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Formulation and *in-vitro* evaluation of novel starch-based tableted microspheres for controlled release of ampicillin ☆

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Abstract

This study reports the preparation of starch-based tableted microspheres that are crosslinked with epichlorohydrin (EPI) using a modified water-in-oil (w/o) emulsification technique. Ampicillin (AMP), a broad spectrum antibiotic was encapsulated up to the extent of 70% into the microspheres. The microspheres were characterized by Fourier transform infrared spectroscopy (FT-IR) to confirm the crosslinking reaction and chemical stability of AMP. Differential scanning calorimetry (DSC) was studied on the placebo and drug-loaded microspheres to confirm the polymorphism of AMP. Results of this study indicated a molecular level dispersion of AMP in the developed microspheres. Scanning electron microscopy (SEM) confirmed the spherical nature and smooth surfaces of the microspheres produced. Mean particle size of the microspheres as measured by laser light scattering ranged between 96 and 158 μm. Diffusion coefficients (D) of water transport through the microspheres were determined using an empirical equation. Values of D decrease with increasing crosslinking as well as increasing content of starch in the microspheres. *In-vitro* release studies were performed in 1.2 and 7.4 pH media to simulate the gastric and intestinal conditions. The results indicated a dependence on the amount of polymer and extent of crosslinking. Release data were fitted to an empirical relation to estimate transport parameters and to understand the transport mechanism. Statistical analyses of release data was performed using analysis of variance (ANOVA) method. Suitable microspheres were selected and compressed into tablets using the directly compressible excipients. SEM photographs of the fractured part of the tablet revealed the presence of discrete microspheres in the tablets, suggesting that the system chosen is ideal for tableting. Tablets significantly lowered the initial burst effect when compared to microsphere formulations. The tablets were effective in releasing the AMP over an extended period of about 24 h.
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Keywords: Ampicillin; Starch; Microspheres; Tablets; Controlled release

1. Introduction

Biodegradable microspheres used as controlled release (CR) systems are important in pharmaceutics. The basic idea to try to accomplish in drug delivery applications is that biodegradable microspheres degrade within the body as a result of natural biological processes, thereby eliminating the need to remove the delivery system after its function is over. Ability of polysaccharides to form a network structure (gel), even at low concentrations has been well known. This property to form a three-dimensional-network structure (gelation) offers an effective means of increasing the chemical stability and mechanical properties of the polymer (Shefer, Shefer, Kost, & Langer, 1992). Starch-based systems are quite promising in several biomedical applications (Espigares et al., 2002; Silva et al., 2004), including drug delivery carriers (Elvira, Mano, San Roman, & Reis, 2002; Malafaya, Elvira, Gallardo, San Roman, & Reis, 2001). Biodegradable starch-based microspheres have been widely investigated in

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the literature for the release of a variety of drugs (Bjoèrk & Edman, 1990).

Starch can be possibly modified chemically through its functional groups (Vansoest & Vliegenthart, 1997). Self association (induced by changes in pH, ionic strength or physical and thermal means), complexation with salts and covalent crosslinking are some of the widely adopted strategies to modify starch. Starch is known to produce low toxicity products that are biodegradable and quite stable in the biological environment (Illum, Farraj, Critchley, & Davis, 1998). Due to the cost-effective attraction of starch-based products, these can be important materials for use in drug delivery applications. Starch microspheres have been investigated before in different drug delivery applications (Leong et al., 2002). However, in oral administration, the native starch is almost completely broken down after its oral ingestion (Vilivalam, Illum, & Iqbal, 2000) by the pancreatic enzymes that lead to subsequent absorption from the small intestine into the systemic circulation. A certain proportion of starch, called resistant starch, escapes the digestion in small intestine and undergoes fermentation by bacteria in the colon (Siew, Basit, & Newton, 2000). Larionova, Ponchel, Duchene, and Larionova (1999) developed the crosslinked starch-protein microcapsules containing proteinase inhibitor in order to allow for oral administration of proteic or peptide drugs. Over the past few decades, several types of peroral CR formulations have been developed to decrease the dosing frequency and enhance the patient compliance, thereby reducing the fluctuation in circular drug release to facilitate a more uniform effect and improve the clinical efficacy of the drug. Such formulations are designed to deliver drugs at controlled and pre-determined rate, thus maintaining their therapeutically effective concentrations in the systemic circulation for a prolonged period of time (Robinson, Li, & Lee, 1987).

