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# Preparation of Biodegradable Polymeric Blend Microspheres for Drug Delivery Application of Anticancer Drug- Flutamide

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**ABSTRACT**

The present study deals with the preparation of flutamide loaded polymeric blend microspheres prepared by solution blending method using soy protein isolate (SPI) and carboxymethylcellulose sodium (Na-CMC). Flutamide is an oral antiandrogen drug primarily used to treat prostate cancer. The purpose of this research is to minimize the frequency of doses, toxicity and to improve the therapeutic efficacy by formulating flutamide loaded polymeric blend microspheres. Microspheres were formulated and characterized by DSC, XRD, FTIR & SEM techniques. Further the drug load microspheres have utilized for drug release studies in terms of drug content, loading efficiency, particle size and *in-vitro* drug release kinetics. From the drug release studies it was observed that microspheres prepared with SPI and Na-CMC ratio 1:1 gives better sustained release for 10hrs as compared to other formulations. The results showed that microspheres were more beneficial as drug delivery system.

## INTRODUCTION

Microspheres are small spherical particles, with diameters in the micrometer range (typically 1 μm to 1000 μm). Microspheres are sometimes referred to as micro particles. Microspheres can be manufactured from various natural and synthetic materials. Glass microspheres, polymer microspheres and ceramic microspheres are commercially available. Solid and hollow microspheres vary widely in density and, therefore, are used for different applications. Hollow microspheres are typically used as additives to lower the density of a material. Solid microspheres have numerous applications depending on what material they are constructed of and what size they are.

A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time thereby causing little toxicity and minimal side effects. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs. Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 μm.

Carboxymethylcellulose (CMC) is an important industrial polymer with a wide range of applications in flocculation, drag reduction, detergents, textiles, paper, foods, drugs, and oil well drilling operation. CMC is a derivative of cellulose and formed by its reaction with sodium hydroxide and chloroaceticacid, it has a number of sodium carboxymethyl groups ($CH_2COONa$). Present in the cellulose molecule which promotes water solubility. The various properties of CMC depend upon three factors: molecular weight of polymer, average number of carboxyl content per anhydroglucose unit, and the distribution of carboxyl substituents along the polymer chains [1-3]. The most important properties of CMC are viscosity building and flocculation. Among all the polysaccharides, CMC is easily available and it is also very cheap and will be useful material for drug delivery application.

Soy protein has variety of functional groups on its polypeptide primary structure, thus offering the ability to be modified with other polymers. However, the soy protein-based materials are

brittle and have poor water holding capacity. These drawbacks limit its applications. To overcome the limitations, blending or grafting with another polymer may be an effective method [4-9]. Particularly, soy protein blending with environmentally sensitive polymers like carboxymethylcellulose sodium through the IPN technology can endow the composites with intelligent features and more biocompatibility, which may be of new potential applications in pharmaceutical field. The soy protein isolate/carboxymethylcellulose sodium through the solution blending can be more biocompatibility. Therefore, the purpose of this work was accomplished by preparing the soy protein isolate/ carboxymethylcellulose sodium blend microspheres to investigate the possibility of the microspheres for controlled drug release.

Prostate cancer is the most common cancer in men in Western countries and it is the second leading cause of cancer death. Flutamide is a potent nonsteroidal pure androgen receptor antagonist used clinically (250mg 3 times daily) for the management of metastatic carcinoma of the prostate [10]. Flutamide undergoes extensive first-pass metabolism [11]. Frequent administration of flutamide is required to reduce the level of testosterone and this may cause hepatotoxicity. The purpose of this study is to reduce the frequency of doses and toxicity and to improve the therapeutic efficacy by formulating flutamide incorporated microspheres of SPI/Na-CMC blend microspheres and to evaluate their particle size, entrapment efficiency, drug release studies. There were no reports in the literature on these systems; we have taken up this study in continuation of our drug delivery studies [12-15].

