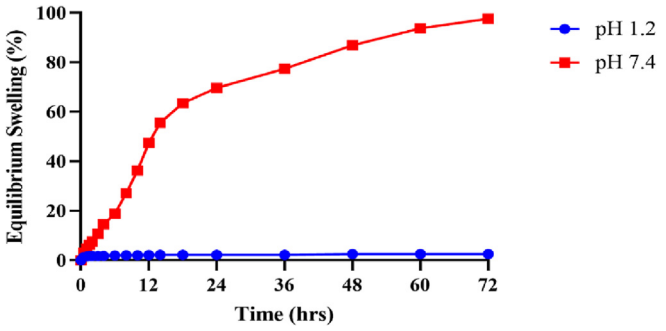

Fig. 8. Equilibrium swelling at pH 1.2 and pH 7.4.

release was more significant at basic pH of 7.4 as compared to acidic pH of 1.2. Results indicated (Fig. 11) that when the quantity of MBA was enhanced from 0.1 g to 0.3 g (IPN1-IPN3), drug release decreased from 83.55 % to 74.22 %. Enhanced quantity of cross-linker resulted in more physical entanglements leading to a denser IPN structure with reduced pore size. The drug release from the polymeric network was hindered as a result of poor penetration of dissolution media. Varaprasad *et al.*, (2014) developed carboxymethyl cellulose and polyacrylamide-based hydrogels. They revealed similar results that upon increasing MBA concentration from 0.032 to 0.081 mM, swelling ratio and drug release were decreased (Varaprasad *et al.*, 2014).

Drug release increased from 78.14% to 89.37%, as the quantity of Pectin increased (IPN4-IPN6) as shown in Fig. 11. This behavior

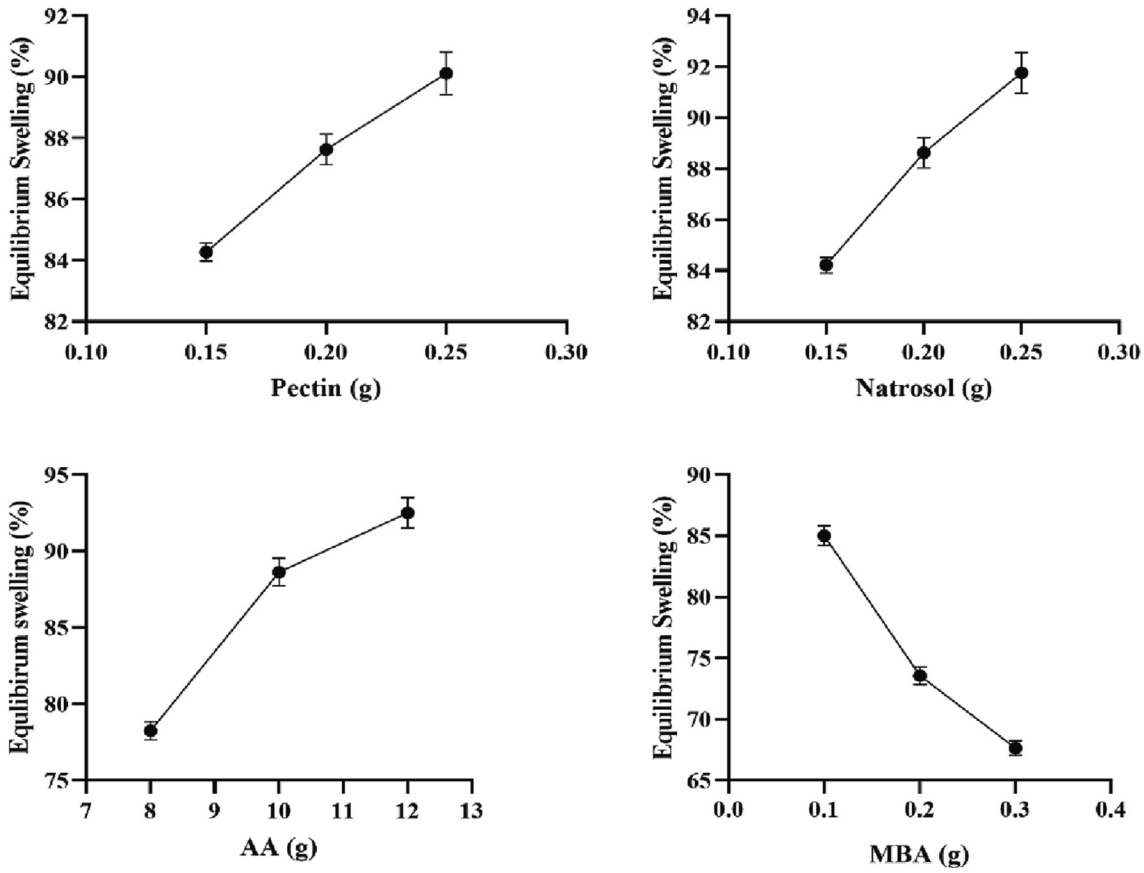

Fig. 9. Effect of formulation ingredients on equilibrium swelling (%).

