FORMULATION AND IN-VIVO EVALUATION OF ACTARIT TABLETS USING CARBOXYMETHYL TAMARIND SEED GUM AND CYCLODEXTRIN NANOSPONGES

Madhavi M[1], Shiva Kumar G[2]

[1]Research Scholar, GITAM School of Pharmacy, GITAM Deemed to be University, Hyderabad – 502329, Telangana, India.

[2]Research Supervisor, GITAM School of Pharmacy, GITAM Deemed to be University, Hyderabad-502329, Telangana, India.

Email: 121965201020@gitam.in

DOI: 10.47750/pnr.2023.14.03.058

Abstract

Actarit tablets of interpenetrating polymer networks were prepared using carboxy methyl tamarind gum and cyclodextrin nanosponges for increasing oral bioavailability. Formulations with different carboxymethyl tamarind gum concentrations were freeze dried to create actarit-loaded interpenetration polymer networks, which were then analysed for drug loading equilibrium swelling and characterised using FTIR, DSC, and XRD. Actarit loaded IPN tablets were prepared and evaluated for in-vitro and in-vivo studies. The drug loading in the IPNs ranged from 59.36 to 65.72%, and swelling in the presence of 0.1N HCl in the IPNs was substantially lower (P 0.05) than in the presence of phosphate buffer pH 6.8. Studies using FTIR, DSC, and XRD confirmed that Actarit and IPNs formed a molecular combination. Actarit loaded IPN tablets' average weight, thickness, hardness, friability, and percentage of drug content were all within acceptable ranges. The drug release was highest for F3 (99.86%) and for marketed 98.67%. From in vivo bioavailability studies, the pure drug's Cmax was 404.34±12.87 ng/ml, significantly higher when compare with marketed product and optimized IPN actarit tablets of 385.21±9.43 and 342.76±13.56 ng/ml respectively. The Tmax of pure drug, marketed and optimized formulation were 1.5±0.05, 2.0±0.06 and 4.0±0.04 h respectively. The formulation of the marketed and optimized formulation exhibited a higher AUC0- ∞ (815±14.23 and 915±28.33 ng.h/ml) than the pure drug (800.76±15.76 ng.h/ml). The optimized tablet formulation showed a significantly higher AUC0-t and MRT than the pure drug and marketed product. The bioavailability of Actarit's cyclodextrin nanosponges based IPNs tablets were significantly improved when compared to the pure drug.

Keywords: Actarit, Nanosponges, Carboxymethyl tamarind seed gum, Tablets, Bioavailability studies.

INTRODUCTION

Polymers have been an important component in the advancement of drug delivery technology by providing controlled release of therapeutic agents in constant doses over long periods, cyclic dosage, and tunable release of variety of drugs [1]. Interpenetrating polymer network (IPN) is an innovative drug delivery system with several advantages like improved mechanical strength, loading capacity, stability, biocompatibility, high swelling capacity and biodegradability which play an important function in targeted and controlled drug delivery. IPNs are polymeric network of two or more polymers that forms a rigid composite network structure by cross-linking of at least one polymer in presence of other [2]. IPN shows better properties for the release of drugs in a controlled manner [3], the phase stability of the end product is eminently enlarged having more biological acceptability and mechanical properties of the products at the final stage are immensely improved, due to union of natural and unnatural polymers [4].

Cyclodextrin nanosponges are a novel hyper-crosslinked synthetic polymer consisting of solid nanoparticles with colloidal sizes and nanosized cavities. These nanosponge looks like a three-dimensional scaffold possessing a long length polymer backbone [5]. The main cross-linking agents used for preparation of this type of nanosponges are active carbonyl compounds such as Diphenyl carbonate, Carbonyl diimidazole and trifosgene [6]. In order to improve the properties of cyclodextrin nanosponges, in this work we have focused on developing the interpenetrating polymer networks based on carboxymethyl tamarind seed gum.

Tamarind gum is a natural polymer which can be used for the preparation of interpenetrating polymer networks with other synthetic polymers [7]. The interpenetrating hydrogels of carboxymethyl tamarind gum and alginate for delivery of acyclovir has been reported in literature [8]. By considering all the above facts, an attempt was made to develop interpenetrating polymer networks of Actarit using carboxy methyl tamarind gum and cyclodextrin nanosponges. The prepared IPNs were

characterized by FTIR, thermal analysis, X-ray diffraction studies.

MATERIALS AND METHODS

Actarit was obtained as a gift sample from Dr. Reddy's Laboratory Ltd., Hyderabad, India. Cyclodextrin nanosponges (NS16) prepared in our laboratory were used for this study. Carboxymethyl tamarind seed gum (Degree of substitution~0.16) was kindly gifted by Tamarind Magic, Hyderabad, India. Glutaraldehyde (25% Aqueous Solution) was purchased from Sigma Aldrich (Milan, Italy). All other chemicals and reagents used in the study were of analytical grade. Milli Q water (Millipore) was used throughout the studies. Commercial tablet of Actarit (Aramact Tablet 10s) was purchased from local market. Dialysis membrane (Molecular weight cut off 12 kDa) was purchased from Hi-media Pvt. Ltd.,

Preparation of interpenetrating polymer networks of Actarit

Actarit loaded interpenetration polymer networks were prepared by slight modification of the by reported methods by freeze drying/lyophilisation (Table 1) [9, 10]

Determination of Actarit loading in IPNs

Accurately weighed quantity (100mg) of Actarit loaded IPNs were dissolved in methanol and sonicated for 10 min to break the complex. The solution was then transferred to 100ml of volumetric flask and volume was adjusted to 100ml with pH 6.8 phosphate buffer. The concentration of actarit was analysed by UV-Visible spectrophotometer at 244 nm. Actarit content was calculated from standard curves. The percent drug loading was calculated using the following formula 1.