## MATERIALS AND METHODS

### Materials

Soy Protein Isolate (SPI) powder was obtained from Honeyville Food Products, Salt Lake City, Utah, USA. According to the manufacturer, the supplied SPI consisted of 90% protein, 4% fat, about 5% ash and 1% remaining unknown constituents. Carboxymethylcellulose sodium salt was purchased from SD-Fine Chemicals, Mumbai. Calcium Chloride was procured from Qualigens Fine Chemicals, Mumbai. Flutamide was supplied by Sun Pharma India Ltd, Mumbai. Double distilled water was used in this research work.

**Preparation of blend microspheres**

SPI was dispersed in distilled water at room temperature, and then basified with 10wt % NaOH aqueous solution to produce a viscous liquid of pH 9-10 containing 3% SPI. Meanwhile, Na-CMC was also dissolved in distilled water to obtain a solution containing 3wt % Na-CMC. Subsequently, the resultant SPI and Na-CMC solutions were mixed by the weight ratio of 1:1, 1:2 and 1:3, respectively. In the mixing solution, the weight ratios of SPI vs. Na-CMC were consistent with the weight ratios of SPI and Na-CMC solution as mixing, namely the SPI content in the total weight of SPI and Na-CMC were 25, 50, 75 wt % , respectively. The mixing solutions were mechanically stirred at room temperature for 2 hours to result in a homogeneous dispersion of SPI and Na-CMC components. At last, the complex microspheres formed by injecting the mixing solutions into 10 % wt $CaCl_2$ aqueous solutions using the syringe equipped with nozzle as well as cross linking –COOH groups in SPI and Na-CMC molecules in the aqueous solution are containing $Ca^{2+}$ for 30 min. The obtained microspheres were washed with distilled water to remove the free ion attached onto the surface and existed into internal holes. According to the SPI content in the solid, the microspheres were coded as SC-1(25 wt % SPI), SC-2(50 wt % SPI), and SC-3 (75 wt % SPI) and the formulations are shown in Table-1. The drug-loaded microspheres were prepared by adding the flutamide with the SPI/ Na-CMC mixing solution as per the weight ratio of 1:1, in which the SC-1 microspheres with best miscibility was used while the content of flutamide is 20 parts with regards to the 100 parts of the whole solid of SPI and Na-CMC. Subsequently, the mixing solution containing flutamide was cross linked by $Ca^{2+}$ and then produced the drug-loaded microspheres. All the drug-loaded microspheres were washed by distilled water to remove the drug attached onto the surface and then dried before the study of drug release.

**Table 1. Formulations of Flutamide loaded polymeric blend microspheres**

| S.No | Batch Code | SPI | Na-CMC | $CaCl_2$ mg | Drug (Flutamide) mg | Drug: Carrier ratios |
|---|---|---|---|---|---|---|
| 1 | SC-1 | 25 | 25 | 10 | 10 | 1:1 |
| 2 | SC-2 | 25 | 50 | 10 | 10 | 1:2 |
| 3 | SC-3 | 25 | 75 | 10 | 10 | 1:3 |

## CHARACTERIZATION

### Fourier transform infrared Spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectra were recorded on a Bomem MB-3000 FTIR spectrometer (Canada). The SPI and Na-CMC powder were taken with the method of KBr pellet and scanned in the range of 4000-400 $cm^{-1}$.

### Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) curves of the plain polymeric blend microspheres (SPI/Na-CMC), plain Flutamide drug and drug loaded polymeric blend microspheres were recorded using a Thermal Analysis instrument sequential thermal analyzer (Model-SDT Q600, USA). Analysis of the samples was performed at heating rate of $10^oC$/min under $N_2$ atmosphere at a purging rate of 100 mL/min.