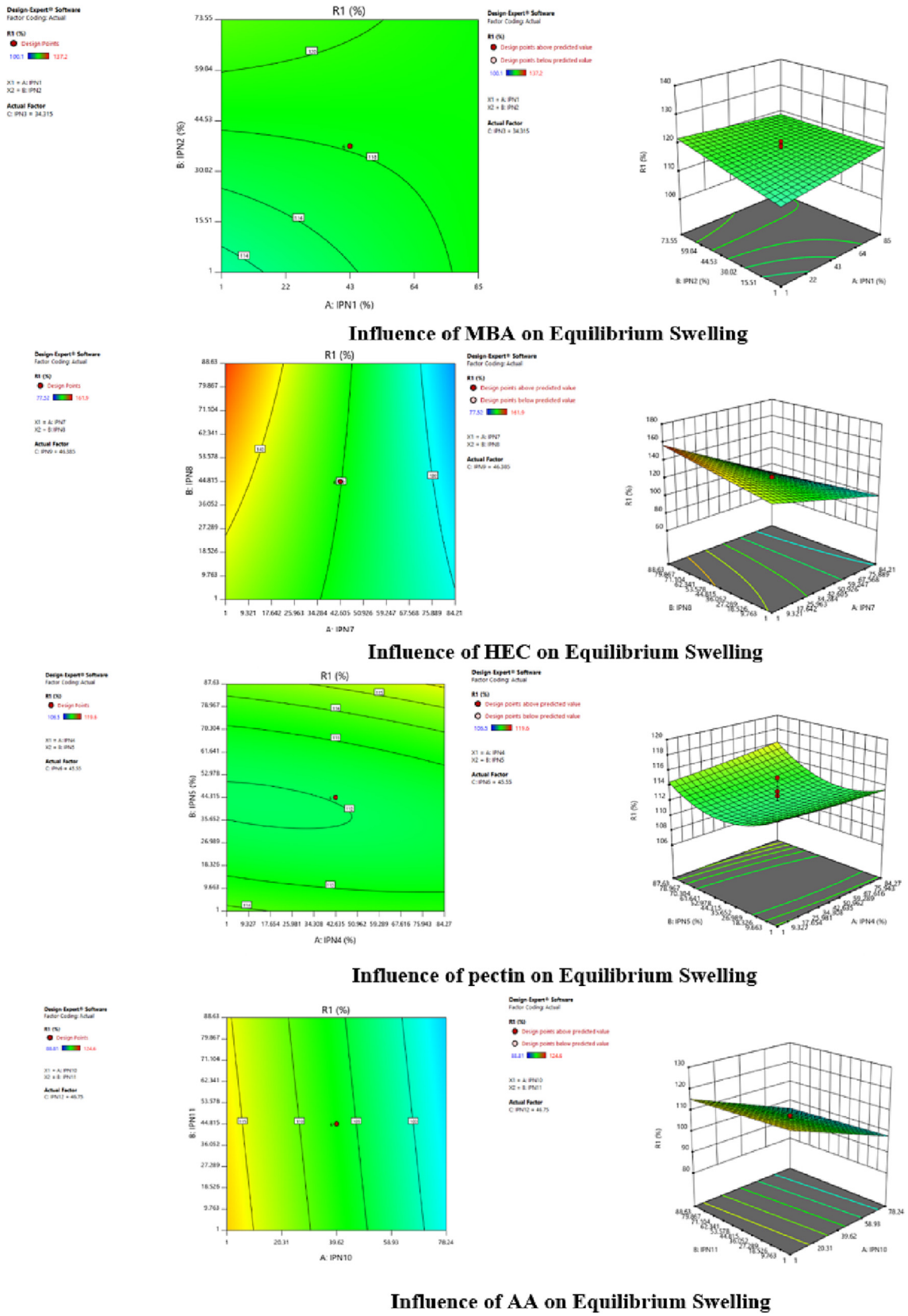

Fig. 10. RSM graphs indicating effect of ingredients on swelling and AA.

was mainly due to the increase in the number of carboxy-methyl groups with an increase in Pectin concentration. A weak barrier for drug release was formed resulting in enhanced drug diffusion from the interpenetrating network. Jaya et al., (2009) developed microcapsules based on Pectin/alginate and studied the effect of alginate and Pectin on drug release properties. Their results also

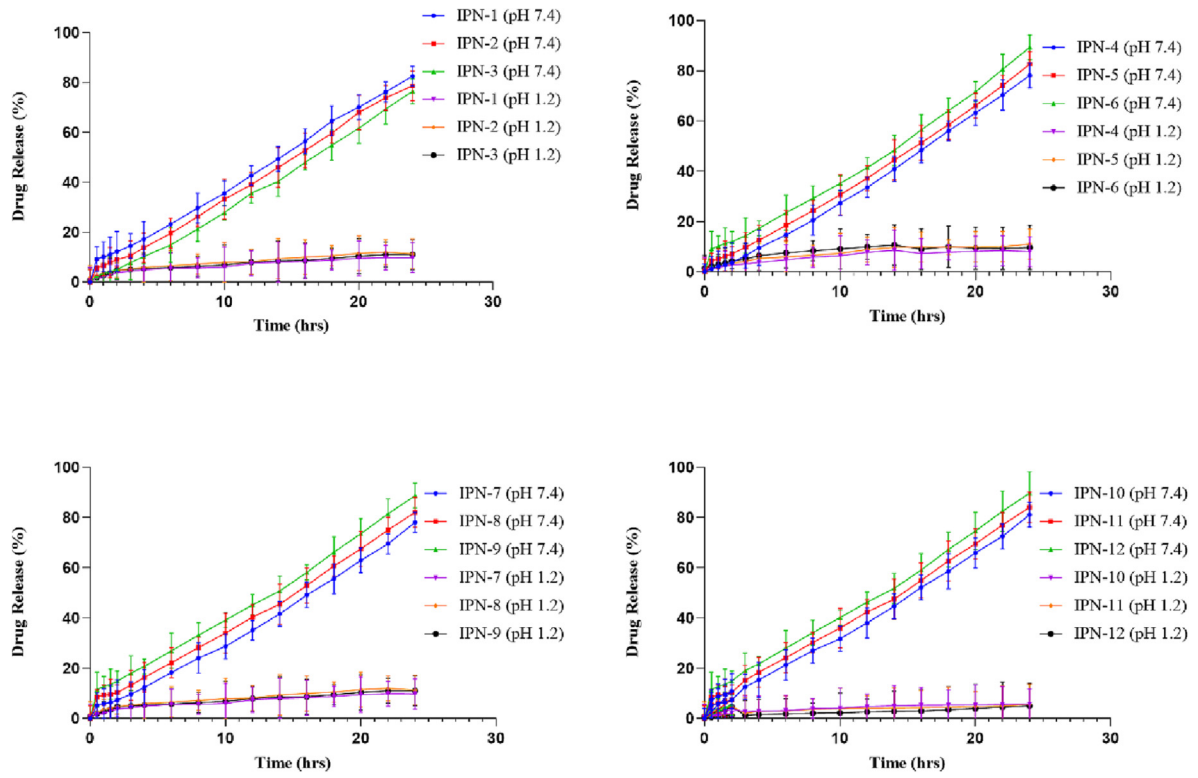

Fig. 11. Results of Release studies at pH 1.2 and pH 7.4.

supported the fact that an increased quantity of Pectin promotes drug release (Jaya et al., 2009).

When the concentration of Natrosol was increased (IPN7-IPN9), release increased to 88.72 % at the simulated intestinal fluid (SIF)) pH 7.4, because of the greater swellability and hydrophilic nature of polymer at 7.4 pH (Fig. 11). With the rise in Natrosol concentration, hydroxyl groups increased. –OH groups increase leads to absorption and retention of more dissolution media ultimately leading to higher drug release at basic pH. At pH 1.2 up to 8.3% Tapentadol Hydrochloride release was observed. Kajjari et al., (2011) developed hydrogel microspheres containing gelatin and Natrosol (HEC). They used Theophylline as a model drug and exhibited similar findings that upon increasing Natrosol concentration swelling and drug release was increased at alkaline pH (Kajjari et al., 2011).

