ejpmr, 2020,7(8), 396-402

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

FORMULATION AND EVALUATION OF IPN MICROSPHERE OF INDOMETHACIN

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Article Received on 28/05/2020 **Article Revised on 17/06/2020** **Article Accepted on 06/07/2020**

ABSTRACT

Interpenetrating polymer network (IPN) is one of the valuable novel biomaterials. An IPN is a combination of at least two polymers and IPN can produce synergistic effect by sharing the properties of both the polymer. IPN microspheres of Indomethacin were prepared using emulsion cross-linking method. IPN Microspheres were prepared using sodium alginate and polyvinyl alcohol (PVA) in the ratio of 1:2, 1:3 and 1:4 using glutaraldehyde as a cross linking agent. Compatibility studies suggested no physicochemical interaction between the drug and the polymers. Microspheres were evaluated for particle size, entrapment efficiency, percentage yield, and equilibrium water up take. By increasing the ratio of poly vinyl alcohol, a slight increase in percent entrapment efficiency was observed. The effect of cross-linking on percent entrapment efficiency was significant. Increase in the amount of cross-linker in the matrices; significantly decrease the percent equilibrium water uptake. In-vitro dissolution study showed 90-98 % drug release at the end of 24 h.

KEYWORDS: Indomethacin, interpenetrating polymer network, sodium alginate, polyvinyl alcohol.

❖ INTRODUCTION

Interpenetrating polymer network (IPN) is a polymer comprising two or more networks which are at least partially interlaced on a polymer scale but not covalently bonded to each other. The network cannot be separated unless chemical bonds are broken.[1]

Controlled release technology has important potential in the fields of medicine, pharmacy and agriculture. In these areas natural polymeric materials have been pre-ferred over synthetic polymers due to their low cost, non-toxicity, easy availability and biodegradability properties. Biodegradable polymers derived from Sodium alginate (NaAlg) and Poly vinyl alcohol (PVA) have shown to be useful in pharmaceutical industries due to their ability for drug release. NaAlg is a biodegradable polymer that has been widely used in controlled release applications of pesticides and drugs. PVA is also a suitable polymer for drug release because of its desirable properties such as nontoxicity and noncarcinogenicity and has been used in many studies due to its biocompatibility. PVA can strongly interact with NaAlg through hydrogen bonding on a molecular level. For this reason in several studies, NaAlg and PVA were chosen for the microsphere formation and successfully crosslinked with glutaraldehyde (GA).[2]

Non steroidal anti inflammatory drugs (NSAID) are used as, analgesic and anti inflammatory agents in various disorders e.g. rheumatoid arthritis, spondylitis, acute gout etc. These are non selective inhibitor of COX I and COX II enzymes that participate in prostaglandin synthesis from arachidonic acid. In case of disease state like arthritis conventional dosage forms are ineffective in delivering the drug in lower GI tract. Among the NSAID Indomethacin is the drug having short biological half life (2 to 3 h), degradation in upper part of GI tract and passes side effect like GI irritation. Also the usual dosage regimen is 25-100 mg three times in day. From the literature survey it was observed that modified release dosage form of Indomethacin was required to be formulating to minimize the side effects like GI irritation and protect the drug from first pass effect.[3]

Hence in the present work an attempt was made to develop IPN microspheres to minimize dosing frequency and to prolong the pharmacological effect.

❖ MATERIALS AND METHODS

Indomethacin was obtained as a gift sample from Sigma life science, Mumbai (India). Sodium alginate, polyvinyl alcohol and glutaraldehyde were procured from Loba chemicals, Pvt. Ltd. Mumbai (India). All chemicals were of analytical grade. Electronic Balance (Mettler), Magnetic Stirrer (Remi equipment), UV Visible spectrophotometer (Shimadzu-1700), USP Dissolution Apparatus (Electrolab), FT-IR spectrophotometer (BRUKER), Stability Testing Chamber (Thermolab

scientific equipment), Differential scanning Calorimetry (METTLER Toledo, Switzerland), Scanning Electron Microscope (JEOL and Tokyo, Japan JSM-6360), X-Ray Diffraction (BRUKER D8 Advanced).