$$\%\ Drug\ loading\ = \frac{Weight\ of\ drug\ loaded\ in\ NS\ formulation}{Initial\ weight\ of\ the\ drug\ fed\ for\ loading} \times 100 \qquad (1)$$

Table 1: Formulation of Actarit loaded interpenetrating polymer networks

Formulation	Nanosponges %w/v	Carboxymethyl tamarind gum %w/v	Actarit %w/v	Glutaraldehyde (ml)
F0	0.5	0.6	0	1
F1	0.5	0.6	0.5	1
F2	0.5	0.6	0.5	2
F3	0.5	0.6	0.5	3
F4	0.5	0.4	0.5	1
F5	0.5	0.8	0.5	1

Physico-chemical Characterization of plain IPNs and Actarit -IPNs

Fourier transformed infrared (FTIR) spectroscopy

The FTIR spectra of Actarit, Cyclodextrin nanosponges, Carboxymethyl tamarind gum, Blank IPNs and Actarit loaded IPNs were carried out by potassium bromide disc method using Tensor 27 FTIR Spectrophotometer (Bruker Optics, Germany) in the region of 4000 to 600 cm−1.

Differential Scanning Calorimetry (DSC)

DSC of Actarit, Cyclodextrin nanosponges, Carboxymethyl tamarind gum, Blank IPNs and Actarit loaded IPNs were carried out using a Perkin Elmer DSC/7 differential scanning calorimeter (Perkin-Elmer, CT-USA) equipped with a TAC 7/DX instrument controller. The instrument was calibrated with indium for melting point and heat of fusion. A heating rate of 10°C/min was employed in the 30-400 °C temperature range. Standard aluminum sample pans (Perkin-Elmer) were used; an empty pan was used as reference standard. Analyses were performed in triplicate on 5 mg samples under nitrogen purge.

X-Ray Powder Diffraction (XRPD)

X-ray powder diffraction patterns of Actarit, Cyclodextrin nanosponges, Carboxymethyl tamarind gum, Blank IPNs and Actarit loaded IPNs were recorded on X-ray diffractometer (Bruker D8 Advance) at a scan rate of 5 °/ min in the 2θ range from 2.5° to 60°.

Equilibrium swelling

Known amount (100mg) of IPNs were transferred to 100ml of 0.1N HCl and phosphate buffer pH 6.8 separately and allowed to swell at room temperature for 24hr. IPNs were separated after 24h and excess water was blotted with filter paper and reweighed again [11]. Finally equilibrium swelling index of formulated batches was calculated by using formula 2:

$$Equilibrium\ Swelling(\%) = \frac{Swollen\ weight\ of\ IPNs - Dry\ weight\ of\ IPNs}{Dry\ weight\ of\ IPNs} \times 100 \quad (2)$$

Preparation of Actarit loaded IPN tablets

An accurately weighed quantities of Actarit loaded IPNs corresponding to 100 mg Actarit and the calculated Avicel PH-102, which was added to attain 300 mg tablet, were mixed for 10 min using mortar and pestle after which the magnesium stearate and talc was added and blended for another 2 min. The final mixtures were compressed using a single punch tablet machine with 8 mm, round, flat-faced single punch.

Evaluation of tablet formulation

Uniformity of weight

Twenty tablets were selected at random, individually weighed in a single pan electronic balance and the average weight was calculated. The uniformity of weight was determined according to specifications of British Pharmacopoeia (BP 2013).

Uniformity of content

The Actarit content of the prepared tablets was carried out according to the reported method

Hardness test

Hardness of the prepared tablets was measured using the tablet hardness tester (Monsanto). Three tablets were selected for testing and results were expressed in kg/cm2.

Friability test

Friability test was done in a digital tablet friability tester apparatus [(VEEGO) model: FT-2D, India], where the tablets were subjected to the combined effect of abrasion and shock by utilizing a plastic chamber that revolves at 25 rpm, while dropping the tablets at a distance of six inches with each revolution. Pre-weighed samples of 20 tablets were placed in the friability chamber, which was operated for 100 revolutions. At the end of rotation, the tablets were removed from the drum, carefully brushed to free them from adhering dust and reweighed. Conventional compressed tablets lose less than 0.5–1.0% of their weight which is generally considered acceptable [12].

The percent friability (% F) is given by the equation 3:

$$\%\ F = \frac{(1 - W_0)}{W} \times 100 \quad (3)$$

where W0 is weight of the tablets before the test and W is the weight of the tablets after test.

In-vitro disintegration test

The time test for in vitro disintegration is a significant feature needed for tablets that are released immediately. The time of dosage forms to disintegrate must be within 25 min. The time for complete disintegration of the tablet was measured in min, with no observable mass remaining in the apparatus [13].

In-vitro release study of Actarit

In vitro release of drug from Actarit loaded IPN tablet and marketed Actarit tablet was performed using USP dissolution test apparatus (Electrolab TDT-06P, India). The drug release was measured in 900 ml acidic medium (pH1.2) for the first 2 hr and in SGF (pH 6.8) until the end of dissolution at 30 °C and 100 rpm. The dissolution medium was added with 0.5% w/v sodium lauryl sulphate (1 ml) to maintain proper sink conditions. At specified time intervals (0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h) the samples were withdrawn and suitably diluted with distilled water to determine the percent drug release. The experiment was carried out in triplicate. [14]

Pharmacokinetic studies of Actarit loaded IPN tablets

Animal preparation

24 New Zealand white rabbits of either sex rabbits were (weighing 4-5 kg) selected for this study, all the animals were healthy during the period of the experiment. Animals were maintained at room temperature 25°C, RH 45%, and 12 h alternate light and dark cycle with 100% fresh air exchange in animal rooms, uninterrupted power and water supply and rabbits were fed with standard diet and water ad libitum. An in vivo pharmacokinetic study was conducted in accordance with the ethical guidelines for investigations in laboratory animals and approved by the Institutional Animal Ethics Committee (IAEC NO: 1447/PO/Re/S/11/CPCSEA-56/A).

