STUDY ON TARGETED DELIVERY OF 5-FLUOROURACIL FROM SUPER PARAMAGNETIC STARCH NANOPARTICLES

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ABSTRACT

This paper deals with the effects of 5-Fluorouracil drug on iron oxide impregnated starch nanoprticles and native starch used in targeted therapy. Such systems have shown potential in releasing chemotherapy drugs controllably. In the present investigation starch nanoparticles were synthesized by emulsion cross linking method and characterized by various techniques such as Fourier transform–infrared spectrometry, Transmission electron microscopy, prepared native starch nanoparticles and Superparamagnetic starch nanopartcles with the average diameter of 6nm to 80nm through W/O emulsification-cross linking method. The release behavior of superparamagnetic starch nanoparticles was studied as a function of various factors such as chemical composition of nanocareer, pH, temperature, biological fluids, applied magnetic field. The results revealed that the iron oxide loaded starch nanoparticles and native starch nanopartcles prove to be an excellent option for controlled and targeted delivery of 5 Floro Uracil by application of an external magnetic field. Therefore, there is a strong incentive to develop a new strategy for the synthesis of starch nanoparticles and research their properties.

Keywords: *5-FU (5-Fluorouracil), Release, Magnetic Nanoparticles, Drug delivery.*

1. INTRODUCTION

A novel approach in this direction has been made by using magnetic nanoparticles as drug carriers and applying magnetic field to achieve drug release at desired rate and targeted sites. This magnetic noparticles based targeted drug delivery is highly efficient, quick-impact technique and may effectively reduce the toxicity and other adverse side effects in the nontarget regions by concentrating drugs at the target sites only[1,2].

The magnetic nanoparticles offer the possibility of being directed towards a specific target in the human body and remaining eventually localised, by means of an applied magnetic field. Obviously, when magnetic nanoparticles are going to be used for in vivo applications, very low values of applied magnetic field are desirable[3].

Polymeric nanoparticles present a higher stability, when in contact with biological fluids and their polymeric nature allows obtaining the desired controlled and sustained release. The advantage that nanoparticles hold over other drug delivery systems is their submicron size which makes extra vasations possible and occlusion of terminal blood vessel unlikely [4].

Starch is unique among carbohydrates because it occurs naturally as discrete particles, called granules. The granules are the primary means of energy storage in green plant over long periods of time, and the shape and size of the granules depend on their origin [5]. Starch being an abundant and inexpensive polysaccharide, is used as a biodegradable polymer. Moreover, the use of starch microspheres has been suggested for immobilization, parenteral and nasal administration[6].

Delivery systems may also provide a range of other advantages, including protection from drug hydrolysis and other types of chemical and enzymatic degradation, reduction of toxicity, controlled drug release rate, and improvement of drug bioavailability [7,8]In the present study, we have synthesized and characterized 5FU containing magnetic starch nanoparticles as potential drug carriers for mediated targeted drug delivery[9]. The objectives of proposed work have been schematically in figure 1.