- **METHOD**

➢ **Formulation of IPN microspheres**

Emulsion cross linking method was selected for preparation of IPN microspheres. IPN microspheres of Indomethacin were prepared using NaAlg and PVA, while GA was used as a cross linking agent. About 60 ml of distilled water was heated at 80^0C and required amount of PVA (Table 1) was dissolved in it in order to make it complete soluble by continuous stirring at 80^oC on magnetic stirrer. Then required amount of NaAlg was dispersed in above homogenous solution and stirred over night on magnetic stirrer to obtain a thick, viscous, homogenous polymeric mass.

Required quantity of Indomethacin was dissolved in small quantity of methanol and added to the above homogenous polymeric mass. The drug loaded homogenous polymeric mass was emulsified into 100 ml light liquid paraffin containing 1 % w/w span 80 to form water in oil emulsion under constant stirring at 900 rpm using high speed mechanical stirrer for 40 min. To this w/o emulsion, 1 ml of 1 N HCL and required amount GA was added drop wise and further stirred for 3 h to harden the microspheres. Microspheres were separated by filtration, washed repeatedly with acetone and distilled water to remove the oil layer as well as excess amount of unreacted surfactant. Finally, the microspheres were washed with 0.1M glycine solution to mask the unreacted glutaraldehyde and dried in an oven at 40^oC for 24 h.[1,4] The detail of composition of each formulation is given in table no.1.

Table 1: Composition of IPN Microspheres.

Formulation Code	Polymer ratio	NaAlg (mg)	PVA (mg)	GA (ml)	Drug loading (mg)
F1	1:2	100	200	3.5	100
F2	1:3	100	300	3.5	100
F3	1:4	100	400	3.5	100
F4	1:2	100	200	4.5	100
F5	1:3	100	300	4.5	100
F6	1:4	100	400	4.5	100
F7	1:2	100	200	5.5	100
F8	1:3	100	300	5.5	100
F9	1:4	100	400	5.5	100

❖ **Evaluation of IPN microspheres**

✓ **Percentage yield**

To calculate percent yield of IPN microspheres, total amounts of microspheres obtained were weighted by using electronic weighing balance (Mettler). The theoretical weight was calculated by taking into consideration the weight of the drug and polymers employed during the preparation. The percent yield was calculated by the following equation[5]

Percentage yield = Amount of microspheres / Amount of drug + Amount of polymers×100.

✓ **Entrapment efficiency**

The actual amount of Indomethacin present in the different formulation batches were estimated by crushing 10 mg swollen microspheres in 100 ml phosphate buffer pH 7.2. The whole system was refluxed for 8 h. After reflux, it was kept for 24 h. These solutions were filtered separately. The drug content was measured using UV-Visible spectrophotometer at 320 nm. Entrapment efficiency was calculated using following formula[5]: % Entrapment Efficiency = Actual drug content/ Theoretical drug content×100

✓ **Particle size analysis**

An optical microscopy technique was used for the measurement of particle size. A standard stage micrometer was used to calibrate the eye-piece micrometer. Dried IPN microspheres were placed on a glass slide and the number of division of the calibrated eye piece was counted. A fifty microspheres were randomly selected from each formulation to measure the particle size. In this analysis, an optical combination of 10X eye-piece and 10X objective was used. The measurements of different formulation of microspheres were done in triplicate and average size of microspheres was calculated using following equation.[6]

$Xg = 10 \times [(ni \times \log Xi) / N]$

Where Xg is mean diameter, ni is number of particles in the range, Xi is the midpoint size range and N is total number of particles analyzed

✓ **Equilibrium water uptake study**

PH-dependent equilibrium water uptake of microspheres was measured by immersing each sample (10 mg) into (100 ml) phosphate buffer solution having pH 7.2. To ensure complete equilibration, microspheres were allowed to swell completely for 24 h to attain equilibrium at 37 ºC. Excess surface adhered liquid droplets of microspheres were removed by blotting with soft tissue papers without pressing microspheres. Hard and swollen microspheres were weighted using single pan balance. Evaluation was carrid out in triplicate for each formulation to obtain reproducible results.[7]

Equilibrium (%) water uptake = Mass of swollen microsphere – mass of dry microsphere / Mass of dry microspheres× 100.