Study Design

Rabbits were randomly divided into 3 groups each group contains six animals (Group A, B and C). The rabbits selected for the study were housed in separate cages and had no medication for two weeks prior to the study. They were denied food and water during the study. The cages of rabbit were placed in 18 h light/6 h dark conditions. The Group A rabbits received pure drug with equivalent dose to animal dose, Group B fed were fed with actarit marketed product, group C actarit optimized formulation.

Blood samples were collected at regular time intervals with syringes from the marginal ear vein. at times 0, 0.50, 1, 1.50, 2,

2.50, 3, 4, 6, 8, 12, 16, 20, 24h post dose and transferred into Eppendrof tubes containing heparin in order to prevent blood clotting. Plasma was separated by centrifugation of the blood at 3400 rpm for 30 min and stored frozen at −20°C until analysis [16].

Instrumentation and Chromatography

The HPLC system consisted of a Jasco–PU 980 intelligent pump (Jasco Ltd., Japan), manual injector port with 20 µl loop (Rheodyne, USA) and Jasco UV/Vis 975 intelligent detector (Jasco Ltd., Japan). The wavelength of the detector was set at 245 nm. Detector output was quantified on Jasco–Borwin (Version 1.50) chromatography software with Hercules 2000 Chromatography Interface (Version 2.0). Separation was carried out on a HiQ Sil C8, 250×4.6 mm i.d., Japan, using methanol:1% acetic acid in the ratio of 50:50 (v/v) as a mobile phase, at a flow rate of 1 ml/min. The mobile phase was filtered through nylon membrane filter (0.45 µm pore size, Pall, Gelman Laboratories) and ultrasonically degassed prior to use. Total analysis time was 10 min [17, 18].

RESULTS AND DISCUSSION

Five batches of IPNs were prepared by lyophilization technique. The percent drug loading in IPN complex was observed in the range of 59.36 to 65.72 as shown in table 2. The loading efficiency of IPN complex was found to be two times more than the Cyclodextrin nanosponges. In case of formulation (F3) high drug entrapment efficiency was noted due to high concentration of glutaraldehyde. The drug entrapment efficiency was found to be increased significantly ($p<0.05$) when concentration of glutaraldehyde was increased.

Table 2: Percent drug loading in IPNs.

S.NO	Name of the formulation	Drug loading (%)
1	F1	59.36
2	F2	62.43*
3	F3	65.72*
4	F4	61.78*
5	F5	63.86*

*Statistically significant ($p< 0.05$) than F1

Figure 1 shows a comparison of FTIR spectra of Carboxymethyl tamarind gum, Cyclodextrin nanosponges (NS16), Actarit, ACNS16 and Actarit loaded IPNs. The FTIR spectra of pure Actarit showed characteristic peaks of Actarit are at around 3311.64, 1695.36, 1637.49, 1602.78, 1544.91, 1502.48, 1396.40, 1377.11, 1292.25, and 700.13 cm-1. In case of drug loaded IPNs, all characteristic peaks of Actarit were observed with insignificant shifting and reduction in intensity. The peak for C=O stretching vibration shifted towards higher frequency indicating hydrogen bonding with IPN network.