Percentage drug release was also increased when Acrylic Acid quantity was enhanced (IPN10-IPN12) at basic pH 7.4 due to the carboxylic groups present in acrylic acid getting ionized at higher pH (greater than4.5). This resulted in more osmotic pressure within IPNs leading to enhanced ionization and drug release at pH 7.4.

Release results were predicted via RSM graph and contour plot as shown in Fig. 12. Central Composite design was employed to investigate the release profile. Release was evaluated by using two independent variables (pH 1.2 and 7.4). The response surface was evaluated via Design Expert software by using coded values of factor values. The regression model was remarkable, and significantly valid for a considered response as indicated by *P values* ($P < 0.05$), R1 and coefficient of variation. These types of plots are used to employ the influence of two factors on the response at one time, while the third factor was kept constant. In formulation IPN1-IPN3 linear relationship was observed and swelling decreased with increase in MBA concentration. However, non-linear and curvilinear relationship of factor X with factor Y was observed for formulation IPN4-IPN6 (Varying Natrosol concentration) and IPN7-IPN9 (varying Pectin concentration). Linear relationship was observed for formulation IPN10-IPN12 which showed increase in drug release with increase in AA content in pH 7.4.

4.14. Evaluation of Tapentadol Hydrochloride release kinetics

Kinetic modeling was applied on Tapentadol Hydrochloride release data by using DDS solver software in order to find out release pattern from Natrosol-Pectin IPN formulations and best fit model. Models such as First order, Zero order, Korsemeyer-Peppas and Higuchi model were applied for obtaining R^2 values of all developed formulations. It was clearly evident from R^2 values that Tapentadol Hydrochloride release from HEC-Pectin IPNs followed Zero order kinetics. While the Korsemeyer-Peppas model revealed mechanism of drug release to be Super case type II transport as value of 'n' was greater than 0.89.

4.15. Oral toxicological evaluation

Oral toxicity and biocompatibility assessments were performed on Natrosol-Pectin IPNs, according to the OECD guidelines. 12 male albino rabbits having a weight ranging between 1500 and 1800 g were selected for this purpose. They were grouped into two subgroups categorized as a control group and a test group (n = 6). We observed when the prepared IPNs were administered to the test group rabbits, no significant ailments such as ocular and dermal toxicity as well as no mortality was seen for 14 days. No noteworthy physical changes were seen in test and control group animals during this observation period. Blood samples were taken from both rabbit groups in order to perform CBC, LFT, RFT and uric acid profiles. Both sets of rabbits (test and control) were sacrificed on the 14th day and their vital organs were removed and sent for

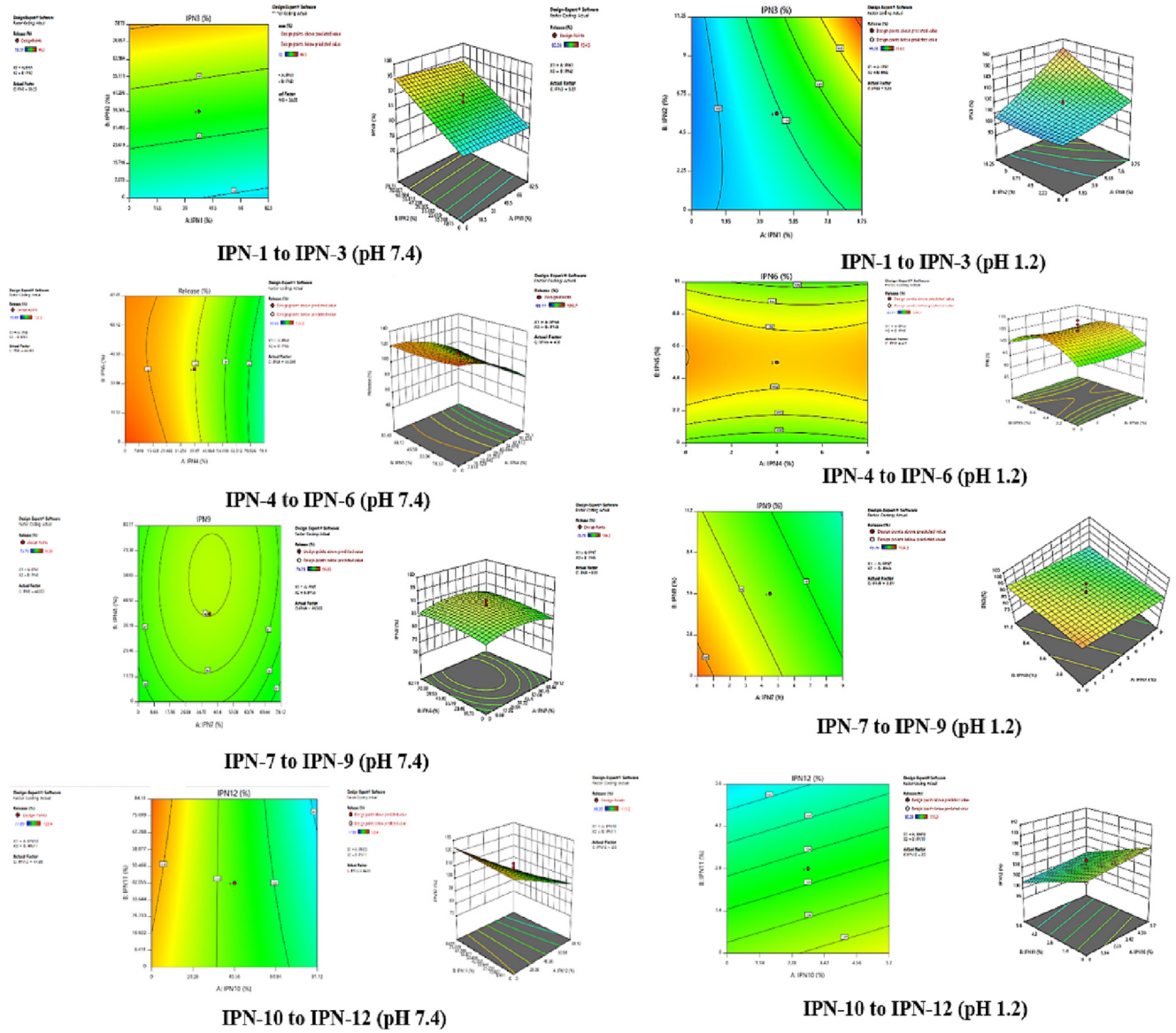

Fig. 12. RSM graphs indicating effect of ingredients on drug release.