✓ **Fourier transformed infrared study (FTIR)**

FTIR spectral analysis was studied to investigate the formation of IPN structure in presence of cross-linking agent.[6,9] FTIR spectrum was recorded by potassium bromide dispersion technique (SHIMADZU) within 4000-400 cm^{-1}.

✓ **Differential scanning calorimetry**

DSC studies were carried out (Mallick et al., 2013) for the pure drug, polymers and selected formulation of microspheres to support drug- excipient compatibility on DSC (METTLER Toledo, Switzerland). About 5 mg of sample was placed in aluminium and scanned over a temperature range (20-300 ^oC). The analysis was performed by heating the samples at the rate of 10^oC/min under an inert nitrogen atmosphere.[6]

✓ **X-ray diffraction (X-RD) studies**

X-ray diffraction analysis was carried out on X-ray diffractometer (BRUKAR D8 Advanced). The dried microspheres of uniform size were mounted on a sample holder and the patterns were recorded in the range 0 to 50 (2θ) at the speed of 5 /min to known crystallinity.[6,10]

✓ **Scanning electron microscopy**

The shape and surface characteristics of IPN microspheres were analyzed[10] with help of scanning electron microscopy (JEOL and Tokyo, Japan JSM-6360).

✓ ***In-vitro* drug release**

In-vitro drug release studies were carried out for all formulation batches on paddle type dissolution apparatus USP XXII. IPN Microspheres equivalent to 25 mg of Indomethacin were transferred to dissolution medium. The dissolution study was performed in 900 ml of PBS pH 7.2. Temperature of dissolution medium was maintained at 37±0.5^0C and the stirring speed was 100 rpm.[5, 11]

1 ml of an aliquot of the dissolution medium was withdrawn at predetermined time intervals of 1h and was replaced by equivalent amount of fresh medium kept at same temperature, sample solutions were filtered through Whatman filter paper no.41. The filtrate was analyzed on UV-Visible spectrophotometer at 320 nm for amount of drug released was determined using standard calibration curve. The dissolution study was carried for 8 h and further percent drug release was calculated. The study was performed in triplicate for each formulation.

The dissolution data was subjected to kinetic treatment. The cumulative amount of Indomethacin released from microspheres was fitted to Zero order, First-order, Higuchi-(matrix) model, Korsmeyer-Peppas model and Hixson-Crowell kinetics.

❖ RESULTS AND DISCUSSION

✓ **Percentage yield**

Total amount of microspheres obtained were weighted and percentage yield was calculated. Percentage yield was improved from 59.56±1.10 to 92.95±1.20 % (Table 2). The impact of concentration of polymer and cross linking agent during the production of drug loaded microspheres was observed. Increase in concentration of cross-linking agent from 3.5 ml to 5.5 ml showed increase in percentage yield of microspheres, this is due to higher extent of cross-linking, which numerously breaks the polymeric chain producing higher quantity of microspheres. It was also observed that increase in the ratio of sodium alginate: PVA gave higher percentage yield of microspheres.

✓ **Entrapment efficiency**

The actual amount of Indomethacin present in the different formulations of IPN microspheres was determined by measuring entrapment efficiency. Drug entrapment efficiency was found in the range of 59.82±0.007 to 91.70±0.003 % for formulation F1 to F9 respectively (Table 2). The percent entrapment efficiency showed a dependence on the ratio of sodium alginate: PVA and extent of crosslinking. By increasing the ratio of sodium alginate and PVA, a slight increase in percent entrapment efficiency was observed. The effect of cross-linking on percent entrapment efficiency was significant. As the concentration of cross linking agent was increased, an increase in percent entrapment efficiency was observed.

✓ **Particle size analysis**

An optical microscopy technique was used for the measurement of size of microspheres. During particle size analysis, it was observed that the size of IPN microspheres depends upon the ratio of two polymers and extent of cross linking. The average size of the microspheres was found between 43.2±0.22 to 102±0.21 µm (Table 2). Thus the data showed a systematic dependence on the amount of cross-linking agent and the ratio of sodium alginate: PVA used during formulating the microspheres.