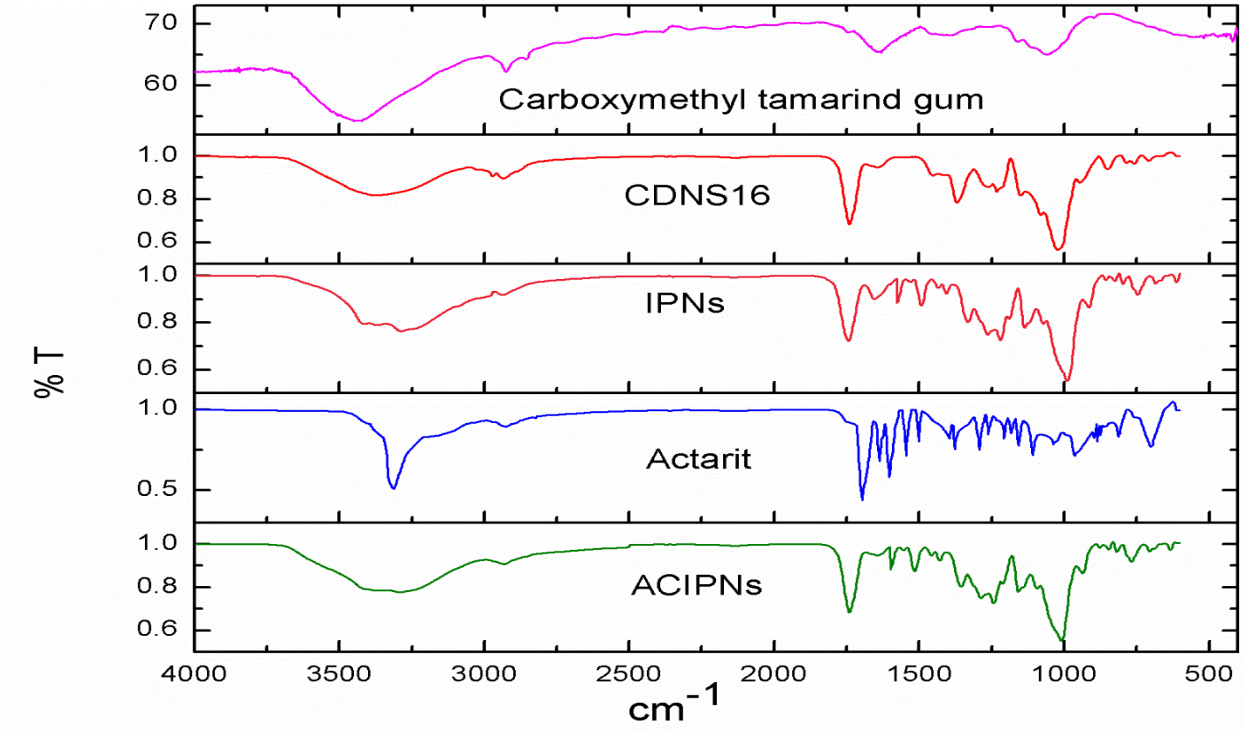

Figure 1: FTIR spectra of carboxymethyl tamarind gum, cyclodextrin nanosponges (CDNS16, Blank IPN, Actarit and Actarit loaded IPN.

The DSC thermogram of NS16 showed exothermic peak at around 350 °C. Carboxymethyl tamarind gum exhibited exothermic peak at around 372 °C. DSC curve of IPN have not shown any characteristic peak. The absence of characteristic peaks both the individual polymer in IPN indicating formation of polymeric cross-linked structure with intercalation of polymeric chains of carboxymethyl tamarind gum and Cyclodextrin nanosponges. The DSC thermogram of free drug Actarit shows a sharp endothermic peak approximately at 176.97 °C indicating the crystalline nature of the drug. Actarit loaded IPN complex have not shown any characteristic peaks of drug and individual polymers.. (Figure 2)

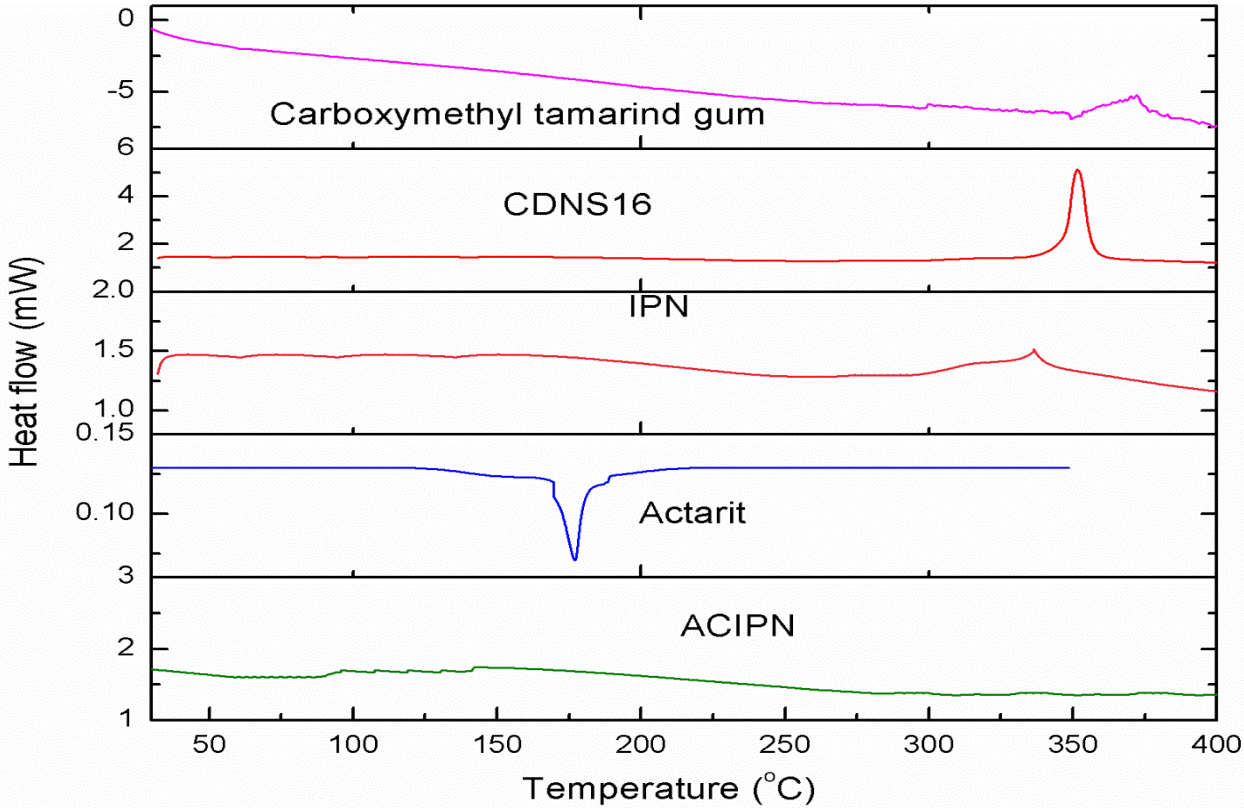

Figure 2: DSC thermograms of carboxymethyl tamarind gum, cyclodextrin nanosponges (CDNS16, Blank IPN, Actarit and Actarit loaded IPN.