Table 4
Clinical monitoring during oral toxicity studies.

Clinical Monitoring	Control Animal Group (CG)	Tested Animal group (TG)
Any illness signs	Nil	Nil
Body Weight (g)		
Before treatment	1643.68 ± 0.4	1748.82 ± 0.4
On 1st Day	1641.53 ± 0.6	1745.44 ± 0.6
On 7th Day	1641.49 ± 0.5	1743.47 ± 0.4
On 14th Day	1640.74 ± 0.2	1743.45 ± 0.5
Food consumption (g)		
Before treatment	73.85 ± 3.145	73.48 ± 2.77
On 1st Day	71.48 ± 2.283	75.83 ± 1.05
On 7th Day	75.47 ± 4.284	68.25 ± 3.03
On 14th Day	67.97 ± 3.779	71.18 ± 3.88
Water intake (ml)		
Before treatment	202.51 ± 2.35	183.61 ± 2.87
On 1st Day	191.44 ± 4.31	189.598 ± 1.65
On 7th Day	196.83 ± 3.38	201.94 ± 3.14
On 14th Day	202.22 ± 2.39	197.66 ± 2.44
Dermal allergy	Nil	Nil
Ocular toxicity	Nil	Nil
Mortality	Nil	Nil

Table 5
Hematological and biochemical analysis of Rabbits blood sample.

Finding parameters	Controlled animal group (A)	Tested animal group (B)
White blood cells (*10^3/µl)	4.3 ± 0.24	5.12 ± 0.28
Red blood cells (*10^6/ µl)	3.78 ± 0.39	4.26 ± 0.61
Hemoglobin (g/dL)	11.43 ± 0.66	12.46 ± 0.59
Platelets (*10^3/ µl)	42.53 ± 0.77	44.52 ± 0.37
Lymphocytes %	62.1 ± 0.48	66.78 ± 0.71
Monocytes %	3.12 ± 0.25	3.09 ± 0.21
Mean corpuscular volume (fL)	61.9 ± 2.09	62.41 ± 2.32
Mean corpuscular hemoglobin (pg)	19.09 ± 0.5	20.93 ± 0.15
Mean corpuscular hemoglobin concentration (g/dL)	35.18 ± 1.1	34.9 ± 1.4

histopathological testing. Table 4 shows the body weight, water and food consumption of rabbits during this time period. The hematological examination is shown in Table 5 and Table 6. Both hematological and histopathological examination signified that

Table 6
Liver and Renal profile of rabbit blood sample.

Finding parameters	Controlled animal group (A)	Tested animal group (B)
ALT(U/L)	51.45 ± 1.325	54.28 ± 1.547
AST(U/L)	121 ± 2.344	135.1 ± 2.845
ALP(U/L)	134.5 ± 1.467	146.2 ± 1.63
ALB(g/L)	5.09 ± 0.405	4.84 ± 0.821
Creatinine(mg/dL)	0.76 ± 0.114	0.7 ± 0.128
Uric acid(mg/dL)	2.04 ± 0.15	2.07 ± 0.24
Urea(mg/dL)	16.82 ± 0.25	13.7 ± 0.08

Natrosol-Pectin IPNs were non-toxic as no specific significant change was observed in the control and test group of animals.

In the histopathological assessment, shown in Fig. 13, the histology slide of each vital organ of both tests and control rabbit were prepared and then their photo-micrographs were documented by single-blind evaluation method. No significant change was seen in test group animals when compared to controlled group animals and hence, Natrosol-Pectin IPNs were found to be non-toxic in nature and were safe to be administered in living tissues. Brain section histopathological examination showed brain cells to be normal and neurons were visible having smooth myelin sheath covering them. Histopathological examination of cardiac tissues of both control and test animals showed normal cardiomyocytes without any signs of hypertrophy and inflammation. Lung cross-section illustrated that alveoli were clearly devoid of any specific sign of inflammation as well as cellular damage. No accumulation of cells was found. Photomicrographs of small intestine histopathological cross-sections also represented normal results in both controls as well as test group animals. Histopathological examination of the liver section of both control and test group animals did not show any specific signs of inflammation and mortification in hepatic cells. Lastly, the kidney cross-section revealed that kidneys were normal in both groups of animals. No cellular accumulation of inflammatory cells was seen and the absence of cellular damage was observed.

5. Discussion

The present study aimed to create an interpenetrating network (IPN) for the controlled delivery of Tapentadol Hydrochloride. This was achieved by developing IPNs of Natrosol-Pectin copolymerized with Acrylic Acid and Methylene bisacrylamide, and analyzing the effects of these ingredients on the physical and chemical characteristics of the IPNs. The findings of this study are significant for the design and optimization of hydrogels for various applications, as different concentrations of Pectin, MBA, AA, and Natrosol can be utilized to develop hydrogel formulations with specific gelation properties that can be tailored to meet specific requirements for targeted applications.

5.1. Sol-gel fraction (%)

The increase in gel fraction with an increase in MBA concentration can be attributed to enhanced physical entanglements and crosslinking in-between polymeric chains, eventually enhancing gel formation. This is consistent with the findings of Ranjha et al. (2011) who developed hydrogels based on Pectin and Acrylic Acid for the controlled delivery of Verapamil and also reported an increase in gel fraction with an increase in MBA concentration. The increase in gel fraction with an increase in Pectin concentration is consistent with the reported greater gelling capability of Pectin. This is supported by the findings of Ali et al. (2020) who also reported an increase in gelation with an increase in Pectin concentration. The increase in gel fraction with an increase in AA concentration can be attributed to the increased availability of carboxylic groups, which can participate in crosslinking. This is consistent with the findings of Ali et al. (2020). The increase in gel fraction with an increase in Natrosol concentration can be

