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Original Article

INTERPENETRATING POLYMER NETWORK MICROSPHERES OF POLY (VINYL ALCOHOL)/METHYL CELLULOSE FOR CONTROLLED RELEASE STUDIES OF 6-THIOGUANINE

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ABSTRACT

Objective: The present study involved the preparation of Interpenetrating Polymer Network (IPN) microspheres formulated from Poly (vinyl alcohol) (PVA) and Methyl Cellulose (MC) for controlled release of an anti-cancer drug, 6-thioguanine.

Methods: The IPN microspheres were prepared by water-in-oil emulsion method using gluteraldehyde as a cross-linker. 6-thioguanine drug was successfully loaded into these microspheres via *in-situ* process. These prepared microspheres were characterized by Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), X-ray diffraction (X-RD), optical microscopy (OM) and Scanning electron microscopy (SEM).

Results: FTIR spectral data results confirmed the cross-linking reaction between IPN microspheres through gluteraldehyde. DSC and X-RD results indicated the molecular level distribution of 6-thioguanine drug in the polymer matrix. SEM images showed the microspheres have spherical shape with rough surface. OM results gave the average size of prepared microspheres that ranged from 40 to 280 µm. Encapsulation efficiency of the drug in these IPN microspheres was found to be 72 %. *In vitro* dissolution studies performed at pH 7.4 buffer medium showed that the drug release was depended on the extent of cross-linker, drug and the percentage of PVA used in the formulations. The drug release was analyzed by an empirical equation and was found to be non-Fickian type diffusion.

Conclusion: This study reveals that the combination of PVA and MC in the form of IPN microspheres may be used successfully for the controlled release of drugs with short plasma half-life. *In vitro* release studies showed the extended release of the drug for more than 12 h.

Keywords: Poly (vinyl alcohol), Methyl Cellulose, 6-thiogauanine, Interpenetrating Polymer Network Microspheres, *In vitro* release studies.

INTRODUCTION

Drug delivery research is primarily focused on targeted delivery of the drug to the desired organ system to minimize toxicity and maximize therapeutic efficacy. As oral route is the most popular route of drug administration, a large emphasis is given on the development of controlled oral drug delivery systems. Drug substances with high water solubility and short half life (elimination half-life 2-3 h) get readily absorbed and eliminated, thus requiring frequent [1]. This may lead to decrease in patient compliance and increase chances of side effects due to dose dumping [2, 3].

Thus, the drugs having high water solubility and short half-life warns extensive research to reduce frequent dosing and dose related side effects by controlling their release rate. Fabrications of drug delivery devices like microparticles or nanospheres are some of the approaches to control the release of highly water soluble drugs. Recently researchers have given a strong emphasis on natural polymer based approaches to develop controlled drug delivery systems. The use of synthetic polymers for drug delivery purposes is of limited application due to problems in biodegradability, use of organic solvents resulting in environmental pollution etc. [4]. However, natural polymers are considered to be much safer since organic solvents are not required for their processing and they are biodegradable in nature [5, 6]. The use of these natural polymers have their own problems like uncontrolled swelling and premature release of loaded drug.

The concept of polymeric blend microspheres is quite effective in overcoming the above problems. Interpenetrating polymer network (IPN) is one such formulation which is considered to be promising in delivery of bioactive molecules, particularly in controlled release applications [7]. IPN's have been recognized as popular responsive polymers having enhanced physical properties, easy fabrication of devices, manipulation of device properties, etc., as compared with conventional blends of their components. This would open up avenues to use IPN's in designing the novel controlled release systems. A combination of judicially selected natural and synthetic polymers based IPN's has been found to be useful in enhancing the release of short half-lived drugs under physiological conditions. In order to achieve this, the blending of natural and synthetic polymers has been modified by grafting, blending and other means [8]. Blending of synthetic polymers with cellulose and modified cellulose are widely accepted [9, 10]. Recently our laboratory developed modified IPN-based carbohydrate polymers for CR systems of various types of drugs [11-13].