✓ **Equilibrium water uptake study**

Equilibrium water uptake study of drug loaded IPN microspheres was performed in phosphate buffer pH 7.2. Results indicated that, as the amount of crosslinking agent in the matrices increases, the percent equilibrium water uptake significantly decreased (Fig. 1). This is due to increased cross-linking density and decreased pore volume of the polymeric network with increasing amount of GA in the matrix. Also, it was found that formulations containing higher ratio of sodium alginate: PVA showed lesser percent of swelling rates than formulations containing lesser ratio of sodium alginate: PVA.

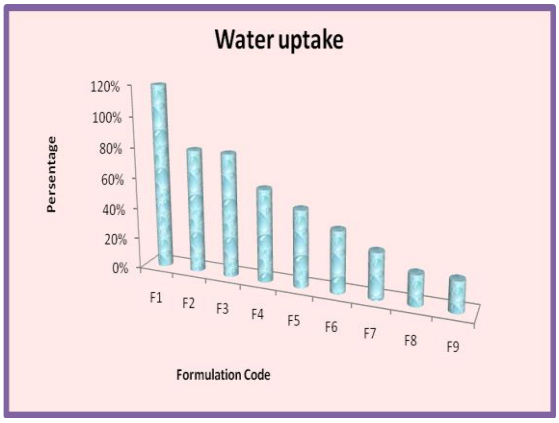

Fig 1: Water uptake study of Indomethacin loaded IPN microspheres.

✓ **FTIR study**

IR spectra of various formulations showed no interaction with in the drug and polymers. Hence drug excipients compatibility was established. There is no shift and no disappearance of characteristics peaks suggesting that there is no interaction between Indomethacin and other excipients or no degradation of drug molecule. Hence drug excipients compatibility was established.

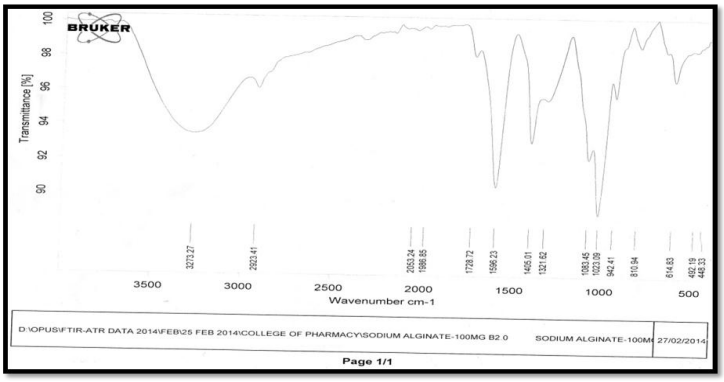

Fig 2: FTIR spectrum of sodium alginate

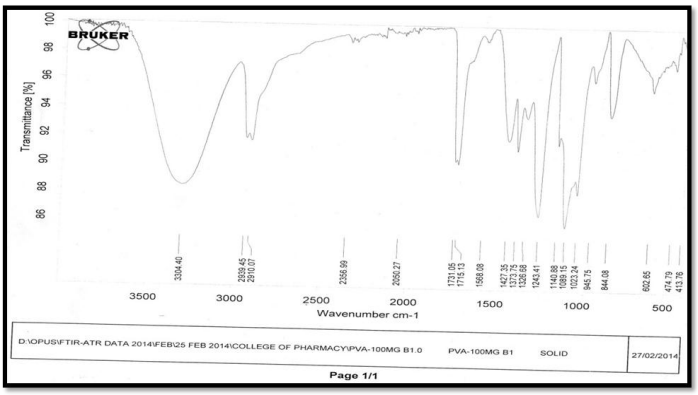

Fig 3: FTIR of poly vinyl alcohol.

