Pharmaceutical Characterisation Of Drug Entrapped Cross- Linked Carboxy Methyl Cellulose Nano-Particles

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ABSTRACT

This study was aimed at investigating the physicochemical properties and pharmaceutical applications of drug entrapped cross linked carboxy methyl cellulose nano-particles (CCMC) prepared by solvent evaporation technique after cross linking CMC with acetic anhydride. Three different batches of CCMC nano particles were made with different polymer concentrations and were labeled as SSK_{10}, SSK_{20} and SSK_{30}. Fulvestrant, a lipophilic drug was used as a candidate drug in this study. Transmission electron microscope (TEM) was used to evaluate the nano-particles morphology, while the zeta potential and the particle size were analyzed with Zetasizer nano ZS using DTS software. The drug loading, in-vitro drug release and percentage entrapped were determined spectrophotometrically. The results showed that all the nano-particles were spherical, smooth surfaced and pH resistant. There is a linear relationship among the polymer concentration, average nano-particle sizes, and drug entrapped (%) within the nano structure. SSK_{30} showed the highest drug loading capability and stability in both acidic and alkaline conditions. The novel polymer SSK_{30}, also shows a gradual and consistent release of the entrapped drug molecule devoid of dose dumping. Thus SSK_{30} offer an alternative, low cost polymeric matrix system suitable for use in the formulation of drug loaded nanoparticles

Key words: Target drug delivery system, carboxy methyl cellulose and nano-particle

INTRODUCTION

Target drug delivery system is an advanced method of delivering drugs to the patients in a manner that increases the concentration of delivered drug to the targeted body part of interest only (organs/tissues/ cells) which in turn improves efficacy of treatment by reducing side effects of drug administration. Basically, targeted drug delivery is to assist the drug molecule to reach preferred or the desired receptor site for therapeutics activity. The inherent advantages of this technique (target drug delivery system) are; reduction in the required drug therapeutic doses and reduction of drug adverse or side effect. This had now formed the backbone of nanopharmaceuticals design and formulation science, (Muller and Keck 2004; Allen and Cullis 2004).

Researchers are continuously exploring the potential of polymeric nanoparticles as target nano drug carriers for therapeutic applications. This might be as a result of their versatility and the fact that they are biodegradable. This class of carriers holds tremendous promise in the areas of cancer therapy and controlled delivery of vaccines, (Hans and Lowman, 2002). The polymeric nanoparticles (PNPs) can be prepared from various biocompatible and biodegradable natural or synthetic polymers in size between 10-1000 nm where the drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed, (Kumaresh *et al 2001;* Mohanraj V. J.and Chen, Y 2007).

Success of polymeric nanoparticles (PNP) formulation and stability however depends on the choice of suitable polymeric system having maximum encapsulation (higher encapsulation efficiency), improvement of systemic bioavailability and retention time.

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These targeting capabilities of polymeric nanoparticle are influenced by particle size, surface charge, surface modification, and hydrophobicity. Among these, the size and size distributions of nanoparticles are most important to determine their interaction with the cell membrane and their penetration across the physiological drug barriers.

Though the list of both natural and synthetic polymers investigated for used in the fabrication of polymeric nanoparticles is endless, however it is required that any polymer selected for use in drug formulation must be compatible, non-toxic, non-reactive (inert) and should be biodegradable, (Raghuvanshi *et al* 2002).This present study is focus on evaluating the physicochemical and the stability of fulvestrant loaded CMC nanoparticle.

MATERIALS AND METHODS

Sodium carboxymethyl cellulose and Fulvestrant were procured from Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, Karnataka, India. Acetic acid anhydride was obtained from S.d Fine Chem. Ltd., Mumbai, Maharastra, India. All other chemicals used were of analytical grade.

Preparation of Cross-linked carboxymethyl cellulose. (CCMC)

30 % w/v dispersion of sodium carboxy methyl cellulose was produced by addition of required quantity of the powder CMC to 100mL of de-ionized water maintained at 60 ^{o}C under constant stirring. 1M acetic acid anhydride was however introduced drop wise into the dispersion until the pH of the dispersion was 4.0. The cross-linked CMC obtained was washed severally with deionized water to remove all impurities , filtered and dried to a constant weight, (Adel, *et al* 2010).