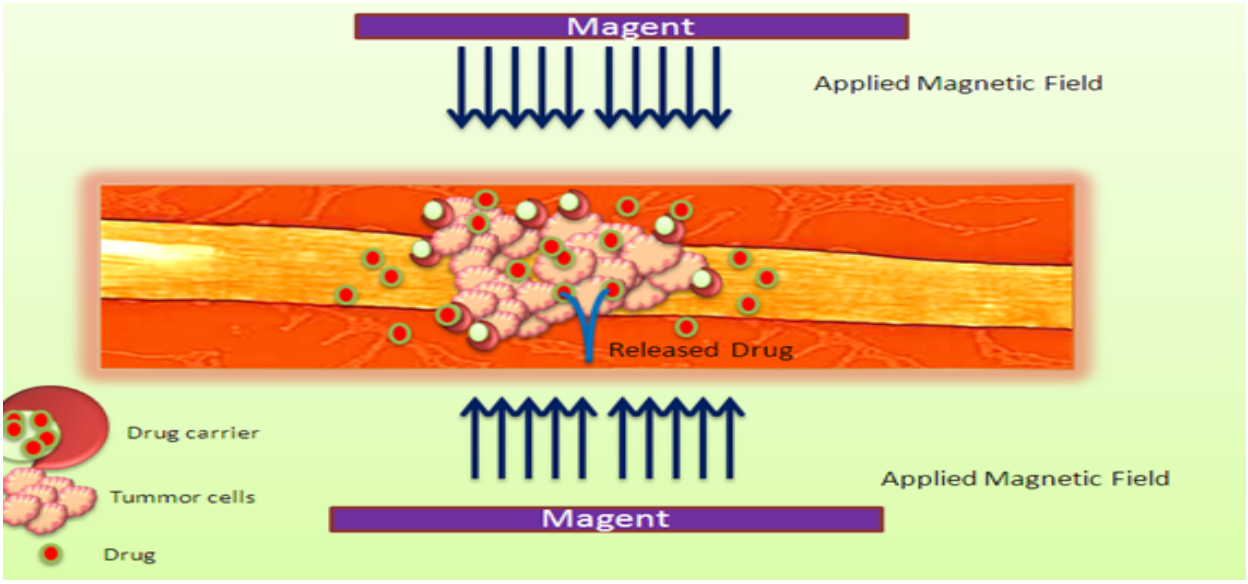


Fig.1. Schematic presentation of possible targeting of cells by released drug due to applied magnetic field.

2. PREPARATION OF MAGNETIC STARCH NANOPARTICLES

The iron oxide impregnated starch nanoparticles were prepared following an emulsion crosslinking method. The most common method for production of magnetic nanoparticles involves co-precipitation of ferrous and ferric salts in an alkaline medium with continuous stirring.

$$2FeCl_3 + FeCl_2 + 8NH_4OH \rightarrow Fe_3O_4 + 8NH_4Cl + 4H_2O$$

In a typical experiment, the 'aqueous phase' was prepared by dissolving a definite amount of starch To this solution, 2g of iron salts and definite volume of silicon oil were added to form the oil phase. The resulting solutions were mixed with vigorous shaking for 1 h to from a stable suspension. Now to this suspension were added NH_4OH and 102.28mM epichlorohydrin emulsions prepared in silicon oil with constant shaking. The crosslinking reaction was allowed to proceed for 5h and the nanoparticles so obtained were successively washed with toluene and acetone.

3. CHARACTERIZATIONS

3.1 FTIR spectral analysis

3.2 FTIR spectral analysis was carried out for structural characterization of nanoparticles. The FTIR spectra of crosslinked nanoparticles were recorded on a FTIR spectrophotometer in the range of 400- 4000cm^{-1} (Perkin-Elmer, 1000 Paragon).

3.2 TEM (Transmission Electron Microscopy) analysis

The TEM of the prepared starch particles were recorded to determine internal particle distribution and structural morphology of nanoparticles. Transmission electron microscopy (TEM) was performed by using a Morgagni-268-D transmission electron microscope with an acceleration voltage of 80.0kv.

4.LOADING OF DRUG ON TO NANOPARTICLES

Drug loading may be done by absorbing the drug after formation of nanoparticles by incubating the carrier with a concentrated drug solution (adsorption/absorption technique). For loading of nanoparticles known volume of drug 5-FU was taken and diluted with appropriate amount of PBS solution and shaken vigorously for mixing of drug and PBS solution. In brief, drug loaded nanoparticles were prepared by allowing 0.1 g of nanoparticles to swell in freshly prepared drug solution (10mL) until equilibrium was reached. The % loading of drug onto nanoparticles was calculated by the following equation-

$$\% \text{ Loading} = \frac{W_d - W_0}{W_0} \times 100 \qquad \text{---------- (1)}$$

where, W_d and W_0 are the weights of loaded and unloaded nanoparticles respectively.