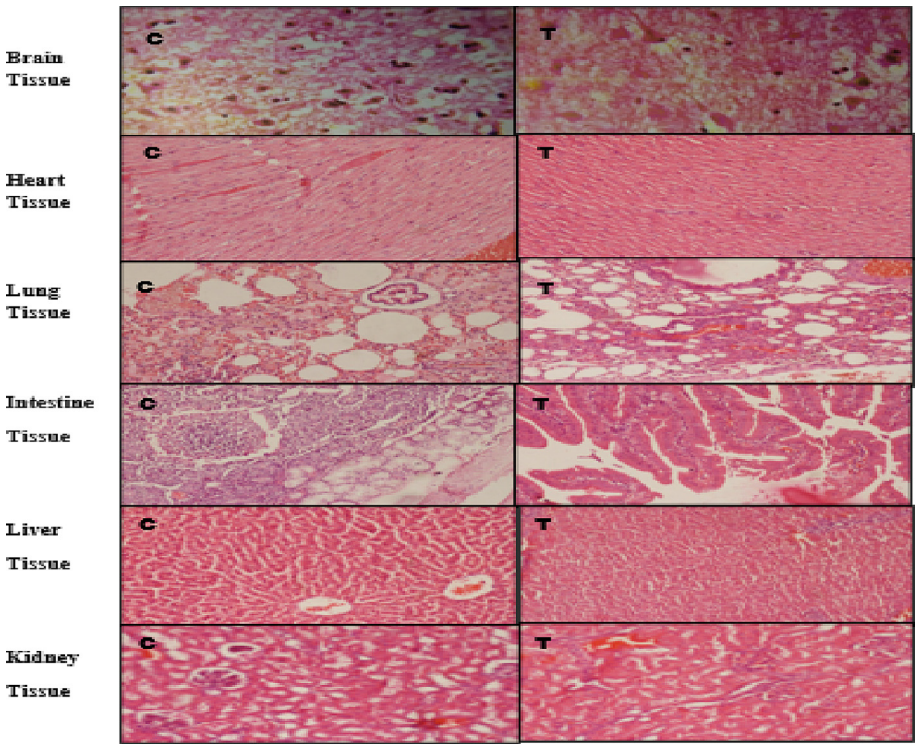

Fig. 13. Histopathological examination of vital organs of Control (C) and Test (T) Rabbits after administration of Natrosol-Pectin IPNs.

attributed to the high gelling capacity of Natrosol in the presence of a cross-linker. This is consistent with the findings of Fekete et al. (2017), who reported an increase in gel fraction with an increase in Natrosol concentration.

5.2. Porosity testing

The results of this study are consistent with previous studies that have investigated the effect of cross-linker concentration, Natrosol content, Pectin ratio, and Acrylic Acid amount on the porosity of hydrogels. For instance, Yoshinobu et al. (1992) found that increasing the cross-linker content in cellulosic hydrogels decreased the porosity percentage, similar to our findings. Bignotti et al. (2017) also observed that an increase in Natrosol content increased the porosity of HEC and polyacrylamide-based hydrogels, supporting our results. Likewise, Bashir et al. (2020) reported that increasing the amount of Pectin and Acrylic Acid in HPMC-Pectin-Acrylic Acid co-polymerized hydrogels increased the porosity proportionally, consistent with our observations.

5.3. Thermal analysis (DSC/TGA)

The DSC/TGA results indicate the thermal stability of pure ingredients, drugs, and developed interpenetrating networks. The endothermic peaks observed in the DSC thermogram of Tapentadol Hydrochloride indicate phase transitions of the drug. The weight loss observed in the TGA thermogram indicates the degradation of the drug. The endothermic peaks observed in the DSC thermogram of Pectin indicate the loss of moisture contents and the breakage of polymeric chains. The exothermic event observed in the DSC thermogram indicates the complete combustion of the polymer. The weight loss observed in the TGA thermogram indicates the degradation of the polymer. The exothermic peaks observed in the DSC thermogram of Natrosol indicate the thermal degradation of polymeric linkages. The weight loss observed in the TGA thermogram indicates the degradation of the polymer. The broad endothermic band observed in the DSC thermogram of Natrosol-Pectin IPNs loaded with model drug Tapentadol Hydrochloride indicates the phase transition of the developed network. The exothermic peaks observed in the DSC thermogram indicate the complete combustion of the developed network. The DSC/TGA studies were useful in determining the thermal stability of the pure ingredients, drug, and developed interpenetrating networks. The thermograms of Tapentadol Hydrochloride indicated the phase transition temperature of the drug and the temperature at which it completely combusted. The thermograms of Pectin and Natrosol showed the temperatures at which these polymers degraded and combusted. The DSC and TGA thermograms of Natrosol-Pectin IPNs loaded with Tapentadol Hydrochloride were compared to those of the individual ingredients. It was found that the formulated IPNs were more stable than the individual ingredients, which indicated the formation of a new interpenetrating network structure.

5.4. Powdered X-ray diffraction (PXRD)

The X-ray diffractograms of Tapentadol hydrochloride, Natrosol, Pectin, and Natrosol/Pectin-co-poly acrylate IPNs were recorded to determine the crystalline or amorphous nature of the test samples (Tian et al., 2020). The diffractogram of Tapentadol hydrochloride showed several evident and sharp peaks at 17.08°, 18.32°, 21.72°, 25.48° and 34.4°, indicating its crystalline nature. The diffractogram of Natrosol exhibited only two prominent peaks at 8.04° and 20.84°, confirming its amorphous nature (Tian et al., 2020). The diffractogram of Pectin presented several sharp peaks at 8.08°, 12.28°, 13.16°, 16.76°, 19.60°, 22.04°, 25.28°, 31.08° and 39.04°, indicating its crystalline nature (Tian et al., 2020). The diffractogram of Natrosol/Pectin-co-poly acrylate IPNs showed fused peaks, indicating the conversion of the crystalline nature of the drug into an amorphous form within the developed IPN (Tian et al., 2020). These results provide valuable insights into the nature of the test samples and can aid in the development of drug delivery systems and other pharmaceutical formulations.

5.5. Energy dispersive spectroscopy

The EDX studies provided valuable information about the composition of the test samples. The absence of chlorine in the unloaded IPN and its presence in the Tapentadol Hydrochloride loaded IPN suggests the successful incorporation of the drug into the IPN. This can be useful in the development of drug delivery systems and other pharmaceutical formulations.

The absence of chlorine in the unloaded IPN and its presence in the Tapentadol Hydrochloride loaded IPN is particularly important as it confirms successful incorporation of the drug into the IPN. The chlorine atoms in the drug molecule provide a distinct signal in the EDX spectrum, allowing for easy detection and quantification of the drug in the IPN. This information can be useful in determining the drug loading efficiency and release kinetics of the formulated IPN. In addition, the presence of sulfur in the unloaded IPN may suggest the use of a sulfur-containing crosslinker or polymer in the formulation. This information can be relevant in understanding the physicochemical properties and stability of the IPN.

5.6. Equilibrium swelling studies

The equilibrium swelling studies revealed that the swelling behavior of the IPN is pH-dependent. This can be attributed to the presence of carboxyl groups in the polymer matrix, which can undergo ionization in different pH environments. At pH 1.2, these carboxyl groups were unable to ionize, resulting in minimal swelling. However, at pH 7.4, the carboxylic groups became ionized, causing the polymer chains to repel each other and allowing for greater uptake of the swelling media. This behavior is consistent with previous reports on pH-responsive polymers (Zhang et al., 2014) and highlights the potential of the developed IPN for targeted drug delivery applications.