### X-Ray diffraction (XRD)

X-Ray diffraction (XRD) patterns were recorded on A Siemens D 5000 powder X-ray diffractometer using Cu K α (1.54056 $A^o$) radiation (35kV, 30mA). All the powder samples were mounted on a sample holder and scanned from $2^o$ to $60^o$ in 2θ at a speed of 10 $min^{-1}$.

### Scanning Electron Microscopy (SEM)

The particle size of the SPI/Na-CMC blend microspheres were photographed using software controlled digital scanning electron microscope (JEOL JSM 5410) with 20 kV as the accelerating voltage. All the microspheres were frozen in liquid nitrogen while some were fractured immediately, and then they were freeze-dried for further characterization.

### Drug release studies

Flutamide was selected for the experiments of drug release because its UV absorption cannot be overlapped with the components of blend microspheres. Similar to the swelling tests again, pH value of 7.4 was adjusted and the experiments carried out at this pH. The drug- loaded SC-1 microspheres were used to study the drug release through *in-vitro* as follows. The drug-loaded microspheres (0.5 g) were incubated into 45 mL solution at pH 7.4 at $20^oC$. After the given intervals, the 5 mL solution was removed for determining the release content of drug, which was

obtained from the absorbance at 286 nm measured on a UV-VIS spectrophotometer (LABINDIA,UV-3000$^{+}$). Subsequently, the 5 mL fresh buffer solution was supplied to keep the total volume of 45mL solutions.

## RESULTS AND DISCUSSION

### FTIR Analysis (Interaction between SPI and Na-CMC components)

Usually, the good miscibility in the blend is driven by the intermolecular hydrogen bond among components. Before discussing the interaction between components, the groups that possibly form hydrogen bonds must be identified. The FTIR spectra of Na-CMC and SPI powder were shown in Fig.1.

Except for the double peaks at 3448 and 3340 cm$^{-1}$ assigned to O-H and N-H stretching vibrations, the SPI powder had two characteristic peaks at 1660 cm$^{-1}$ (amide I) and 1588 cm$^{-1}$ (amide II), which can reflect the formation or cleavage of hydrogen bonds. The O-H groups in Na-CMC can also anticipate into hydrogen bonding, and its stretching vibration located at 3450 cm$^{-1}$. In addition, the –COOH group can be cross linked with $Ca^{2+}$ , which constructs the structure of microspheres and plays a stabilizing role. The –COOH has asymmetrical and symmetrical stretching vibrations located at 1660 cm$^{-1}$ and 1588 cm$^{-1}$ respectively; the former is stronger and wider while the latter is sharp.

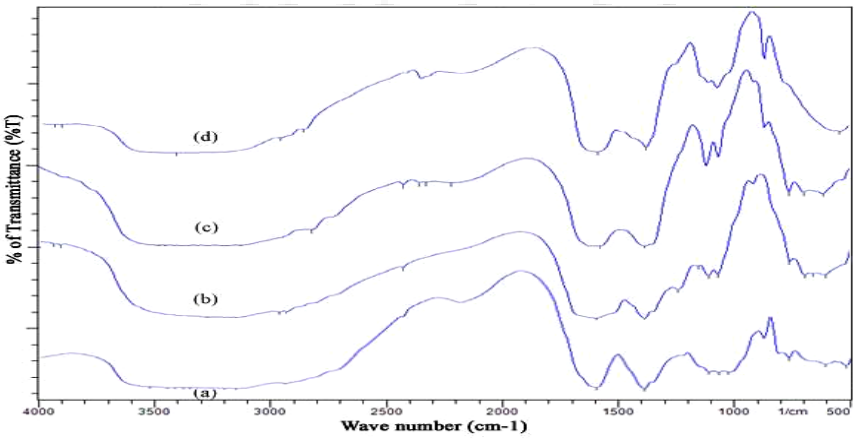


**Figure 1. FTIR spectra of (a) Pure Na-CMC, (b) Pure SPI, (c) SC microspheres with drug (d) SC microspheres without drug**