Methylcellulose (MC) is a cellulose derivative that has been extensively investigated for biomedical applications. It is a food additive used as a thickening agent and is unique due to its thermoreversibility characteristics. It exhibits low viscosity at low temperatures and a higher viscosity at higher temperatures as the polymer chains dehydrate and interact with each other. MC is biocompatible and its gelling properties have been well studied [14, 15, 16-18]. MC has been proven to support some nerve regeneration and can also be linked with proteins which encourage axonal extension [19]. Mixtures of MC with hyaluronan have recently been shown to change the properties of methylcellulose and provide a new hydrogel with regenerative abilities [15].

Poly (vinyl alcohol) (PVA) has been used widely in a variety of fields since its discovery in 1924 [20] due to its desirable properties such as non-toxicity and non-carcinogenicity [17]. It finds extensive applications as biomaterials [21-23], such as contact lenses, artificial blood vessels, artificial intestines [20], and kidneys [22]. Drug release studies have also been carried on PVA-based hydrogels, a biocompatible, chemically stable, and desirable for bioseparations and cell encapsulations [21, 23, 24]. However, PVA is a highly hydrophilic polymer and has poor stability in water, so its solubility must be prevented for use in aqueous systems. To overcome this problem, PVA should be insolubilized by blending [25], copolymerization [26], grafting [27, 28] and cross-linking [29].

Hence in the present study PVA is blended with a natural polymer, MC and incorporated with an anticancer drug for drug release study.

6-thioguanine, chemically known as 2-amino-6-mercaptopurine belongs to the thiopurine family of drugs which is an example of antimetabolite. It is a purine analogue of the nucleobase guanine and used as an anticancer drug. Its drug usage was originally as a cytostatic agent in chemotherapy for the treatment of acute lymphoblasic leukemia in children [30]. The plasma half-life of thioguanine is short, due to the rapid uptake into liver and blood cells. From a perusal of the literature it was found that no reports are available on MC/PVA IPN microspheres for CR of 6-thioguanine drug. This prompted us to incorporate water soluble 6-thioguanine drug into MC/PVA IPN microspheres. The authors successfully studied the release profiles for different formulations by varying PVA content in MC/PVA blend, cross-linking agent and drug concentration.

MATERIALS AND METHODS

Materials

Methyl cellulose (MC) ($\overline{M_n}$ ≈ 40,000, degree of methylation = 1.6-1.9), Poly (vinyl alcohol) (PVA) ($\overline{M_n}$ ≈ 1,25,000) and 6-thioguanine were purchased from Sigma – Aldrich, USA. Analytical reagent grade of Gluteraldehyde solution (25% v/v), n-hexane, petroleum ether (B. P = 60 – 80 ^{0}C), Span-80, hydrochloric acid (HCl) and Paraffin Oil (light) were purchased from S. D. Fine chemicals, Mumbai, India.

Preparation of IPN Microspheres

IPN microspheres of MC and PVA were prepared by W/O emulsion cross – linking method. In brief, known amount of PVA was dissolved in distilled water by continuously stirring at 80 ^{0}C until a homogeneous solution was obtained. After cooling to ambient temperature, known amount of MC solution (obtained by dissolving separately in water by stirring overnight) was added in the above PVA solution and stirred overnight to obtain a clear solution. Then, known amount of 6-thioguanine drug dissolved in water was added to the blend polymer solution.

This solution was slowly added to a mixture of petroleum ether and light liquid paraffin (40:60, w/w) containing Span-80 (1% w/w) under constant stirring at 400 rpm speed for 10 min. To this emulsion, 1 mL of 0.1 M HCl and the known amount of GA was added slowly and further stirred for another 30 min. The hardened microspheres were separated by filtration, washed with n-hexane and water to remove oil and excess amount of unreacted GA. These microspheres were dried under vacuum at 40 ^{0}C for 24 h and stored in desiccator for further analysis and characterization. In total, seven formulations were prepared by varying the drug concentration, cross – linker (GA) and % of PVA according to the Table 1. Representation of IPN microsphere is shown in Scheme 1.