Preparation of Fulvestrant loaded CCMC nanoparticles.

The preparation of the fulvestrant loaded CCMC nanoparticle follows solvent evaporation technique, 5mg of the drug (fulvestrant) and 10g of CCMC were completely dissolved in 50ml of chloroform and this was transfer into a 250ml round bottom flask. 1mL of tween 80 and 3mg soya lecithin were however dispersed in 15mL (50:50) mixture of acetone and water. The drug polymer mixture in the round bottom flask was place in a bath sonicator (Sonix AS300 Mubai, India) operated at 1500 rpm. After an hour of sonication, the acetone/ water mixture containing tween 80 and lecithin was added drop wise into the drug /polymer dispersion while sonication continue until the nanoparticle are formed and all the organ solvents are completely evaporated. The nanoparticle obtained were carefully removed from the round bottom flask and lyophilized for about 48 h. SSK_{20} and SSK_{30} were similarly produced but with different quantities of material as in table1.

Table 1: batch formulation of Fulvestrant loaded CCMC

Formulation code	CCMC (mg)	Drug (mg)	Lecithin (mg)	Tween 80 (mL)
SSK_{10}	10	5.00	3.00	1.00
SSK_{20}	20	5.00	3.00	1.00
SSK_{30}	30	5.00	3.00	1.00

Characterization of fulvestrant loaded CCMC nanoparticles.

The obtained Fulvestrant loaded nano particle CCMC were characterized using the following parameters;

I. Study of drug excipients interaction.

The pure drug, CMC and mixture of drug with CMC mixed separately with IR grade KBr in the ratio 1:100 and corresponding pellets were prepared by applying 5.5 metric ton of pressure in a hydraulic press. The pellets were scanned over a wave number range of 4000 to 400 cm−1 in Magna IR 750 Series II (Nicolet, Madison, WI, USA) Fourier transform infra-red (FTIR) instrument.

II. Determination of Zeta potential

Zeta potentials of formulations were measured by the instrument Zetasizer nano ZS using DTS software (Malvern Instrument Limited, UK) using M3-PALS technology. The experimental formulations were taken in lyophilized form in 2 mL eppendrof tube and the samples were suspended in phosphate buffer, pH 7.4 and then introduced in the instrument following the guideline of the manufacturer, the results were then read and the determinations were in triplicate for each formulation, mean particle size and zeta potential were equally determined from the data obtained.

III. Determination of polydispersibility (PDI)

Polydispersibility was performed with a Zetasizer nano ZS with DTS software (Malvern Instrument Limited, UK). NIBS® (noninvasive backscatter optics) technology was used for measurements of particles. The formulations were taken in lyophilized form in micro-

centrifuge tubes, suspended in a freshly prepared phosphate buffer, pH 7.4 and introduced in the instrument to read the results. PDI was calculated from the equation below;

$$PDI = \left(\frac{\sigma}{d}\right)^2$$

Where σ is the standard deviation and *d* is the mean particle sizes of the nanoparticles.

IV. Study of morphology of nanoparticles by transmission electron microscopy (TEM)

The grid for TEM analysis was prepared by placing a drop of the nanoparticle suspension on a carbon-coated copper grid and allowing the water to evaporate inside a vacuum dryer. The grid containing fulvestrant nanoparticles was scanned at 100 kV voltage and 300 000 magnification.by a Transmission Electron Microscope (FEI (Czech Republic), type FP 5018/40 TECHNAI G^2SPIRIT BioTWIN)

V. Drug loading study

A known amount of drug-loaded nanoparticles were added to a solution 50mL of methanol. After a definite period of time, the dispersed phase was separated from the continuous phase by means of centrifugation. Then the supernatant was collected and released drug was assayed spectrophotometrically.

VI. Drug entrapment efficiency

$$\text{Percentage drug entrapped} = \frac{di - du}{di}$$

Where *d*i is the initial weight of the drug loaded and *du* is the amount of drug entrapped in the supernant layer.