FTIR spectra of the freeze-dried microspheres with different SPI and Na-CMC content and their blend microspheres with and without drug loaded were shown in Fig.1. After the SC microspheres formed by the $Ca^{2+}$ cross linking, the absorption of O-H stretching vibration at3450 $cm^{-1}$ of pure Na-CMC powder was shifted down to3379 $cm^{-1}$, indicating that there existed stronger hydrogen bonding. Meanwhile, the cross linking of $Ca^{2+}$ caused two peaks assigned to asymmetrical and symmetrical stretching vibration of –COOH approached each other after ionization (from $–COO^-$), and located at 1660 and 1588 $cm^{-1}$. The changes of SPI content in complex microspheres can be observed by the intensity changes of characteristic peak of SPI component, namely the intensities of peaks at 1660 and 1588 $cm^{-1}$ assigned to Na-CMC component decreased. Introducing SPI containing N-H groups resulted in the shift of absorption above 3000 $cm^{-1}$ to high wave number for the complex microspheres, shown as the peak position at 3450 $cm^{-1}$ in FTIR spectra of (b) microspheres. Meanwhile, the C=O stretching vibration assigned to SPI shifted to low wave number of 1660 $cm^{-1}$ in contrast to SPI powder, indicating that the SPI component in (c) microspheres participated in the formation of hydrogen bonds.

**Differential Scanning Calorimetry**

DSC tracings of the Tolterodine (a), SC blend microspheres (b) and Tolterodine drug loaded SC microspheres {25% of SPI (SC1) and 50% of SPI (SC2)} are displayed in Fig.2. The onset melting peak of Tolterodine was observed at 100$^oC$ (Pure drug). However, no characteristic peak of drug in the drug loaded (SC1 & SC2) and plain SC blend microspheres, suggesting that the drug particles are molecularly dispersed in the polymer matrix.

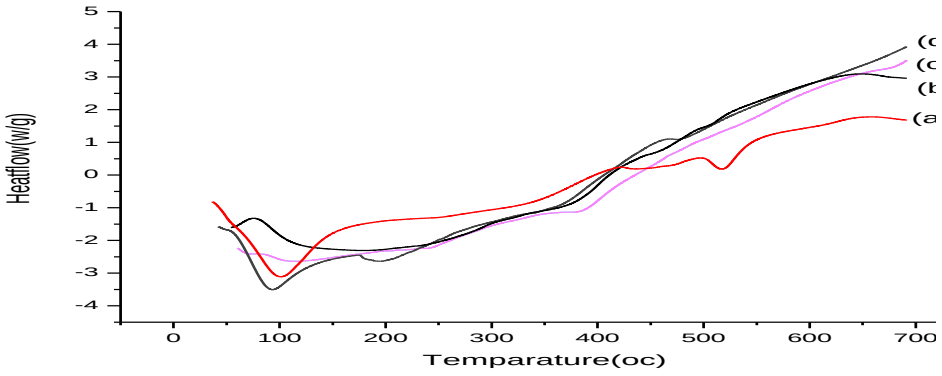


**Figure 2. DSC thermo grams of pure drug (a), pure blend (b), drug loaded blend microspheres at 25% of SPI (C) and at 50% of SPI (d), respectively.**

**XRD analysis**

XRD analysis provides a clue about the crystallinity of drugs in the SC polymeric blend microspheres. The X-ray diffraction patterns of (a) Pure SPI, (b) Pure Na-CMC, (c) SC polymeric blend microspheres with Flutamide drug of SC1, (d) pure Flutamide drug. SC1 microspheres XRD patterns are presented in Fig.3. From Fig.3.(d) it is noticed that Pure Flutamide drug peaks observed at 2θ of 20.0$^o$ and 22.0$^o$ are due to crystalline nature of drug, whereas these peaks were observed at 2θ of 20.9$^o$ case of in drug loaded SC1 polymeric microspheres (Fig.3.c). But from Fig.3. (a) & Fig.3. (b) it is observed that the drug peaks are not found. This indicates that the Flutamide drug particles are dispersed at molecular level in the polymer matrix, hence semi-crystalline nature of the drug was observed in the case of drug loaded microspheres.