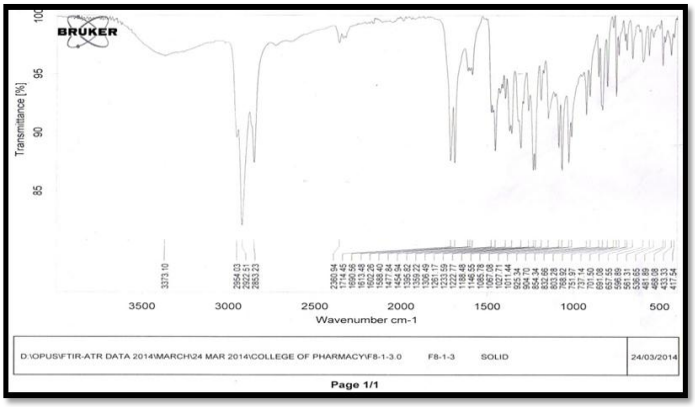

Fig 4: FTIR of Indomethacin.

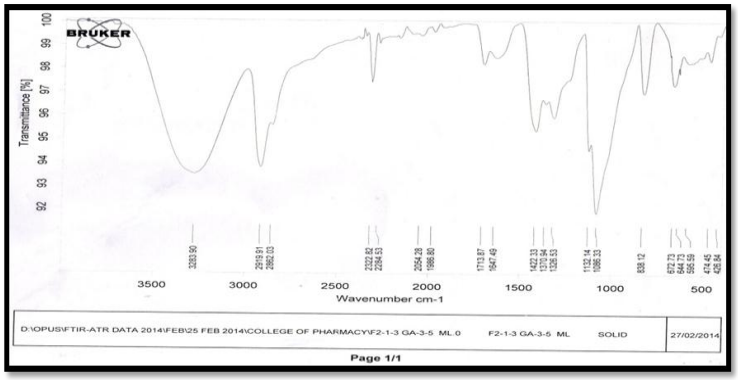

Fig 5: FTIR of Indomethacin loaded IPN microsphere.

✓ **Differential scanning calorimetry**

The DSC curve of Indomethacin showed sharp endothermic peaks at 161.67 ^{0}c corresponding to melting point of Indomethacin as shown in Figure No.2. The DSC curve of IPN microsphere containing Indomethacin exhibited characteristic peaks at 189.81oC as shown in Figure 2. The disappearance of melting point endothermic peak of drug in IPN microspheres indicate that the drug might have dispersed or converted in to amorphous form during preparation of IPN microspheres.

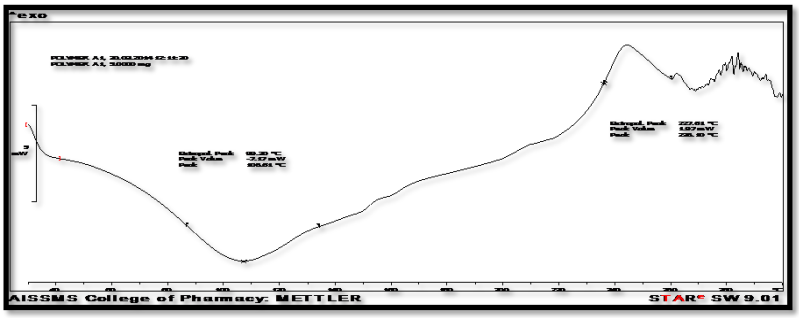

Fig 6: DSC of sodium alginate.

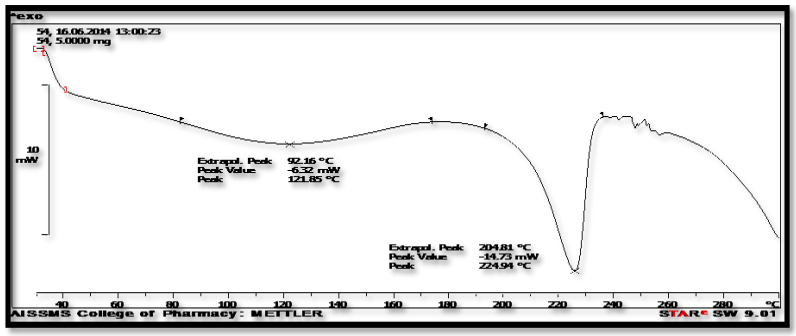

Fig 7: DSC of poly vinyl alcohol.