Fourier Transform Infrared (FTIR) Spectroscopy

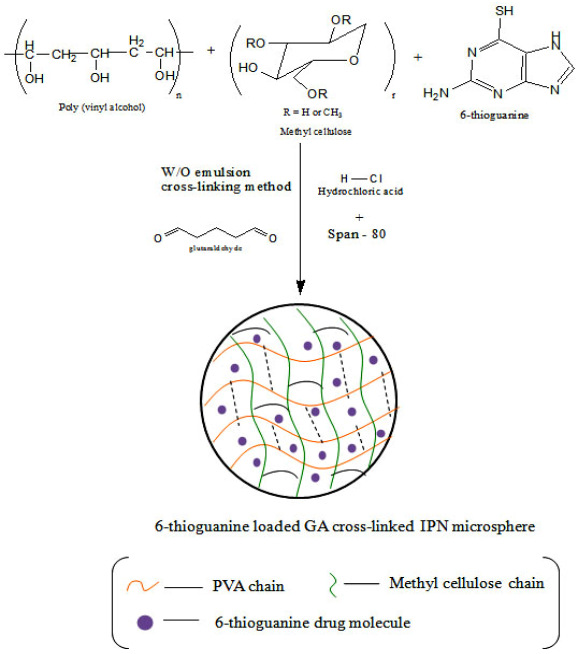
The FTIR spectra of pure PVA, pure MC, pure drug, pure IPN microspheres and drug- loaded IPN microspheres were recorded using FTIR spectrophotometer (Model impact 410, Wisconsin, MI, USA). The samples were crushed finely with potassium bromide (KBr) to make pellets under hydraulic pressure of 700 dynes/m^2 and scanned between 4000 and 400 cm^{-1}.

Differential scanning colorimetry

DSC curves of pure MC/PVA microspheres, drug-loaded MC/PVA microspheres and pure drug were recorded using a DSC (Model – SDT Q600, USA) by heating from 40 to 600 ^{0}C at a heating rate of 10 ^{0}C/min under nitrogen atmosphere (flow rate, 20 ml/min).

X-ray diffraction studies

X-Ray diffraction (X-RD) patterns of pure MC/PVA microspheres, drug-loaded MC/PVA microspheres and pure drug were recorded using a Rigaku Giegerflex diffractometer (Tokyo, Japan) equipped with Ni-filtered CuKα radiation (λ = 1.5418 A^0) at 40 kV and 50 mA. The dried microspheres of uniform size were mounted on a sample holder, and the patterns were recorded in the 2θ range of 0- 60^0



Scheme 1: Formation of 6-thioguanine encapsulated IPN microsphere by the cross – linking reaction between PVA and MC using GA.

Particle Size and Scanning Electron Microscopy

To determine the particle size and size distribution, ~ 100-200 microspheres were taken on a glace slide and their sizes were measured using an optical microscope under regular polarized light. Scanning electron microscope (SEM) micrographs of IPN microspheres were obtained using JEOL Model, JSM-6360, Kyoto, Japan at the required resolution (Mag 100 x 30 kv).

Estimation of Drug Loading and Encapsulation Efficiency

The drug-loaded microspheres (10 mg) were pulverized and incubated in 10 mL of phosphate buffer (pH 7.4) at room temperature for 24 h. The suspension was agitated with agate mortar and filtered through filter paper. The drug solution was assayed spectrophotometrically for 6-thioguanine content at the wavelength of 340 nm. The results of % drug loading and encapsulation efficiency were calculated using following equations

$$\% \text{ Drug loading} = \left[\frac{\text{Amount of drug in microspheres}}{\text{Amount of microspheres}}\right] \times 100 \quad \ldots\ldots\ldots\ldots (1)$$

$$\% \text{ Encapsulation efficiency} = \left[\frac{\text{Actual loading}}{\text{Theoretical loading}}\right] \times 100 \ldots\ldots\ldots\ldots (2)$$

In vitro release studies

In vitro dissolution studies were carried out in phosphate buffer solution (pH 7.4) using Tablet dissolution tester (Lab India, Mumbai, India) equipped with eight baskets. Dissolution rates were measured at 37 ± 0.5 ^{0}C at constant speed of 100 rpm. At regular time intervals, aliquot samples were withdrawn and analyzed using UV spectrophotometer (Lab India, Mumbai, India) at the fixed λ_{max} value of 340 nm. After each sample collection, the same amount of fresh medium at the same temperature was added to the release medium to maintain the sink condition. All measurements were carried out in triplicate, and values were plotted with standard deviation errors.