5 Kinetic Study

5.1 Release Kinetics

The drug loaded nanoparticles suspension was shaken for 3.5 h to study kinetics of the release process. The kinetic data were analyzed with the help of the following equation, which could be helpful in determining the nature of the release process,

$$\frac{W_t}{W_\infty} = Kt^n \qquad \text{---------- (2)}$$

where W_t and W_∞ are the amount of the drug release at time t and at infinity time (equilibrium amount of drug released), respectively, and K is rate constant. The exponent n, called diffusional exponent is an important indicator of the mechanism of drug transport and, in general, has a value between 0.43 and

0.85. The numerical value of n indicates the nature of the release process, i.e. when n = 0.43, release is Fickian in nature, when n=0.85, release is of case II type and when n lies between 0 and 0.85, the release process becomes anomalous in nature. For evaluating the diffusion constant of loaded drugs, the following equation can be used:

$$\frac{W_t}{W_\infty} = 4\left(\frac{Dt}{\pi L^2}\right)^{0.5} \qquad \text{---------- (3)}$$

where, D is the diffusion constant of the drug and L being the diameter of the dry nanoparticles.

6. RESULT AND DISCUSSION

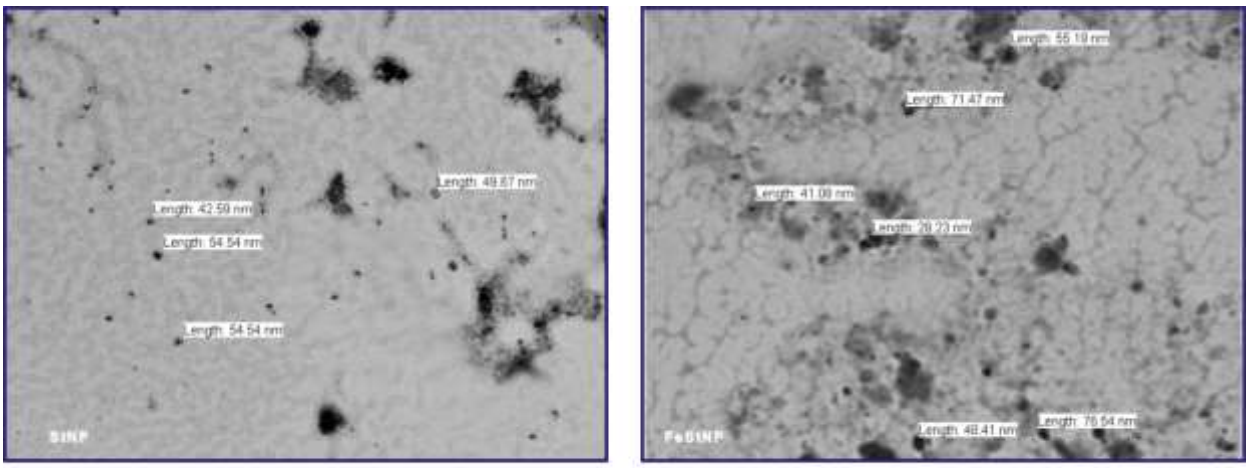
6.1FT-IR spectral analysis

The FTIR spectra shown in Fig. 2 (a) clearly mark the evidence for the presence of native starch observed by the peaks at 3581cm^{-1} due to the O-H stretching vibration. Additional multiple bands appear at a lower frequency in the range 3500-3000 cm^{-1} at the expense of the starch hydroxyl groups, C-H stretching at 2920 cm^{-1}, O-H bending at 1259cm^{-1}, C-O stretching at 1026cm^{-1} and a prominent C-O-C stretching of the glycosides bond at 1105 cm^{-1} (typical for starch). The spectra shown in Fig 2 (b) shows iron oxide impregnated starch nanoparticles the presence of iron oxide also as evident from the peaks observed at 526- 418 cm^{-1}.Another spectra shows the presence of 5 FU in the drug loaded nanoparticles as evident from the two sharp peaks observed at 677 cm^{-1} and 538 cm^{-1} (due to torsional oscillation of –NH_3^+ group).

Fig.2. FTIR spectra of - (a) starch nanoparticles (b) iron oxide impregnated starch

6.2 TEM analysis

The transmission electron micrograph (TEM) of native starch nanoparticles and IOISNPs is shown in Fig 3(A) and (B), respectively. It is clear from the Fig that the size of native starch and IOISNPs varies from 18 nm to 80 nm and 10 to 100 nm, respectively and the morphology of nanoparticles was non uniform and heterogeneous in appearance[10].



(A) (B)

Fig.3 TEM image of-(a) starch nanoparticles, and (b) IOISNPs.