To study the physical nature of Actarit with in the IPNs, X-ray diffraction pattern of pure Actarit, blank IPNs and Actarit loaded IPNs complexes were investigated. The x-ray diffractograms of plain Actarit exhibited sharp intense peaks at 2θ values of 10.8°, 13.3°, 16.1°, 18.9°, 22.1°, 23.6°, 25.4°, 27.6° and 31.2 ° with different peak intensity indicating crystalline nature of drug as shown in figure 3. However, there were no characteristics peak of pure Actarit were observed in IPN complex. The absence of such crystalline peaks of Actarit in IPN complex clearly indicates that the amorphization of drug throughout the polymer network of the IPNs. FTIR, DSC and XRD studies confirmed the formation of molecular complex of Actarit with IPNs.

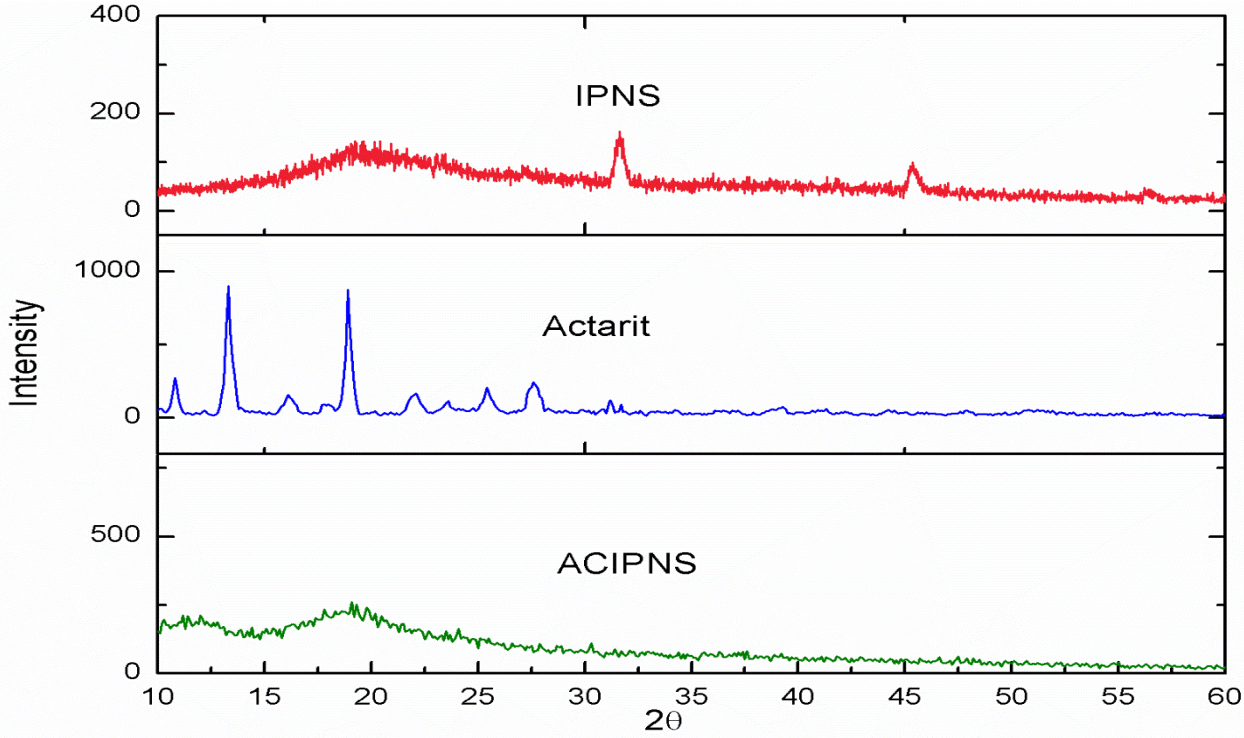

Figure 3: XRPD pattern of Blank IPNS, Actarit and Actarit loaded IPNS.

IPNs showed significantly low ($P<0.05$) swelling in 0.1N HCl than the phosphate buffer pH 6.8. carboxymethyl tamarind gum is anionic polymer which is in unionized state in the acidic environment. The pH of 0.1N HCl solution is less than the pKa of the carboxyl group present in carboxymethyl tamarind gum leading to decreased electrostatic repulsion due to protonation of carboxylic groups (COOH). This retards swelling owing to reduction in water uptake capacity of IPNs. (Figure 4)