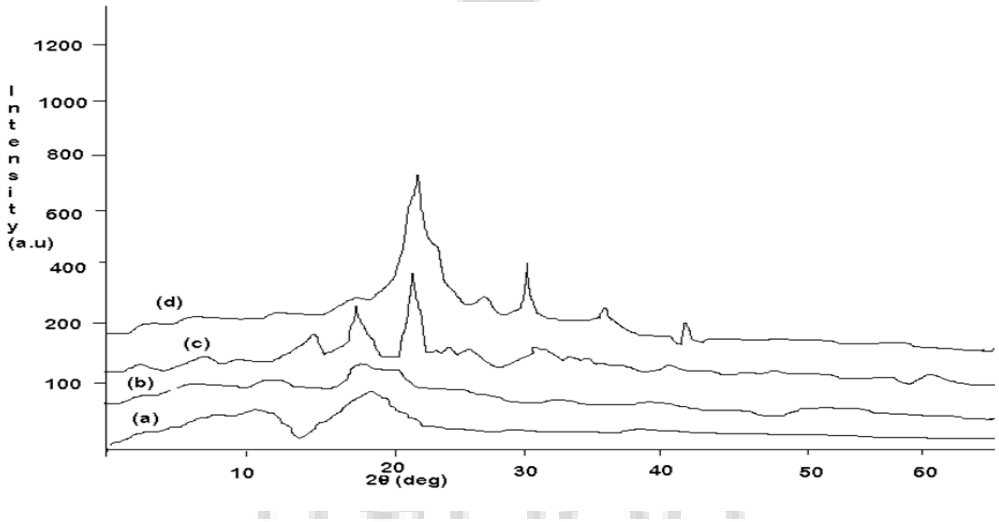


**Figure 3. (a) Pure SPI, (b) Pure Na-CMC, (c) SC1 polymeric blend microsphere with flutamide drug (d) pure Flutamide**

**Scanning electron microscopic (SEM) studies**

SEM images of the microspheres were recorded using a software controlled digital scanning electron microscope (JEOL JSM 5410) with 20 kV as the accelerating voltage. Fig. 4 shows the SEM micrograph of Flutamide drug loaded SPI/Na-CMC polymeric blend microspheres, and they are spherical in nature.

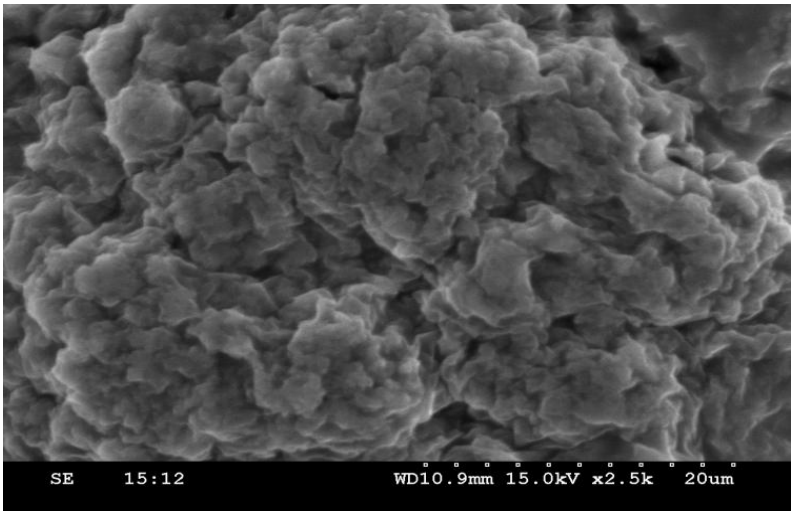


**Figure 4. Scanning electron micrograph of Flutamide drug loaded SPI/Na-CMC polymeric blend microspheres**

**Particle size analysis**

The particle size of the Flutamide incorporated SPI/Na-CMC polymeric blend microspheres were evaluated by scanning electron microscope for 3 formulations SC-1, SC-2, SC-3. And these values are presented in Table 2. The microspheres used in preparing drug loaded formulations were uniformly dispersed and they were ranging from 334µm to 619µm. The size of the particle varied depending on the flutamide loaded polymeric blend microspheres Table 2.