6.3 Effect of % loading on methotrexate release

The loading of drug involves swelling of preweighed nanoparticles into the drug solution in the concentration range from 10% - 80%. The release profiles clearly indicate that initially up to 20% drug loading cumulative release increases, while beyond this there is a fall in the release rate. The drug (5FU) loaded IOISNPs may be visualized as a three dimensional network of starch macromolecules which accommodate drug molecules into the space available between the network chains. When such drug loaded nanoparticles are allowed to swell in a suitable release medium the solvent (normally water) molecules enter into the nanoparticles network as a result of their diffusion into the nanoparticles matrix and subsequent relaxation of polymer chains take place [11].

6.4 Effect of starch on 5FU release

The effect of starch on the amount of release drug has been investigated by varying its amount in the range of 3.0 to 6.0 g in the feed mixture. The release study was carried out in both conditions (with and without MF). The release and swelling results are displayed in Fig.4 (a) which clearly indicates that the cumulative release of 5FU increases with increasing starch content in the range of 3.0-5.0 g and, thereafter, a decrease in release was noticed.The results may be attributed to the fact that starch is a hydrophilic biopolymer, and its increasing amount in the particles will obviously increase the hydrophilicity of the nanoparticles and thus an increase in drug release is expected[12]. However, beyond 5.0 g of starch content, the observed decrease in drug release may be due to an enhanced compactness of the particles and greater interaction between macromolecular chains of starch nanoparticles.

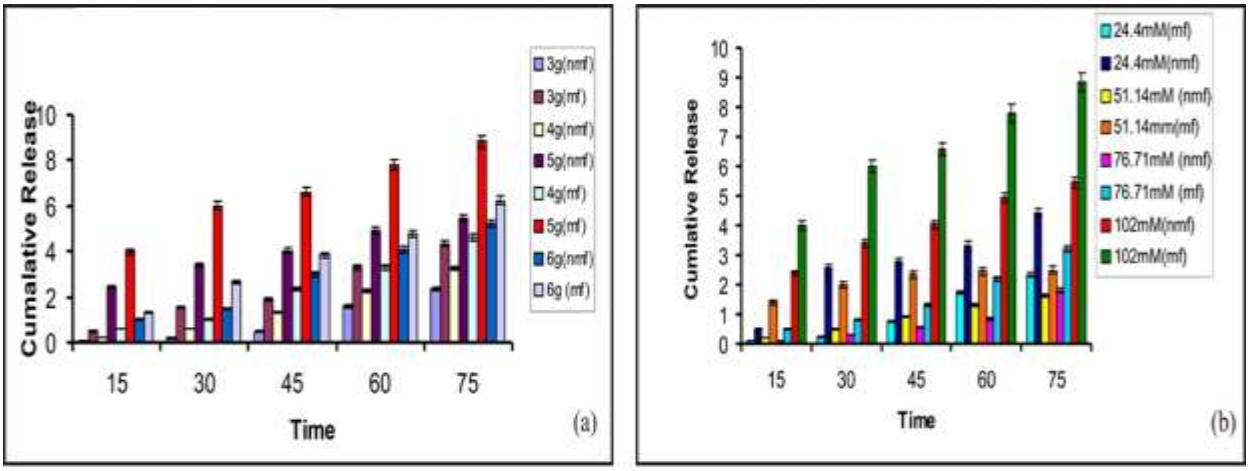


Fig.4.(A) Effect of starch content of the nanoparticles on the release profile of 5FU (B) Effect of epichlorohydrin on the release profile of 5FU.

6.5 Effect of crosslinker on release of 5FU

In the present study epichlorohydrin has been used to crosslink starch in the concentration range of 24.4mM to 102.28mM in the feed mixture. The results are depicted in Fig. 4(b) which shows an increase in drug release with increase in concentration of crosslinker.The observed increase in released drug is due to the fact that epichlorohydrin is a low molecular weight crosslinking agent of starch, which at its two terminals crosslink with the hydroxyl groups of starch[13]. Thus, a crosslinked starch network could be imagined as ultrahigh molecular weight starch molecules that contains wide pore sizes in its structure and, therefore, possesses an abnormal capacity of accommodating into the network. Thus, capacity to imbibe increasing number of drug molecules results in an increased release.