VII. In- vitro drug release study

SSK_{10} formulations (5 mg) were weighed and suspended in 1mL of PBS pH 7.2 and the product sonicated to re-disperse the nanoparticles. This was thereafter transferred into a dialysis membrane (0.01micro) with both end sealed. The sealed dialysis membrane was the suspended in 100mL beaker containing 50mL PBS maintained at 37 ^oC with constant shaking. Samples were withdrawn at regular time interval up to ten hours. All aliquots withdrawn were subjected to centrifugation and appropriately dilution prior to UV spectrophotomeric analysis at 520 nm (UV-1601 Shimadzu Columbia, Maryland USA).Experiments were performed in triplicates on all formulations.

RESULTS

TEM photograph (Figure 1) shows that the prepared nanoparticles for all the formulations were spherical in shape and all were in nano size range. This was further confirmed by particle size analysis, which showed that size distribution varied from 38 nm to 113 nm. There were clear distributions of both the small and large particles in all the formulations. The calculated average particle size for SSK_{10} was, 43nm, while SSK_{20} and SSK_{30} were 63 and 83 nm respectively. Table 1 depicts the various physicochemical properties of the prepared CCMC loaded nanoparticles. The polydispersibility indices also showed the similar patterns of dispersibilities i.e., formulation SSK_{10} had the least value while SSK_{30} had the maximum value. All the formulations had negative zeta potential values which varied between −3.301 to −4.300 mV. SSK_{30} had the highest value of −3.301 mV as compared to other formulations.

Table 2: Characterization of Fulvestrant loaded CCMC nanoparticles.

Formulation code	Average particle size(nm) ±SD	Polydispersibility	Zeta potential (mV)	Load capacity (%) ±SD
SSK_{10}	43±0.001	0.029	-43.00	19.10±0.001
SSK_{20}	63±0.000	0.038	-33.01	24.90±0.001
SSK_{30}	83±0.002	0.051	-48.10	35.72±0.000

The drug loading capabilities were observed to be significantly different for each of the formulation ($p<0.05$). SSK_{30} formulation showed a higher drug loading value of 35.72% as compared to 19.1 and 24.9 % for SSK_{20} and SSK_{10} formulations respectively.

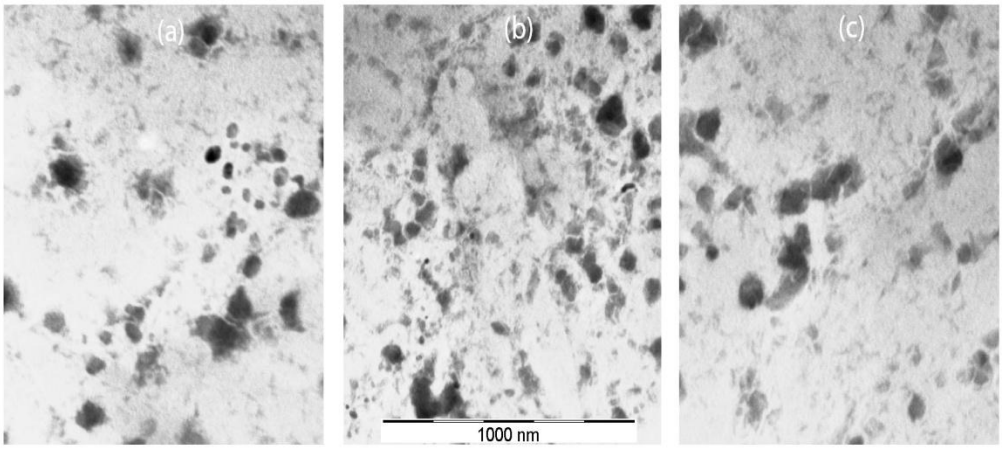

Figure I: TEM photography of SSK_{10}, (a) SSK_{20} (b) and SSK_{30} (c)