5.7. Effect of cross-linker concentration on swelling

The results of the swelling studies indicate that the concentration of MBA plays a significant role in determining the swelling behavior of the IPN. Increasing the concentration of MBA resulted in decreased swelling due to the development of a more rigid network, which limited the ability of the chains to elongate and expand. This observation is consistent with previous reports by Ibrahim et al. (2019), who also observed a similar effect of MBA content on swelling behavior.

The reduced swelling behavior of the IPN at higher MBA concentrations can be attributed to the high crosslinking density of the network, which masks the carboxylic groups and reduces the electrostatic repulsion for chain elongation. This effect is more pronounced at pH 7.4, where the carboxylic groups are ionized into carboxylate ions, promoting higher uptake of the swelling media and subsequently displaying higher swelling. At pH 1.2, the carboxyl groups are unable to get ionized, resulting in minimal swelling. Overall, these results suggest that the swelling behavior of the IPN can be modulated by varying the concentration of MBA, which can be useful in designing drug delivery systems with desired release profiles.

5.8. Effect of pectin contents on swelling

The results showed that increasing the concentration of Pectin had a significant effect on the swelling behavior of IPN at pH 7.4 but not at pH 1.2. The ionization of carboxymethyl groups in Pectin at basic pH 7.4 led to enhanced electrostatic repulsions and subsequently increased swelling. Additionally, the increased porosity of IPN by increasing the concentration of Pectin at simulated intestinal fluid (basic pH 7.4) led to a higher solvent flux into the IPN formulations, resulting in higher swelling. These findings are consistent with the results reported by Sarioglu et al. (2019) in their study on Theophylline-loaded Pectin-based hydrogels, where the swelling was promoted parallel to Pectin contents at basic pH (Sarioglu et al., 2019).

5.9. Effect of Natrosol contents on swelling

The results indicate that Natrosol is highly hydrophilic, which is consistent with its ability to imbibe swelling media, resulting in better swelling characteristics of the IPNs. The carboxylic acid groups present in Natrosol are converted to carboxylate ions at basic pH, which contributes to the increased swelling observed in IPNs with higher concentrations of Natrosol at pH 7.4. The lack of significant swelling at acidic pH can be attributed to the other components used in the IPN formulation, which retard swelling at low pH values. These findings are in line with the results reported by Ijaz et al. (2018), who developed IPNs containing Natrosol and Acrylic Acid for controlled delivery of Perindopril Erbumine and observed similar results. Ijaz and Tulain, (2019) observed similar findings that when the percentage of HEC was increased from 2 to 4%, swelling was also promoted from 53.76 to 55.41% at alkaline pH.

5.10. Effect of acrylic acid contents on swelling

The authors note that the observed increase in swelling at pH 7.4 was likely due to the ionization of carboxylic groups in Acrylic Acid, which promotes the expansion of IPNs. By increasing the availability of carboxylic groups and their conversion into carboxylate ions, higher swelling was observed. The lack of significant swelling change at pH 1.2 was not discussed in detail. Jalil et al., (2017) fabricated highly porous co-polymeric hydrogel composites of sodium alginate and Acrylic Acid by utilizing free radical polymerization technique for controlled delivery of Diclofenac potassium. Their findings showed that increase in pH and Acrylic Acid contents caused a simultaneous increase in swelling proportion of developed hydrogels (Jalil et al., 2017).

5.11. In-vitro release studies

In the present study, drug release from drug-loaded IPN discs was evaluated in buffer solutions of simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4) separately. Results indicated that drug release was more significant at the basic pH of 7.4 compared to the acidic pH of 1.2. This behavior can be attributed to the swelling behavior of IPNs in different pH conditions. At a basic pH, IPNs swell more due to the presence of more hydrophilic functional groups, resulting in increased drug release (Varaprasad et al., 2014). The effect of feed contents on drug release was also investigated. It was observed that when the quantity of MBA was increased, drug release decreased. This was due to the formation of a denser IPN structure with reduced pore size, which hindered the drug release by reducing the penetration of dissolution media. Similarly, Varaprasad et al. (2014) reported that increasing the MBA concentration resulted in a decrease in swelling ratio and drug release. On the other hand, an increase in Pectin concentration resulted in enhanced drug release due to the formation of a weak barrier for drug release. The carboxy-methyl groups in Pectin increased with an increase in Pectin concentration, leading to increased drug diffusion from the interpenetrating network. This finding is consistent with the study conducted by Jaya et al. (2009), who reported that an increased quantity of Pectin promotes drug release. The effect of Natrosol concentration on drug release was also investigated. It was observed that drug release increased as the concentration of Natrosol increased, especially at a basic pH of 7.4. This behavior can be attributed to the greater swellability and hydrophilic nature of the polymer at a basic pH, leading to increased absorption and retention of more dissolution media and ultimately resulting in higher drug release. This finding is consistent with the study conducted by Kajjari et al. (2011), who reported that increasing Natrosol concentration resulted in increased swelling and drug release at alkaline pH. Lastly, an increase in the quantity of acrylic acid also resulted in increased drug release at a basic pH of 7.4 due to the ionization of carboxylic groups present in acrylic acid at higher pH. This behavior resulted in more osmotic pressure within IPNs, leading to enhanced ionization and drug release at pH 7.4.

5.12. Oral toxicological evaluation

The histopathological assessment is a crucial step in evaluating the safety of any new drug or material. In this study, the authors conducted a histopathological assessment of vital organs (brain, heart, lung, small intestine, liver, and kidney) to determine the toxicity of Natrosol-Pectin IPNs. The results of the histopathological assessment showed that there were no significant changes in the test group animals when compared to the control group animals, indicating that the Natrosol-Pectin IPNs were non-toxic and safe to be administered in living tissues. These results are consistent with previous studies that have investigated the safety of IPNs. For example, Wang et al. (2012) conducted a histopathological examination of IPNs based on gelatin and chitosan and found them to be non-toxic and safe for in vivo applications. Similarly, Fan et al. (2019) evaluated the toxicity of IPNs based on polyvinyl alcohol and sodium alginate and found them to be non-toxic and safe for use in biomedical applications. Overall, the results of this histopathological assessment suggest that Natrosol-Pectin IPNs are safe for use in living tissues and may have potential biomedical applications.