RESULTS AND DISCUSSIONS

Fourier transform infrared spectroscopy

Fig 1 shows the FTIR spectral analysis of pure microspheres (a). Drug loaded microspheres (b) and pure drug (c). These spectra were used to examine the stability of the drug in the formulation and to confirm the formation of IPN matrix structure.

FTIR spectra of pure microspheres and drug loaded microspheres (Fig 1a, 1b) shows the characteristic absorption band at 3440 cm^{-1} due to the – O – H stretching vibration and a peak at 2962 cm^{-1} was due to the – C –H stretching vibrations. The peak at 1134 cm^{-1} in the pure microspheres (Fig 1a) indicates the formation of acetal between PVA and MC due to crosslinking by GA. Similar finding has been observed elsewhere [31].

The FTIR bands (Fig 1c) present at 1482 cm^{-1} and 1535 cm^{-1} in 6-thioguanine are assigned to aromatic C – C symmetric and asymmetric stretching vibrations, the IR bands observed at 3263 cm^{-1} and 3309 cm^{-1} were assigned to N-H symmetric and asymmetric vibrations. The bands at 1373 cm^{-1} and 1434 cm^{-1} are assigned to C-N symmetric and asymmetric stretching vibrations [32]. In the case of drug loaded microspheres (Fig 1b) all the peaks corresponding to pure microspheres and 6-thioguanine were present, which provided the physical stability of drug in the formulation.

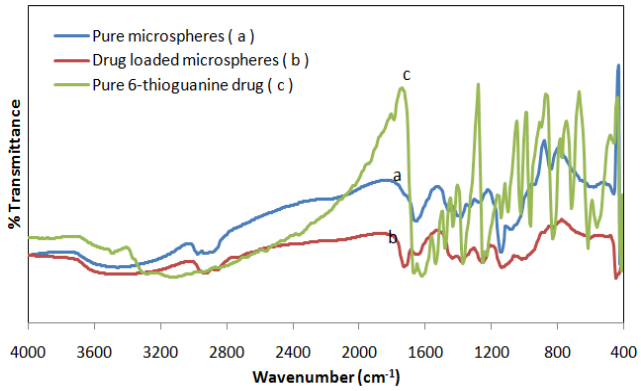


Fig. 1: FTIR spectra of (a) pure microspheres, (b) drug loaded microspheres and (c) pure 6-thioguanine.

DSC Analysis

DSC thermograms of the pure 6-thioguanine drug (a), pure microspheres (b), and drug loaded microspheres (c) are displayed in Fig 2. The onset melting peak of 6-thioguanine was observed at 384.35 ^{0}C (Fig 2a). However, no characteristic peak of 6-thioguanine was observed in the DSC curves of the drug loaded microspheres, suggesting that the drug is molecularly dispersed in the polymer matrix.

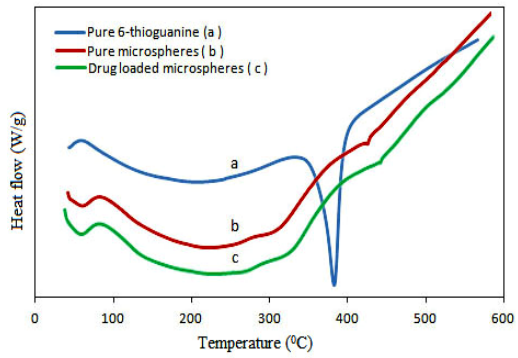


Fig. 2: DSC thermograms of (a) pure 6-thioguanine, (b) pure microspheres and (c) drug loaded microspheres.

Particle Size and Scanning Electron Microscopic Studies

SEM images of few drug loaded blend IPN microspheres are shown in Fig 3. The microspheres are spherical without forming agglomeration and their surfaces are slightly rough. The results of particle size of microspheres were in the range of 40 – 280 µm. The variation of particle size with polymer composition and GA content are shown in Fig 4a, 4b respectively. As % of PVA increases the average size of microspheres decreased. This may be due to the fact that PVA produces a compact network of macromolecular chains in the blend IPN microspheres, which tightens the network and hence decrease in size was observed. As GA content increases the average size of microspheres decreases. This was due to the increased resistance to the water diffusing out from the microspheres during microsphere formation.