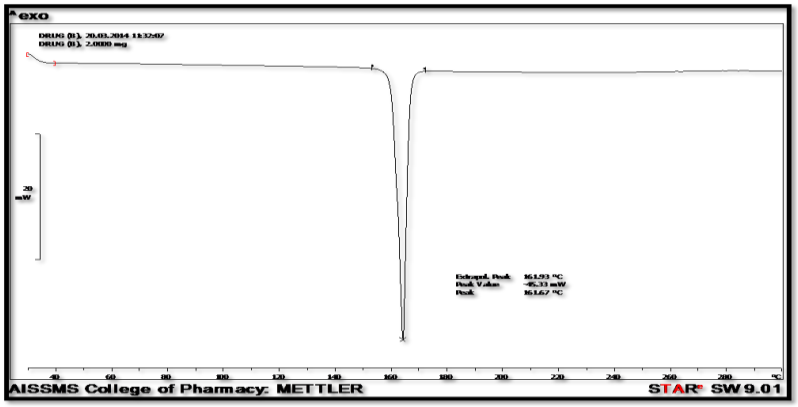

Fig 8: DSC of Indomethacin.

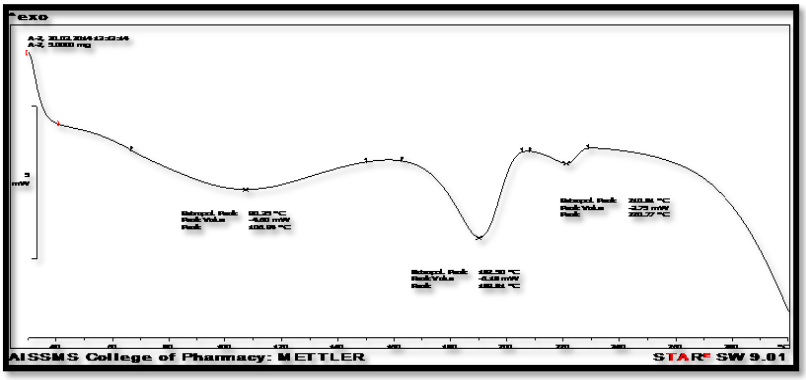

Fig 9: DSC of Indomethacin loaded IPN microsphere.

✓ **X-ray diffraction studies**

The X-ray diffraction (X-RD) pattern of drug loaded microspheres showed crystalline nature of microsphere. The result of X-RD studies are shown in Figure No.3. Indomethacin has shown intense peaks between 15 to 35^{o} (2θ) due to its crystalline nature. XRD pattern of drug loaded IPN microspheres showed characteristics peak but at less intensity than that of the drug.

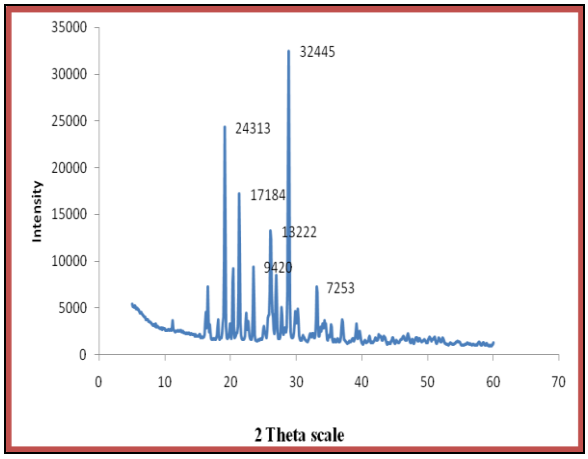

Fig 10: XRD of Indomethacin.