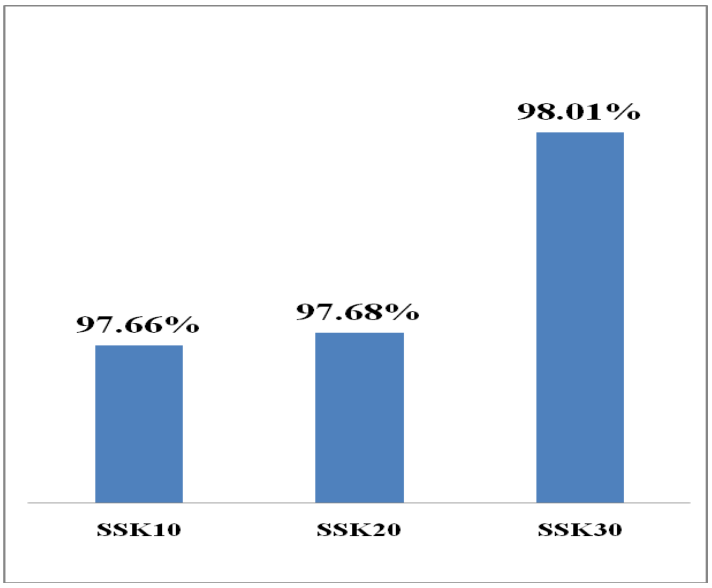

Figure II: Drug entrapment efficiency of various formulations

The drug entrapment efficiency of three formulations is as shown in figure II, and was ranked, SSK_{10} <SSK_{20}<SSK_{30} .There appeared to be no significant difference among the formulations ($P< 0.05$). Drug release study was carried out to understand the in-vitro release pattern from the three formulations. When the cumulative percentages of the drug release from the experimental formulations were plotted against time, SSK_{10} was found to achieve 100% release of the active medicament in about 6 hours, SSK_{20} in 8 hours and SSK_{30} in 12 hours. The sustained released of SSK30 was found to be gradual and extended over a period of 12hrs, thereby enabling twice daily dosing. SSK_{30} was thereafter selected for further release studies at different pH values. The effect of pH on the cumulative release of fulvestrant from SSK_{30} is as presented in figure IV. The difference observed at each time interval and pH values were insignificant ($p< 0.05$)

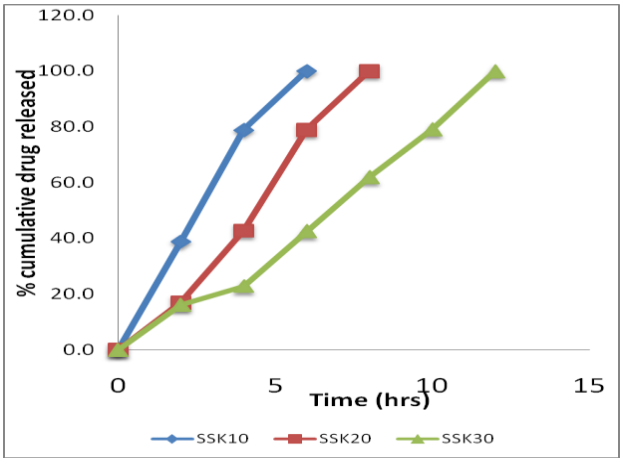

Figure III: Comparative in-vitro dissolution release of SKK_{10}, SSK_{20} and SSK_{30} @ pH 7.2

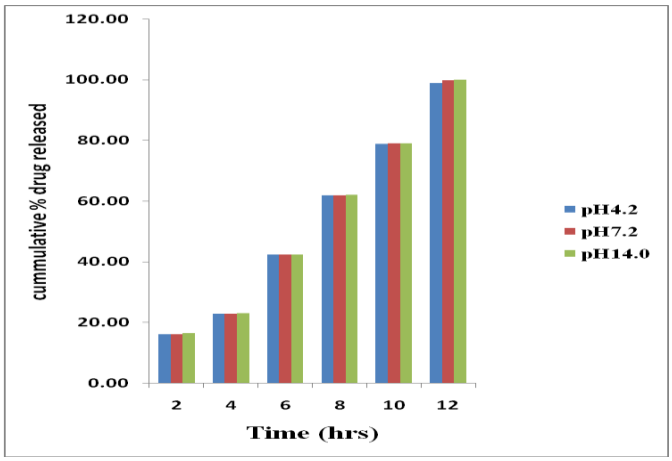

Figure IV: Comparative in-vitro dissolution release of SSK_{30} @ pH 4.2, pH 7.2 and pH14.0

DISCUSSION

In the present study, CCMC was selected to formulate fulvestrant-loaded nanoparticles, because of its biodegradable, readily available at low cost, and low physiological toxicity, (WHO, 2003).