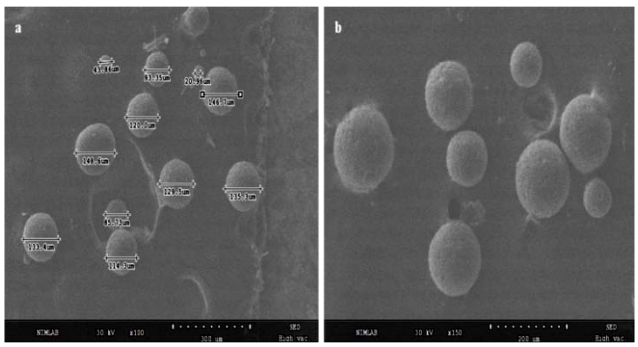


Fig. 3: SEM micrograph of drug loaded IPN microspheres.

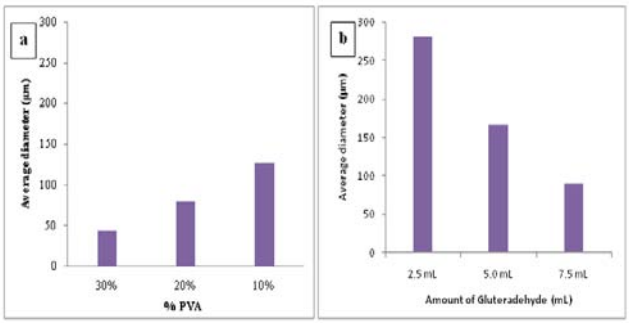


Fig. 4: Size and size distribution of IPN microspheres (a) effect of PVA, and (b) effect of GA content.

X-Ray Diffraction Studies

X-ray diffractograms of (a) pure 6-thioguanine, (b) pure microspheres, and (c) drug loaded microspheres are showed in Fig 5. These studies are useful to investigate the crystallinity of drug in the cross linked microspheres. The diffractogram of pure drug has shown characteristic intense peaks between 2θ of 10^0 and 27^0 due to its crystalline nature.

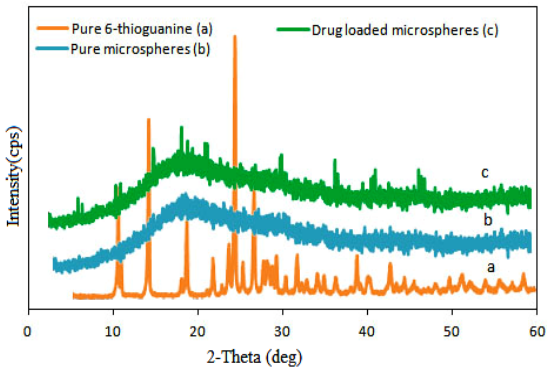


Fig. 5: X-RD patterns of (a) pure 6-thioguanine, (b) pure microspheres and (c) drug loaded microspheres.

However, these peaks have disappeared in the drug loaded microspheres, but only peaks observed in pure microspheres were seen. This indicates the molecular dispersion of the drug after incorporation into the IPN microspheres. These results are in accordance with the conclusions drawn above from DSC results explained earlier.

Encapsulation Efficiency

Effects of GA and PVA content on encapsulation efficiency of drug-loaded microspheres are given in Table 1. The encapsulation efficiency of 6-thioguanine drug decreases with increasing PVA content in the formulations. This is due to the fact that at higher concentrations, PVA produced a compact network of macromolecular chains during the microspheres formation. Hence, increasing content of PVA in the PVA/MC blend microspheres decrease trend in drug encapsulation efficiency was observed. Microspheres cross-linked with 2.5, 5 and 7.5 mL of GA, encapsulation efficiencies are 72%, 63%, and 61% respectively. Such decreasing trend is due to an increase in cross-link density, resulting in the formation of a more rigid network structure, which reduces the free volume spaces within the polymer matrix and hence, a reduction in encapsulation efficiency is observed.