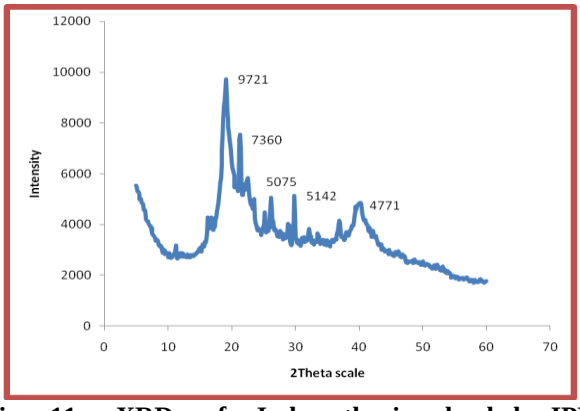

Fig 11: XRD of Indomethacin loaded IPN microsphere.

✓ **In-*vitro* drug release studies**

In- vitro drug release studies were performed on Paddle type dissolution apparatus USP XXII (Electrolab TDT 08L) at 37±0.5^{0}C and 50 rpm. The percentage of drug released was determined in phosphate buffer pH 7.2 for 8 hours.

The amount of Indomethacin released from IPN microspheres was fitted to Zero order, First-order, Higuchi-(matrix) model, Korsemeyer-Peppas model and Hixon-crowell models to determine the kinetics of drug release. Formulations F1to F3 and F7-F9 followed Korsmeyer Peppas model while F4-F6 followed Hixson Crowell model.

Batch code	Zero Order		First Order		Higuchi Matrix		Hixon-crowell		Korsmeyer Peppas	
	R^2	K	R^2	K	R^2	K	R^2	K	R^2	K
F1	0.024	6.50	0.450	0.16	0.748	28.15	0.656	6.21	0.933	43.17
F2	0.531	5.98	0.535	-0.12	0.811	25.59	0.433	0.003	0.956	36.87
F3	0.339	6.53	0.580	0.17	0.691	49.65	0.432	0.003	0.961	49.65
F4	0.561	5.81	0.730	0.12	0.896	24.10	0.985	0.003	0.635	0.003
F5	0.536	5.36	0.632	0.25	0.695	24.11	0.995	0.003	0.635	0.12
F6	0.562	5.32	0.653	0.26	0.635	22.36	0.896	0.16	0.536	23.21
F7	0.431	6.53	0.580	0.17	0.861	28.27	0.339	0.13	0.961	49.65
F8	0.339	6.50	0.450	0.16	0.748	28.15	0.459	0.03	0.933	43.17
F9	0.338	6.56	0.613	0.17	0.791	28.23	0.148	0.03	0.959	42.17

Fig 12: *In- vitro* drug release study of Indomethacin IPN microspheres.

✓ **Scanning electron microscopy**

SEM micrographs showed that microspheres were not perfectly spherical as shown in Figure15. The rigid matrix structure of IPN network may be due to higher extent of cross-linking.

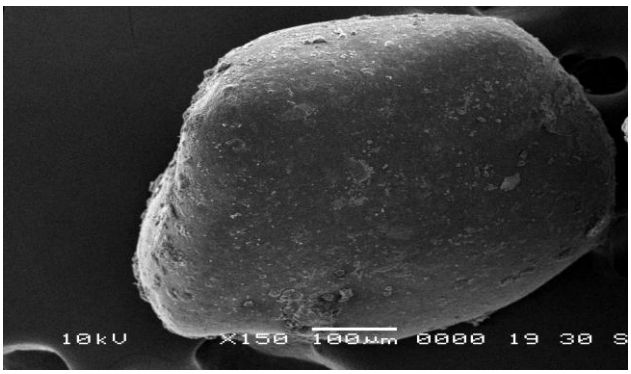

Fig 13: SEM photograph of Indomethacin IPN microsphere.

❖ CONCLUSION

This work demonstrate the effective encapsulation of indomethacin into NaAlg and PVA to produce IPN Microsphere by emulsification crosslinking method. The IPN microsphere demonstrated better controlled release results than pure NaAlg, indicating the suitability of IPN for microsphere preparation. The crosslink density was signicantly affected by the amount GA and the polymers in the formulations. The release of Indomethacin was found to be dependent on the extent of crosslinking, the amount of drug loading and polymer content of the matrix. The Fickian behavior. It can concluded that microspheres prepared in this study can be effectively used as a controlled release for the release of Indomethacin.

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