Polydispersity varies from formulations to formulations. Reports have shown that the polydispersity variation existed from 100 nm to even 20 µm (Panyam *et al* 2003; Qaddoumi *et al* 2004; Han *et al* 2005). Variation of few hundred nm range is also very common and permissible (Song *et al* 1997).

In this study, the of zeta potential obtained are given in Table 2. All formulations had negative values and can be ranked, SSK_{20}< SSK_{10}< SSK_{30}. Particles with zeta potentials values above +30 mV and more negative than −30 mV are normally considered stable for colloidal dispersion (Yue *et al* 2008). From the data it is evident that all the formulations are stable in the colloidal state. SSK_{30} will therefore be expected to demonstrate highest drug stability. This also suggests that nano particles can be stored in a liquid suspension form, or better still they can be stored in a lyophilized state, to be reconstituted immediately before administration. Reports suggest that formulations stored in colloidal stage cause more stability problem than in the dry form (Abdelwahed *et al* 2006).

The observed increase in the average particle sizes, and Polydispersibility of the nanoparticles with increasing polymer concentration is consistent with earlier finds, and may not be unconnected to coalescence phenomena during formation of nanoparticles, (Uttandaraman and Macosko 1995; Utsav C. Rathod *et al* 2012 ;Jagtap, *et al* 2012) .

Various researchers had reported various factors affecting drug loading of nanoparticle among which are formulation compositions, methodology, process parameters etc.
In this study, the drug loading significantly increased with increasing polymer concentration, this is in agreement with the finding of earlier researchers, and this may be due to larger particle sizes at higher polymer concentration which invariably accommodating more drug molecules within it core, (Basarkar *et al* 2007 ; Mukherjee *et al* 2008).

It was equally observed that the concentration of the polymer do not have any significant control over the amount of drug molecule entrapped, which is consistent with earlier reports, (Vyas and Khar 2002). Figure II and Figure III summarize the findings of in-vitro drug release study. Particles with smaller average diameter showed rapid release of the active ingredient, translating to 100% release of drug within 8hrs for formulation SSK_{10}, 10 hrs for formulation SSK_{20} and 12hrs for SSK_{30}. Generally, cross linked polymers such as cross linked carboxymethyl cellulose possess reduced hydrophilicity, due to higher molecular weight. Enhanced hydrophobicity is also associated with decreases polymer degradation and slower release of the entrapped drug molecules; which may be due to longer diffusion pathways the drug molecule have to travel. Appropriate blend of SSK_{10} and SSK_{30} may however be necessary in the final formulation, since SSK_{10} will rapidly release the entrapped drug molecule to initial therapeutic action and SSK_{30} will ensure longer and constant sustained availability of drug molecule at the receptor site, this is crucial to hormonal responsive breast chemotherapy. Also insignificant effect of pH on the in-vitro release of fulvestrant in SSK_{30} formulation is a good indication of oral stability of fulvestrant loaded CCMC nanoparticle.

CONCLUSION

CCMC polymer can be used to prepare nanoparticles containing Fulvestrant and other similar lipophilic drug molecule. Narrow-ranged densely-dispersed nanoparticles having a good drug- loading capacity and slow and sustained drug release characteristics could be developed using this simple technology. From the data provided, it is evident that nanoparticles prepared with 30% concentration of CCMC (SSK_{30}) gave an optimal performance in term of drug loading, stability, and in-vitro drug release. SSK_{30} formulation can be selected for in-vivo and other investigations to demonstrate its stability in biological system.

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