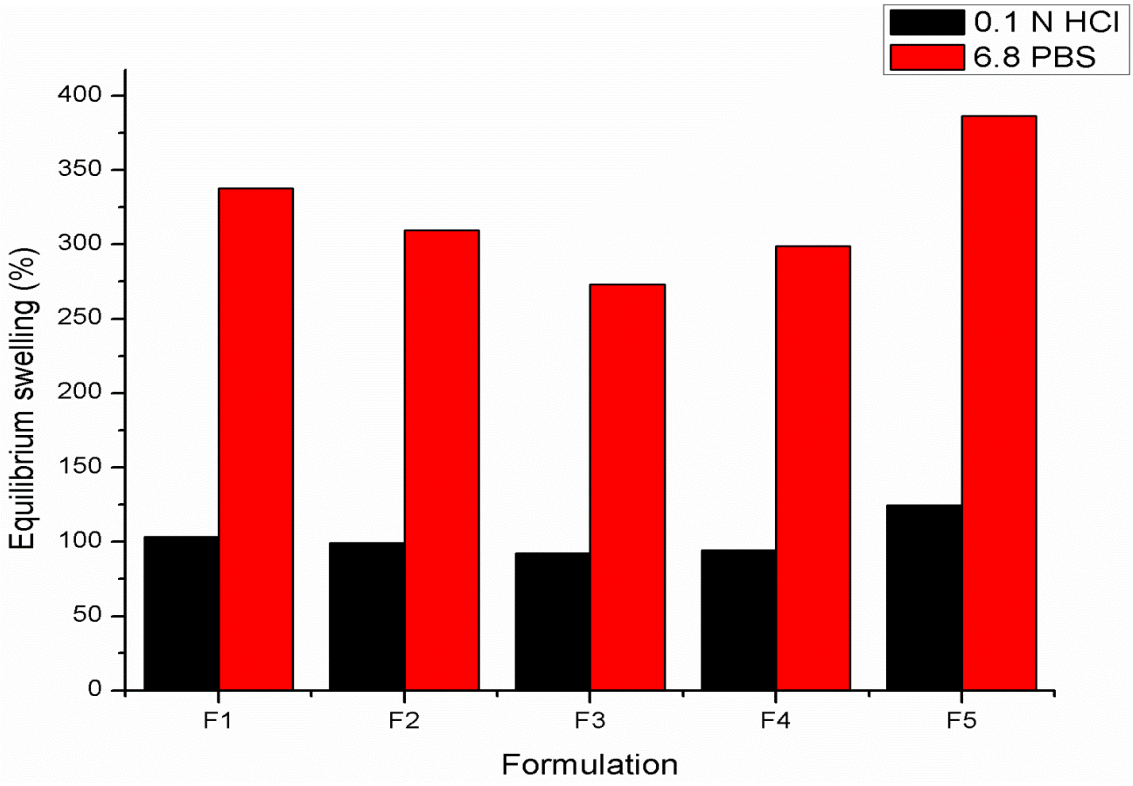

Figure 4: Equilibrium swelling (%) of IPNs in 0.1N HCl and phosphate buffer pH 6.8.

The mean weight ranged from 298.24 mg ± 0.32 to 301.56 mg ± 0.86. The mean thickness ranges from 5.12 mm ± 0.43 to 5.53 mm±0.82. The mean hardness ranges from 5.46 kg/cm2 ± 0.65 to 5.85 kg/cm2 ± 0.32. The mean friability values ranges from 0.27 % ± 0.34 to 0.42 % ± 0.18 and the average percentage drug content ranges from 99.12 % ± 0.23 to 99.71% ± 0.61, as shown in Table 3.

Table 3: Evaluation parameters of actarit loaded IPN tablets.

Formulation	Weight (mg)	Thickness (mm)	Hardness (kg/cm^2)	Friability (%)	Drug content (%)
F1	300.45	5.12	5.42	0.32	99.44
F2	301.56	5.35	5.46	0.30	99.12
F3	300.26	5.26	5.63	0.27	99.71
F4	298.24	5.53	5.58	0.29	99.37
F5	299.73	5.49	5.85	0.34	99.58

In vitro release study

The marketed tablet of actarit was completed its release within one hour as shown in Figure 5. A biphasic release pattern of actarit from the prepared IPN tablets was observed. The IPNs tablets showed less than 10% of drug release in the acidic pH 1.2 at the end of 2h due to the partial swelling in an acidic environment. The release was ranged from 12.46 % ± 1.1 to 19.23 % ± 1.56 followed by sustained release of the drug for 12 h in SGF (pH 6.8). The percent of actarit released from IPN tablets after 9 h ranged between 89.44 % ± 4.06 to about 98.33 % ± 4.12.