Table 1: Results of % of encapsulation efficiency and release kinetic parameters of different formulations

Formulation Codes	% of MC	% of PVA	% of Drug	Amount of GA added (mL)	% of E. E ± S. D	n	k	Correlation coefficient, r
PM-1	90	**10**	10	5	70.3±0.4	0.71	0.0136	0.9612
PM-2	80	**20**	10	5	68.1±1.2	0.64	0.0152	0.9833
PM-3	70	**30**	**10**	**5**	63.9±0.7	0.59	0.0242	0.9454
PM-4	70	30	**20**	5	66.4±1.1	0.55	0.0311	0.9758
PM-5	70	30	**30**	5	68.7±0.8	0.48	0.0167	0.9879
PM-6	70	30	10	**7.5**	61.6±2.7	0.66	0.0174	0.9918
PM-7	70	30	10	**2.5**	72.3±1.6	0.62	0.0565	0.9445

E. E = Encapsulation efficiency; S. D = Standard deviation

Drug Release Kinetics

Drug release kinetics was analyzed by plotting cumulative release data versus time and fitting these data to the exponential equation of the type [33]:

$$\frac{M_t}{M_\infty} = kt^n \quad \text{.....(3)}$$

Here M_t/M_∞ represents the fractional drug release at time t; k is a constant characteristic of the drug-polymer system and n is an exponent parameter characterized the release mechanism. Using the least squares procedure, we have estimated the values on n and k for all the formulations, and these values are given in Table 1. If n = 0.5, drug diffuses and releases out of the polymer matrix following the Fickian diffusion. For n > 0.5, anomalous or non-Fickian type drug diffusion occurs, if n = 1, a completely non Fickian or case II release kinetics is operative. The intermediary values ranging between 0.5 and 1.0 are attributed to the anomalous type of diffusive transport [33].

In this study, the values of k and n have shown a dependence on the extent of cross-linking, % drug loading, and PVA content of the matrix. Values of n for microspheres prepared by varying the amount of PVA in the microspheres of 10, 20, and 30 wt% by keeping 6-thioguanine (10 wt%) and GA (5 mL) constant, ranged from 0.59 to 0.71 (see Table 1) leading to the drug diffuses and erosion controlled release from the polymer matrix following a non-Fickian type diffusion.

This may be due to the reduction in the regions of low microviscosity and closure of microcavities in the swollen state of the polymer. Similar findings have been observed elsewhere [34]. Correlation coefficients, r, obtained while fitting the release data are in the range from of 0.9445 to 0.9918.

***In vitro* drug release studies**

Effect of Drug Content

The release profiles of 6-thioguanine drug loaded MC/PVA IPN microspheres at different amounts of drug loading are shown in Fig 5. Release data showed that PM-5 formulation containing a higher amount of the drug (30%) displayed fast and higher release rates compared to the formulation PM-3, which is having small amount of the drug (10%). In other words, with a decreasing amount of drug in the matrix, it is observed that the release rate becomes quite slower due to the availability of more free void spaces through which a lesser number of drug molecules could transport.

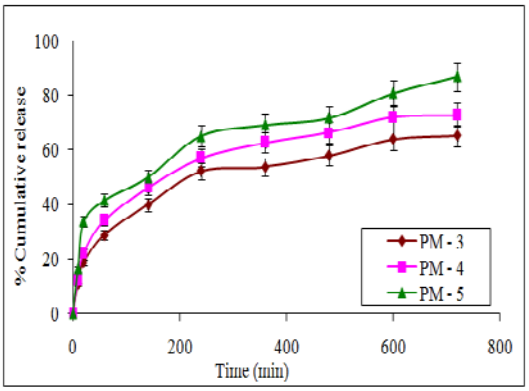


Fig. 5: % Cumulative release of 6-thioguanine through IPN microspheres containing 70:30 ratio of MC/PVA with different amount of 6-thioguanine (PM-3) 10%, (PM-4) 20%, and (PM-5) 30%.