6. Conclusion

Natrosol-Pectin IPNs were successfully fabricated by means of chemical cross-linking method i.e. free radical polymerization method. Except MBA, incremental rise in Natrosol, pectin and acrylic acid contents promoted percent swelling of IPNs and Tapentadol Hydrochloride release. FTIR, SEM, DSC, TGA and XRD tests substantiated the complex formation of IPNs, which was having enhanced thermal stability as compared to individual components of IPN. Gelling percentage was also directly proportional to reactants. Acute oral toxicity studies validated safety and biocompatibility of the fabricated IPNs towards living tissues. So, fabricated IPNs presented pH responsive character that can be effectively employed in the management of pain using opioid analgesics such as for controlled delivery of Tapentadol hydrochloride.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project no. (IFKSUOR3–067-3).

References

Ali, L., Ahmad, M., Aamir, M.N., Minhas, M.U., Shah, H.H., Shah, M.A., 2020. Cross-linked pH-sensitive Pectin and Acrylic Acid based hydrogels for controlled delivery of metformin. Pak. J. Pharm. Sci. 33, 1483–1491. https://doi.org/10.36721/PJPS.2020.33.4.REG.1483-1491.1.

Arjunan, V., Santhanam, R., Marchewka, M.K., Mohan, S., Yang, H., 2015. Structure activity studies of an analgesic drug tapentadol hydrochloride by spectroscopic and quantum chemical methods. J. Mol. Struct. 1100, 188–202. https://doi.org/10.1016/j.molstruc.2015.07.040.

Azam, F., Ijaz, H., Qureshi, J., 2021. Functionalized crosslinked interpenetrating polymeric network for pH responsive colonic drug delivery. Int. J. Polym. Mater. Polym. 70 (9), 646–655. https://doi.org/10.1080/00914037.2020.1740995.

Bashir, S., Zafar, N., Lebaz, N., Mahmood, A., Elaissari, A., 2020. Hydroxypropyl Methylcellulose-Based Hydrogel Copolymeric for Controlled Delivery of Galantamine Hydrobromide in Dementia. Processes 8, 1350. https://doi.org/10.3390/pr8111350.

Bignotti, F., Agnelli, S., Baldi, F., Sartore, L., Peroni, I., 2017. Macroporous polyacrylamide hydrogels with tailored porosity and mechanical properties via microphase separation in the presence of hydroxyethylcellulose. Polym. Eng. Sci. 57, 764–771. https://doi.org/10.1002/pen.24624.

Danish, Z., Ijaz, H., Razzaque, G., Aslam, M.M., 2021. Facile synthesis of three-dimensional porous hydrogel and its evaluation. Polym. Bull. 79, 7407–7428. https://doi.org/10.1007/s00289-021-03855-y.

Dash, S., Murthy, P.N., Nath, L., Chowdhury, P., 2010. Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol. Pharm. 67, 217–223.

El Fawal, G.F., Abu-Serie, M.M., Hassan, M.A., Elnouby, M.S., 2018. Hydroxyethyl cellulose hydrogel for wound dressing: Fabrication, characterization and in vitro evaluation. Int. J. Biol. Macromol. 111, 649–659. https://doi.org/10.1016/j.ijbiomac.2018.01.040.

El-Sayed, N.S., Awad, H., El-Sayed, G.M., Nagieb, Z.A., Kamel, S., 2020. Synthesis and characterization of biocompatible hydrogel based on hydroxyethyl cellulose-g-poly (hydroxyethyl methacrylate). Polym. Bull. 77, 6333–6347. https://doi.org/10.1007/s00289-019-02962-1.

Fekete, T., Borsa, J., Takács, E., Wojnárovits, L., 2017. Synthesis and characterization of superabsorbent hydrogels based on hydroxyethylcellulose and Acrylic Acid. Carbohydr. Polym. 166, 300–308. https://doi.org/10.1016/j.carbpol.2017.02.108.

Feng, L., Yang, H., Dong, X., Lei, H., Chen, D., 2018. pH-sensitive polymeric particles as smart carriers for rebar inhibitors delivery in alkaline condition. J. Appl. Polym. Sci. 135, 45886. https://doi.org/10.1002/app.45886.

Grande, D., Rohman, G., 2019. Oligoester-derivatized (semi-) interpenetrating polymer networks as nanostructured precursors to porous materials with tunable porosity. Chem. Afr. 2, 253–265. https://doi.org/10.1007/s42250-019-00044-3.

Gupta, P., Vermani, K., Garg, S., 2002. Hydrogels: from controlled release to pH-responsive drug delivery. Drug Discov. Today 7, 569–579. https://doi.org/10.1016/S1359-6446(02)02255-9.

Harsoor, S., 2011. Emerging concepts in post-operative pain management. Indian J. Anaesth. 55 (101–103). https://doi.org/10.4103/0019-5049.79872.

Ibrahim, A.G., Sayed, A.Z., El-Wahab, H., Sayah, M.M., 2019. Synthesis of poly (acrylamide-graft-chitosan) hydrogel: optimization of the grafting parameters and swelling studies. Am. J. Polym. Sci. Technol. 5, 55–62. https://doi.org/10.11648/j.ajpst.20190502.13.

Ichibouji, T., Miyazaki, T., Ishida, E., Sugino, A., Ohtsuki, C., 2009. Apatite mineralization abilities and mechanical properties of covalently cross-linked Pectin hydrogels. Materials Science and Engineering: C. 29, 1765–1769. https://doi.org/10.1016/j.msec.2009.01.027.

Ijaz, H., Tulain, U.R., 2019. Development of interpenetrating polymeric network for controlled drug delivery and its evaluation. Int. J. Polym. Mater. Polym. 68, 1099–1107. https://doi.org/10.1080/00914037.2018.1534110.

Ijaz, H., Tulain, U.R., Minhas, M.U., Mahmood, A., Sarfraz, R.M., Erum, A., Danish, Z., 2022. Design and in vitro evaluation of pH-sensitive crosslinked chitosan-grafted Acrylic Acid copolymer (CS-co-AA) for targeted drug delivery. Int. J. Polym. Mater. Polym. 71, 336–348. https://doi.org/10.1080/00914037.2020.1833011.

Jalil, A., Khan, S., Naeem, F., Haider, M.S., Sarwar, S., Riaz, A., 2017. The structural, morphological and thermal properties of grafted pH-sensitive interpenetrating highly porous polymeric composites of sodium alginate/Acrylic Acid copolymers for controlled delivery of diclofenac potassium. Des. Monomers Polym. 20, 308–324. https://doi.org/10.1080/15685551.2016.1259834.

Jaya, S., Durance, T., Wang, R., 2009. Effect of alginate-Pectin composition on drug release characteristics of microcapsules. J. Microencapsul. 26, 143–153. https://doi.org/10.1080/02652040802211345.