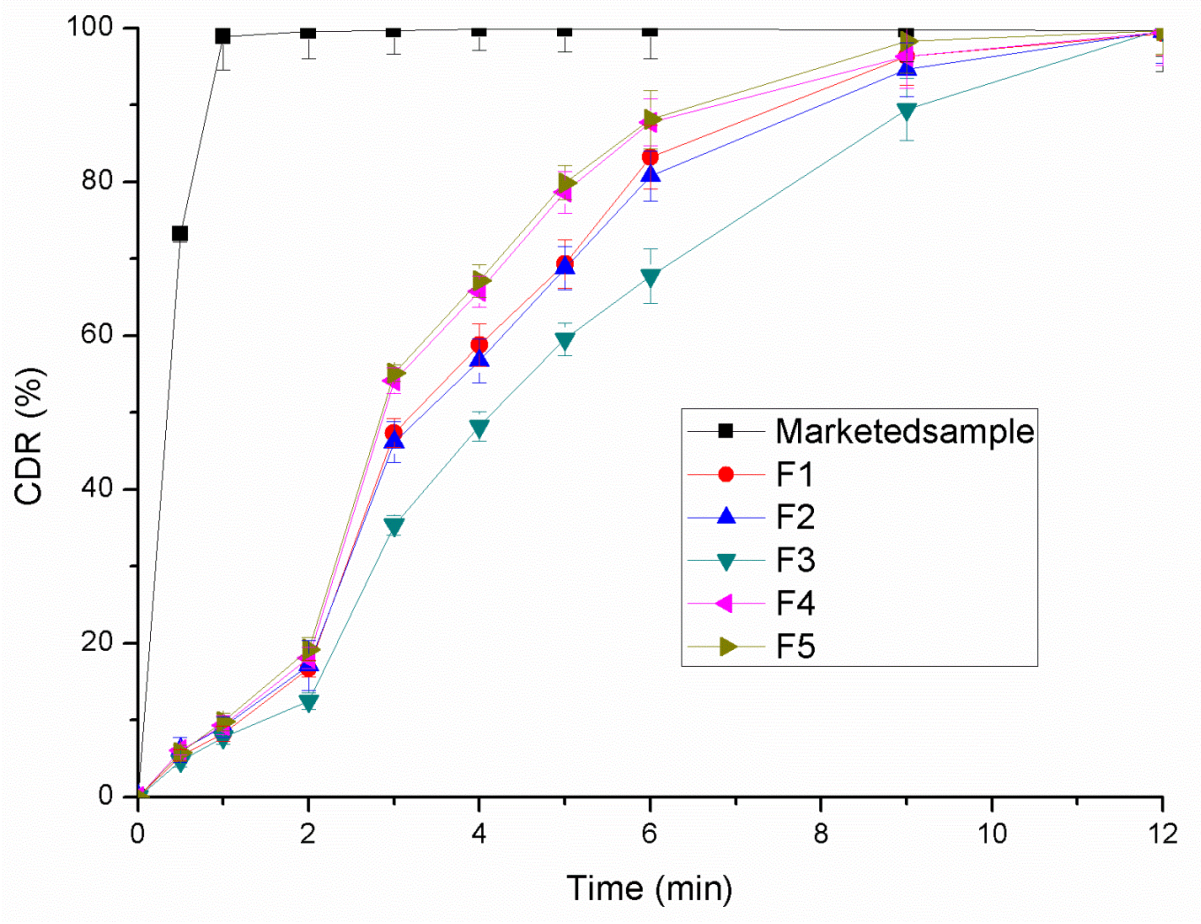

Figure 5: In vitro release of Actarit IPN tablets and marketed tablets

Pharmacokinetic studies

Figure 6 shows the plasma concentration–time curve in rabbits after a single oral dose of Actarit optimized formulation, as compared to Actarit pure drug and marketed formulation.

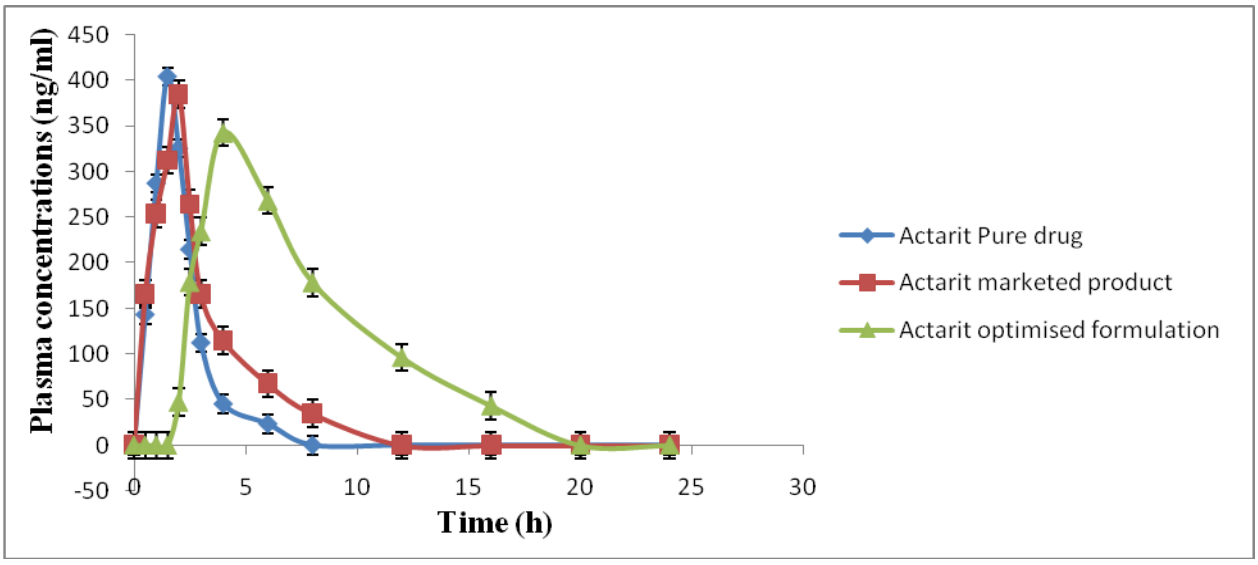

Figure 6: Mean plasma concentration-time profiles for actarit pure drug, actarit marketed product and actarit optimized formulation in rabbits (n=6).

The pure drug's Cmax was 404.34±12.87 ng/ml, significantly higher when compare with marketed product and optimized IPN actarit tablets of 385.21±9.43 and 342.76±13.56 ng/ml respectively. Tmax of pure drug, marketed and optimized formulation was 1.5±0.05, 2.0±0.06 and 4.0±0.04h, respectively. AUC0-∞ infinity for marketed product and optimized formulation was higher (815±14.23 and 915±28.33 ng.h/ml) than the pure drug 800.76±15.76 ng.h/ml. AUC0-t of the marketed and optimized

tablet's formulation (714±18.24 and 834±19.14 ng.h/ml) was significantly higher as compared to pure drug (696.54±18.43 ng.h/ml). Higher amount of drug concentration in blood indicated better systemic absorption of Actarit from optimized formulation as compared to the pure drug. The t 1/2 and MRT of optimized formulation were higher as compared to pure drug and marketed product. (Table 4)

Table 4: Mean pharmacokinetic parameters of actarit pure drug, actarit marketed product and actarit optimised formulation

Pharmacokinetic parameters	Actarit Pure drug	Actarit marketed product	Actarit optimised formulation
C_{max} (ng/ml)	404.34±12.87	385.21±9.43	342.76±13.56
AUC_{0-t} (ng.h/ml)	696.54±18.43	714±18.24	834±19.14
AUC_{0-inf} (ng.h/ml)	800.76±15.76	815±14.23	915±28.33
T_{max}(h)	1.5±0.05	2.0±0.06	4.0±0.04
$t_{1/2}$ (h)	1.76±0.83	2.34±0.53	4.35±0.12
K_{el}	0.3937±0.001	0.2961±0.004	0.159±0.001
MRT (h)	2.52±0.12	3.02±0.14	5.21±0.15