**Table 2. Flutamide loaded polymeric blend microspheres of Particle size and Percentage of Entrapment Efficiency**

| S.No | Batch Code | Drug : Polymer Ratios | Particle Size (µm) | Entrapment Efficiency (%) |
|---|---|---|---|---|
| 1 | SC-1 | 1:1 | 334 | 61.60±0.3 |
| 2 | SC-2 | 1:2 | 455 | 68.62±0.2 |
| 3 | SC-3 | 1:3 | 619 | 73.12±0.7 |

***In vitro* Release Studies**

To understand the release patterns of Flutamide, all the formulations prepared and performed *in-vitro* release studies in a Phosphate buffer solution (pH-7.4) at $37^0$C. These experiments were carried out in triplicate and the results presented are averaged. The formulations loaded with 10, 15 and 20 w/w % Flutamide are graphically shown in Fig. 5, Fig. 6 and Fig. 7 in Table 3.

**Table: 3 Formulations of Different percentage of Flutamide drug loaded polymeric blend microspheres**

| S.No | Batch Code | SPI | Na-CMC | $CaCl_2$ mg | Drug (Flutamide) mg | Drug: Carrier ratios |
|---|---|---|---|---|---|---|
| 1 | SC-4 | 25 | 25 | 10 | 10 | 1: 1 |
| 2 | SC-5 | 50 | 25 | 10 | 15 | 2:1 |
| 3 | SC-6 | 75 | 25 | 10 | 20 | 3:1 |

**Effect of SPI content**

Release profiles of SC microspheres prepared with different amounts of SPI & Na-CMC are displayed in Fig.5. It was found that SC polymeric blend microspheres produced up to 76-94% cumulative release in 10h. The increase in drug release could be due to the fact that during dissolution, microspheres might have systematically swollen with an increasing amount of SPI (hydrophilic) due to the formation of loosely cross linked network chains of SPI. Thus, a relaxation-type response of the polymeric chains might be possible due to stresses induced by the surrounding solvent medium during the dissolution, resulting in an increase of chain dimension (radius of gyration) of the polymer; leading to increase in the molecular volume of hydrated polymer due to increased swelling of SPI component of the polymer matrix, thereby reducing the void volume of the matrix. Notice that the nature of release profiles showed almost similar trend in all the formulations containing different amounts of SPI, indicating that swelling SPI has established a liner relationship with the release profiles.