6.6 Effect Of pH

At low pH of 1.8, the FeOH groups of the iron oxide nanoparticles present on the surfaces and bulk of starch nanoparticles matrix get protonated thus producing $FeOH_2^+$ ions which due to mutual repulsion tends to relax the starch chains and result in higher swelling ratio [14, 15]. As the pH of the medium increases, the existing repulsion forces goes on decreasing which consequently produces a decrease in release of the drug. This fall in water sorption capacity continues till the zero point charge of iron oxide which is reported to lie in the range 7-8. At the point of zero charge, an equal number of $FeOH_2^+$ and $FeOH^-$ ionic groups exists which causes a minimum repulsion between the macromolecular chains of starch and result in a minimum swelling. A slight increase in swelling, however, is observed upon further increasing pH of the medium which may be attributed to the reason that due to the repulsion between the anionic groups FeO^- swelling ratio slightly increases.

7. IN VITRO RELEASE KINETICS

The drug molecules get dissolved into water and release out through water permeation channels present in the macromolecular network. The diffusion of drug molecules and relaxation of nanoparticles chains determine the type of release mechanism being followed by the drug molecules. It has been laid down by Higuchi equation [16] that if n = 0.43, the release is diffusion controlled (Fickian), when n = 0.84 the release is non-Fickian (or case II), and for n lying in the range 0.43 to 0.84, the mechanism becomes anomalous. In some cases n has been found to exceed 0.84 and the mechanism is known as super case II [17]. The values of diffusion coefficient (D) and release exponent (n) calculated as

described above are summarized in Table.1 The data clearly reveal that the value of n is near to 0.43 in almost all cases, and the release of drug may, therefore, be considered as Fickian and diffusion-controlled.

Table.1 Data showing the release exponents and diffusion coefficients obtained under varying experimental conditions.

Sample No	Starch (g)	Epichlorohydri (mM)	pH	Diffusion Coefficient $D\times10^{-3}$ (Cm^2/S)	n*	Mechanism	R^2
1	3.0	102.28	7.4	3.42 (.1026)	0.10(±0.003)	Fickian	0.95
2	4.0	102.28	7.4	3.06 (.091)	0.49(±0.014)	Fickian	0.92
3	5.0	102.28	7.4	3.30(.099)	0.21(±0.006)	Fickian	0.91
4	6.0	102.28	7.4	2.87 (.085)	0.40(±0.012)	Fickian	0.94
5	5.0	24.4	7.4	9.51 (.285)	0.36(±0.010)	Fickian	0.94
6	5.0	51.14	7.4	3.11(.093)	0.11(±0.003)	Fickian	0.96
7	5.0	76.71	7.4	4.55 (.136)	0.70(±0.021)	Nonfickian	0.79
8	5.0	127.85	7.4	1.5 (.045)	0.46(±0.013)	Fickian	0.92
9	5.0	102.28	1.8	3.61(.1083)	0.35(±0.010)	Fickian	0.91
10	5.0	102.28	8.6	3.13(.0939)	0.10(±0.003)	Fickian	0.93

8. CONCLUSIONS

In the present study, we have demonstrated a novel method for synthesizing homogeneously dispersed iron oxide impregnated superparamagnetic starch nanoparticles. The magnetite nanoparticles were synthesized by using the in-situ precipitation of magnetic iron oxide nanoparticles by a wet chemical method. The nanoparticles were characterized by various analytical techniques, such as FTIR spectroscopy and TEM analysis which confirm the in-situ impregnation of nano sized iron oxide within the matrix of starch nanoparticles. The polymeric nanoparticles clearly show the presence of characteristic groups of starch and iron oxide as confirmed by their FTIR spectra. The TEM of the nanoparticles provide information about their semi crystalline nature and nanosize of the particles, respectively.

With an increase in percentage loading of drug on to the nanoparticles, the swelling and release of 5FU constantly increases.The release of drug considered as Fickian and diffusion-controlled.The amount of 5FU released also increases with increasing intensity of magnetic field and iron oxide content. The pH of the release medium significantly affects the release profile and it is noticed that at in both acidic (pH 1.8) and alkaline (pH 8.6) medium the cumulative release of drug constantly decreasses in the whole studied range.

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