CONCLUSION

The interpenetrating networks of carboxymethyl tamarind gum and cyclodextrin nanosponges were successfully developed were prepared using the freeze-drying method and evaluated. The dissolution of IPN actarit nanosponges tablets was substantially greater (99.86%) than that of the marketed drug (98.67%) due to the decreased drug particle size, the formation of a high-energy amorphous state, and intermolecular hydrogen bonding. The drug may be maintained and released relatively slowly by the nanosponge structure. FTIR, DSC, and XRD analyses indicated the development of a actarit inclusion complex with nanosponges. The Cmax of the marketed, optimized tablet formulation 385.21±9.43 and 342.76±13.56 ng/ml respectively which was significant as compared to the pure drug 404.34±12.87 ng/ml. The Tmax for the pure drug, marketed and the optimized formulation were 1.5±0.05, 2.0±0.06 and 4.0±0.04h, respectively. The AUC0-inf of the marketed and optimized formulation (815±14.23 and 915±28.33 ng.h/ml)was higher than that of the pure drug suspension (800.76±15.76 ng.h/ml). Hence, when compared to the pure drug, optimized Actarit loaded cyclodextrin nanosponges based IPN tablets had a higher bioavailability and MRT.

REFERENCES

1. Liechty WB, Kryscio DR, Slaughter BV, Peppas NA. Polymers for drug delivery systems. Annu Rev Chem Biomol Eng 2010; 1:149-173.
2. Boppana R, Kulkarni R V, Mutalik SS, Setty CM, Sa B. Interpenetrating network hydrogel beads of carboxymethylcellulose and egg albumin for controlled release of lipid lowering drug. J Microencapsul 2010; 27:337–44.
3. Pater RH (1990) IPNs high performance. VCH Pub, New York, pp 377–401
4. Jain N, Banik A, Gupta BN. Novel IPN microspheres of Lepidium sativum and poly(vinyl alcohol) for the controlled release of simvastatin. Int J Pharm Sci 2013(5):125–130
5. Trotta F, Cavalli R. Characterization and application of new hyper-cross-linked cyclodextrins. Compos. Interfaces 2009; 16: 39–48.
6. Cavalli R, Trotta F, Tumiatti W. Cyclodextrin-based nanosponges for drug delivery. J. Incl. Phenom. Macrocycl. Chem. 2006;56:209–213.
7. Rao P, Srivastava H, Tamarind, Whistler R, BeMiller J. Ind. Gums Polysaccharides Their Deriv., 2nd ed., Academic Press, New York, 1973, pp.369–411.
8. Jana S, Saha A, Nayak AK, Sen KK, Basu SK. Aceclofenac loaded chitosan-tamarind seed polysaccharide interpenetrating polymeric network microparticles. Colloids Surfaces B Biointerfaces 2013; 105:303–9.
9. Kulkarni RV, Mutalik S, Mangond BS, Nayak UY. Novel interpenetrated polymer network microbeads of natural polysaccharides for modified release of water-soluble drug: Invitro and in-vivo evaluation. J Pharm Pharmacol 2012; 64:530–40.
10. Swaminathan S, Pastero L, Serpe L, Trotta F, Vavia P, Aquilano D, Trotta M, Zara G, Cavalli R. Cyclodextrin-based nanosponges encapsulating camptothecin: Physicochemical characterization, stability and cytotoxicity. Eur. J. Pharm. Biopharm 2010;74:193–201.
11. Jana S, Banerjee A, Sen KK, Maiti S. Gelatin-carboxymethyl tamarind gum biocomposites: In vitro characterization & anti-inflammatory pharmacodynamics. Mater Sci Eng C 2016;69: 478–85.
12. Darwish MKM, Abu El-Enin ASM, Mohammed KHA. Formulation, optimization, and evaluation of raft forming formulations containing Nizatidine. Drug Dev Ind Pharm 2019;45:651–663
13. Abioye AO, Kola-Mustapha A. Formulation studies on ibuprofen sodium-cationic dextran conjugate: effect on tableting and dissolution characteristics of ibuprofen. Drug Dev Ind Pharm 2016;42(1):39-59.
14. Shaw GS, Biswal D, B A, Banerjee I, Pramanik K, Anis A, Pal K. Preparation, characterization and assessment of the novel gelatin–tamarind gum/carboxymethyl tamarind gum-based phase-separated films for skin tissue engineering applications. Polym Plast Technol Eng 2017; 56:141–52.
15. Omar SM, Ibrahim F, Ismail A. Formulation and evaluation of cyclodextrin-based nanosponges of griseofulvin as pediatric oral liquid dosage form for enhancing bioavailability and masking bitter taste. Saudi Pharm J 2020;28(3):349-361
16. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother 2010; 1:87-93

17. Loya, P, Saraf MN. Determination of actarit from human plasma for bioequivalence studies. Indian journal of pharmaceutical sciences 2010; 72(6):726–731.
18. Li, Yuenan Pan, Hao Duan, Hongliang Chen, Jianting Zhu, Zhihong Fan, Jingxin Li, Pingfei Yang, Xinggang Pan, Weisan. Double-layered osmotic pump-controlled release tablets of actarit: In vitro and in vivo evaluation. Asian Journal of Pharmaceutical Sciences. 2018;14:10.