Kajjari, P.B., Manjeshwar, L.S., Aminabhavi, T.M., 2011. Semi-interpenetrating polymer network hydrogel blend microspheres of gelatin and hydroxyethyl cellulose for controlled release of theophylline. Ind. Eng. Chem. Res. 50, 7833–7840. https://doi.org/10.1021/ie200516k.

Khanum, H., Ullah, K., Murtaza, G., Khan, S.A., 2018. Fabrication and in vitro characterization of HPMC-g-poly (AMPS) hydrogels loaded with loxoprofen sodium. Int. J. Biol. Macromol. 120, 1624–1631. https://doi.org/10.1016/j.ijbiomac.2018.09.184.

Liu, L., Fishman, M.L., Hicks, K.B., 2007. Pectin in controlled drug delivery–a review. Cellulose 14, 15–24. https://doi.org/10.1007/s10570-006-9095-7.

Mahmood, A., Amara Sharif, F.M., Sarfraz, R.M., Abrar, M.A., Qaisar, M.N., Anwer, N., 2019. Development and in vitro evaluation of (β-cyclodextrin-g-methAcrylic Acid)/Na+-montmorillonite nanocomposite hydrogels for controlled delivery of lovastatin. Int. J. Nanomed. 14, 5397–5413. https://doi.org/10.2147/IJN.S209662.

Minhas, M.U., Ahmad, M., Khan, K.U., Sohail, M., Khalid, I., 2020. Functionalized Pectin hydrogels by cross-linking with monomer: synthesis, characterization, drug release and Pectinase degradation studies. Polym. Bull. 77, 339–356. https://doi.org/10.1007/s00289-019-02745-8.

Naik, S., Krishna, T.V., Abhinaya, N., Pai, G.K., 2020. Hydrogels for cancer drug delivery. Res. J. Pharm. Technol. 13, 4023–4027. https://doi.org/10.5958/0974-360X.2020.00711.8.

Padmasri, B., Nagaraju, R., Prasanth, D., 2020. A comprehensive review on in situ gels. Int. J. Appl. Pharm. 12, 24–33. https://doi.org/10.22159/ijap.2020v12i6.38918.

Pizzorusso, G., Fratini, E., Eiblmeier, J., Giorgi, R., Chelazzi, D., Chevalier, A., et al., 2012. Physicochemical characterization of acrylamide/bisacrylamide hydrogels and their application for the conservation of easel paintings. Langmuir 28, 3952–3961. https://doi.org/10.1021/la2044619.

Ranjha, N.M., Mudassir, J., Zubair, S.Z., 2011. Synthesis and characterization of pH-sensitive Pectin/Acrylic Acid hydrogels for verapamil release study. Iranian. Polym. J. 20, 147–159. http://doi.magiran.com/p829950.

Ranjha, N.M., Qureshi, U.F., 2014. Preparation and characterization of crosslinked Acrylic Acid/hydroxypropyl methylcellulose hydrogels for drug delivery. Int. J. Pharm. Pharm. Sci. 6, 400–410.

Rehmani, S., Ahmad, M., Minhas, M.U., Anwar, H., Sohail, M., 2017. Development of natural and synthetic polymer-based semi-interpenetrating polymer network for controlled drug delivery: optimization and in vitro evaluation studies. Polym. Bull. 74, 737–761. https://doi.org/10.1007/s00289-016-1743-y.

Sarioglu, E., Arabacioglu, K.B., Turan, D., Batirel, S., Guner, F.S., 2019. Theophylline-loaded Pectin-based hydrogels. II. Effect of concentration of initial Pectin solution, cross-linker type and cation concentration of external solution on drug release profile. J. Appl. Polym. Sci. 136, 48155. https://doi.org/10.1002/app.48155.

Shin, M.S., Kim, S.J., Kim, I.Y., Kim, N.G., Song, C.G., Kim, S.I., 2002. Swollen behavior of crosslinked network hydrogels based on poly (vinyl alcohol) and polydimethylsiloxane. J. Appl. Polym. Sci. 85, 957–964. https://doi.org/10.1002/app.10409.

Singh, D.R., Nag, K., Shetti, A.N., Krishnaveni, N., 2013. Tapentadol hydrochloride: A novel analgesic. Saudi J. Anaesth. 7, 322–326. https://doi.org/10.4103/1658-354X.115319.

Sundar Raj, A. A., Rubila S., Jayabalan R., Ranganathan T. V., 2012. A Review on Pectin: Chemistry due to General Properties of Pectin and its Pharmaceutical Uses. 1, 1-4. doi:10.4172/scientiicreports.550.

Tian, L., Singh, A., Singh, A.V., 2020. Synthesis and characterization of Pectin-chitosan conjugate for biomedical application. Int. J. Biol. Macromol. 153, 533–538. https://doi.org/10.1016/j.ijbiomac.2020.02.313.

Torrest, R., 1982. Rheological properties of aqueous solutions of the polymer Natrosol 250HHR. J. Rheol. 26, 143–151. https://doi.org/10.1122/1.549661.

Vera-Graziano, P.-A., Martínez-Richa, A., Barceló, F., Halachev, T., Castaño, V., 2003. On the structure and physicochemical properties of acrylic compounds. Int. J. Polym. Mater. 52, 85–95. https://doi.org/10.1080/714975922.

Wang, T., Chen, L., Shen, T., Wu, D., 2016. Preparation and properties of a novel thermo-sensitive hydrogel based on chitosan/hydroxypropyl methylcellulose/glycerol. Int. J. Biol. Macromol. 93, 775–782. https://doi.org/10.1016/j.ijbiomac.2016.09.038.

Wong, R.S.H., Dodou, K., 2017. Effect of drug loading method and drug physicochemical properties on the material and drug release properties of poly (ethylene oxide) hydrogels for transdermal delivery. Polymers 9, 286. https://doi.org/10.3390/polym9070286.

Yoshinobu, M., Morita, M., Sakata, I., 1992. Porous structure and rheological properties of hydrogels of highly water-absorptive cellulose graft copolymers. J. Appl. Polym. Sci. 45, 805–812. https://doi.org/10.1002/app.1992.070450506.

Zhang, M., Cheng, Z., Zhao, T., Liu, M., Hu, M., Li, J., 2014. Synthesis, characterization, and swelling behaviors of salt-sensitive maize bran–poly (Acrylic Acid) superabsorbent hydrogel. J. Agric. Food Chem. 62, 8867–8874. https://doi.org/10.1021/jf5021279.