Effect of Crosslinking Agent

The cumulative release vs. Time curves for varying amounts of GA (2.5, 5, 7.5 mL) at a fixed amount of drug (10 wt %), MC/PVA microspheres PM-7, PM-3, and PM-6 are displayed in Fig 6. The % of cumulative release is quite faster and larger at lower amount of GA (2.5 mL) (PM-7), whereas the release is quite slower at higher amount of GA (i. e. 7.5 mL) (PM-6). The cumulative release is slower when the microparticles containing the higher amount of GA, it may be due to the polymeric chains become rigid due to the contraction of microvoids, thus decreasing the % cumulative release of 6-thioguanine drug through the microspheres.

Effect of PVA Content

To understand the effect of PVA content on the release profiles of 6-thioguanine from cross linked MC/PVA blend microspheres, *In vitro* release studies was carried out in pH 7.4 phosphate buffer solution at 37 ^{0}C. Fig 7 shows the cumulative release of drug (10 wt%) through microspheres containing different ratios of MC/PVA, at a constant GA (5%) concentration. From Fig 7 it was found that the highest cumulative release is obtained for PM-1 formulation, which has 10 % PVA ratio. On the other hand, the least cumulative release is obtained in PM-3, which has 30 % PVA ratio.

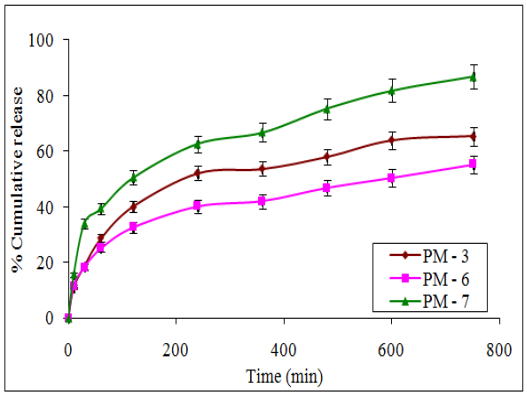


Fig. 6: % Cumulative release of 6-thioguanine through IPN microspheres containing 70:30 ratio of MC/PVA and 10% drug with different amount of GA (PM-7) 2.5 mL, (PM-3) 5.0 mL, and (PM-6) 7.5 mL.

This is due to the fact that PVA is virtually a linear polymer with small hydrate groups and thus produces a compact network of macromolecular chains in the blend microspheres, where as MC is a natural water-soluble polymer and contains residual –OH groups, which imparts hydrophilicity to the macro-molecule. Hence, with an increase of PVA in the PVA/MC blend microspheres a decrease in % of cumulative release is observed.

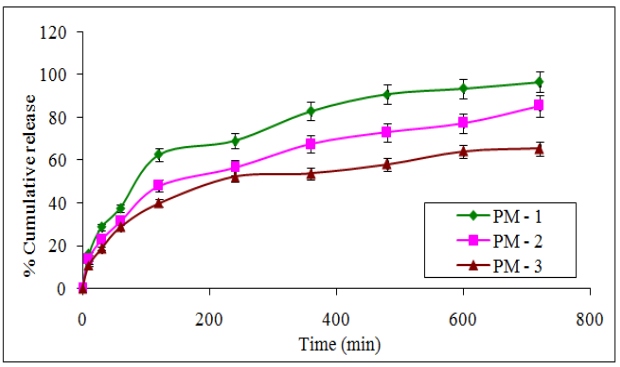


Fig. 7: % Cumulative release of 6-thioguanine through IPN microspheres containing 10% of drug and 5 mL of GA with variation of PVA (PM-1) 10%, (PM-2) 20%, and (PM-3) 30%.

CONCLUSION

MC/PVA IPN blend microspheres loaded with 6-thioguanine were prepared by W/O emulsion method using span-80 as the surfactant. The molecular level dispersion of drug, in the drug loaded microspheres was confirmed by DSC analysis. SEM images have shown the formation of spherical microspheres with the rough surface. Particle size measurements using optical microscopy gave an average size of 40-240 µm. The encapsulation efficiency was found to vary between 61% and 72% depending upon the blend composition, cross-linking and the amount of drug loading. Drug release studies indicated controlled release of 6-thioguanine for more than 10 h from IPN microspheres.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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