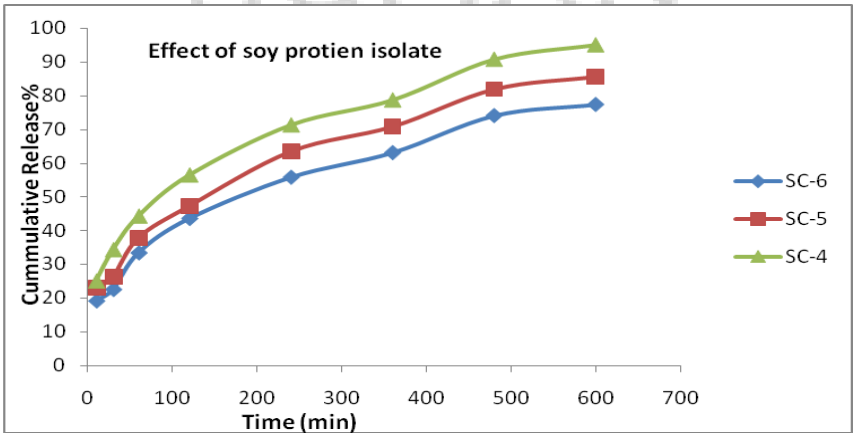


**Figure 5. % of Cumulative drug release with time for various SPI content in different SC formulations**

**Effect of Na-CMC content**

Release profiles of SC microspheres prepared with different amounts of Na-CMC are displayed in Fig.6. It was found that SC polymeric blend microspheres produced up to 60-90% cumulative release in 10h. The increase in drug release could be due to the fact that during dissolution, microspheres might have systematically swollen but with an increasing amount of Na-CMC (hydrophilic) the swelling nature is also more in these cases. Comparatively with increase of Na-CMC content the drug release increased. This may also be due to swelling nature of Na-CMC. This is evident from the Fig.6.

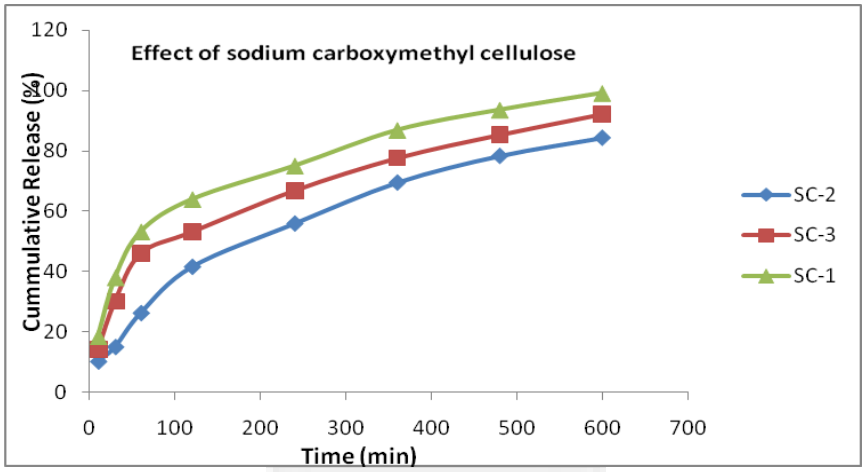


**Figure 6. % of Cumulative drug release with time for various Na-CMC content in different SC formulations**

**Effect of Flutamide drug content**

The other parameter that affects the Flutamide release is its concentration. For this purpose the cumulative drug release % is shown with reference to time by varying the drug content in SC formulation studied and presented in Fig.7. The Fig.7 illustrates that the drug release from SC polymeric blend microspheres increases with increase in drug content. The cumulative release (%) of the microspheres containing 20% drug showed 100% release within the studied period of time (10h), whereas the microspheres containing 10% drug showed 68% release in 10h. Higher initial load of the drug causes the faster movement of water penetration on the surface of the microspheres, this further facilitate the relaxation of the polymer chains.

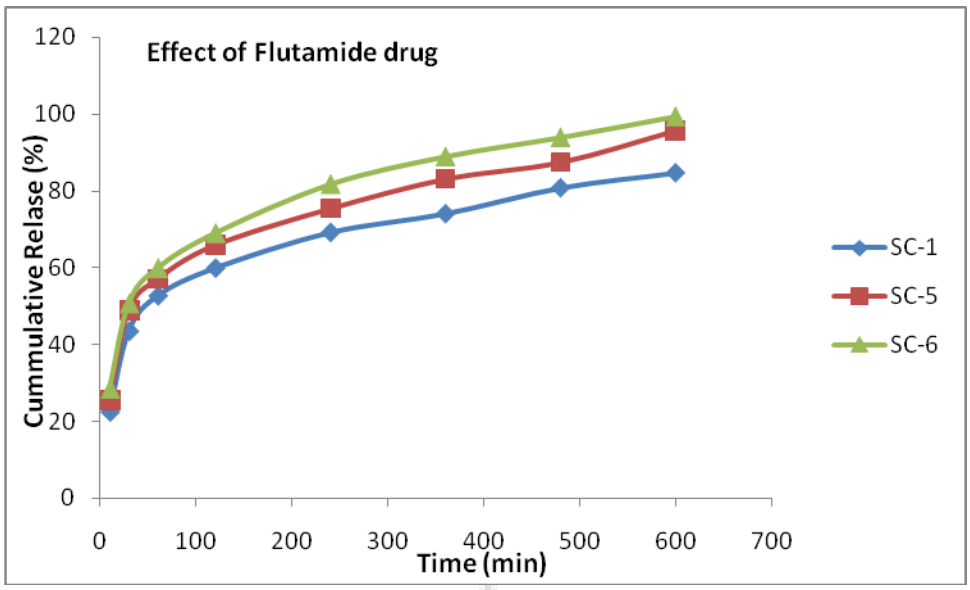


**Figure 7. % of Cumulative release of drug with time for various Flutamide drug content in different SC formulations**

**Drug Release Kinetics**

Drug release kinetics was analyzed by plotting the cumulative release data versus time by fitting to the following empirical equation [16].

$$(M_t/M_\infty) = kt^n \quad \text{--------} \ (1)$$

Here, $M_t/M_\infty$ represents the fractional drug release at time t, k is a constant characteristic of the drug, polymer system, and 'n' is an empirical parameter characterizing the release mechanism. Using the least squares procedure, we have calculated the values of 'n' and 'k' for all the formulations developed. If n=0.5, drug diffuses and release out of the polymer matrix following a Fickian diffusion or case I. For n >0.5, anomalous or non-Fickian transport occurs. If n=0.5, non-Fickian or case II release kinetics is prevalent. The calculated values of 'n' for the present study have been presented in Table.4.

**Table 4. Release kinetic parameters of different formulations**

| S.no | Formulation codes | "K" values | "n" values |
|---|---|---|---|
| 1 | SC-1 | 0.153 | 0.540 |
| 2 | SC-2 | 0.206 | 0.206 |
| 3 | SC-3 | 0.239 | 0.195 |
| 4 | SC-4 | 0.385 | 0.385 |
| 5 | SC-5 | 0.156 | 0.298 |
| 6 | SC-6 | 0.919 | 0.334 |

In the present research, the values of 'k' and 'n' show a dependence on the extent of cross-linking, % drug loading and SPI content. Values of 'n' for microspheres prepared by using the varying amounts of SPI and Na-CMC keeping Flutamide constant have ranged from 0.153 to 0.540, producing a slight deviation from the Fickian mode of transport. The Flutamide loaded microspheres have 'n' values ranging from 0.195 to 0.540 giving a shift from erosion type release to swelling-sustained non-Fickian transport. Similar findings have been observed elsewhere [17], wherein, the effect of different polymer ratios on the dissolution kinetics was investigated. The 'n' value for formulations containing different amounts of SPI, Flutamide, and Na-CMC is 0.5, which indicates the non – Fickian diffusion transport, i.e., slight deviation from the Fickian trend.

## CONCLUSION

In summary, a potential drug-delivery system based on Na-CMC and SPI is developed, and these microspheres are stabilized by the cross linking of $Ca^{2+}$. The best miscible microspheres contain the weight ratio of SPI and Na-CMC by 1:1, and present a uniform and smooth structure.

Furthermore, the strong interaction among components, such as hydrogen bonding, is the main driving force to promote the miscibility of their polymer in these blend microspheres. Different from the rapid cleavage of pure Na-CMC microspheres in the conditions of pH 7.4, flutamide release through the modified microspheres continued up to 10h. Such complex microspheres may be helpful to facilitate the prompt release of drugs in the case of treatment of prostate cancer in the human body (pH 7.4).

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