Oral CR multiple unit dosage forms such as microparticles, beads and pellets are gaining considerable importance in recent years in view of their added advantages over the conventional single unit formulations. Once the tablet or capsule containing multiple units disintegrates, particles are spread uniformly throughout the gastrointestinal tract (GIT). This will avoid the release of drug at a particular site, thus avoiding the risk of toxicity caused by locally restrained tablet within the GIT. Uniform distribution of multiple units in GIT thus resulting a more reproducible absorption and will reduces the risk of local irritations as compared to single unit systems. These systems distribute more uniformly in the GIT, thus resulting in more uniform drug absorption and reducing the patient-to-patient variability (Celik, 1994; Soppimath, Kulkarni, & Aminabhavi, 2001).

Tableting of microparticles has been reported to reduce the release of drugs, which results in a CR formulation (Soppimath et al., 2001; Vilivalam & Adeyeye, 1994). After tableting of microparticles, the particles may remain intact within the tablet without undergoing merging or rupturing and hence, drug release will take place from the individual microparticles; if not, the microparticles may merge or rupture to become bigger compacts. In such cases, the release will occur from compacts in the tablet formulation. Ideally, the drug release should occur from individual particles, which should not be affected from the compression process. However, the excipients used in tableting should provide a sufficient cushioning effect to withstand the compression force and thereby, prevent the merging or rupturing of microparticles. In recent years, a number of studies have been made on the compression of microparticles, such as microspheres, microcapsules and microsponges into tablets; effects of excipients and compression on drug release rates from such formulations have been investigated (Soppimath et al., 2001; Vilivalam & Adeyeye, 1994; Sveinsson, Kristmundsdottir, & Ingvarsdottir, 1993). In continuation of this study, we describe here the development and characterization of starch-based microspheres using a novel emulsion crosslinking technique to encapsulate ampicillin (AMP). AMP i.e., (2*S*,5*R*,6*R*)-6-[[(2*R*)-Aminophenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid, a broad spectrum antibiotic, was chosen as a model drug for the study. It is used in systemic therapy as well as locally for gastric or intestinal infections, due to its acid resistance property, it is generally given orally. It has a short biological half-life of 45–90 min. In order to make the application of AMP more effective, research has been directed to design CR formulations (Anal & Stevens, 2005). The drug-loaded formulations have been characterized by Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), dynamic laser particle size analyzer for understanding their interactions, morphology and size. Drug release characteristics have been investigated in depth to study the variations of drug/polymer concentration and the amount of crosslinking agent. The ideal formulations were selected and compressed into tablets using different direct tableting excipients such as microcrystalline cellulose, poly(vinyl pyrrolidone), magnesium stearate and tablets were analyzed for thickness, weight variation, hardness and disintegration test.

2. Materials and methods

2.1. Materials

Ampicillin trihydrate was received as a gift sample from Medreich Sterilab Ltd. (Bangalore, India). Starch (soluble), light liquid paraffin oil, analytical reagent grade epichlorohydrin (EPI), Span 80® and sodium hydroxide (NaOH) were procured from s.d. fine chemicals (Mumbai, India). Directly compressible microcrystalline cellulose (RanQ 102, RanQ Pharmaceuticals and Excipients Pvt. Ltd., Mumbai, India), poly(vinyl pyrrolidone) K-30 and magnesium stearate were all received as gift samples from Himalaya Drug Co. (Bangalore, India). Double-distilled water was used throughout the work. All the chemicals were used without further purification.

2.2. Methods

2.2.1. Preparation of microspheres

Crosslinked starch microspheres have been prepared by modified emulsion crosslinking method (Hamdi, Ponchel, & Ducheãne, 1998; Tabata & Ikada, 1989). Briefly, starch was dissolved in 20 mL alkaline solution (2 N NaOH) by continuously stirring until a homogeneous solution was obtained. Then, accurately weighed amount of AMP was dissolved in the polymer solution. This solution was added slowly to a jacketed flask containing 100 mL mixture of petroleum ether and light liquid paraffin (40:60, w/w) and 1% (w/w) Span®-80 under constant stirring at 400 rpm speed using Eurostar (IKA Labortechnik, Germany) high speed stirrer for about 10 min. To this w/o emulsion, required amounts of EPI were added under mechanical stirring and the entire assembly was maintained at 40 °C for 6 h by circulating water from the water bath (Grant, Model GR 150, GP 200, UK).

Microspheres with different extents of crosslinking were prepared by taking 4.0 and 8.0 mL of EPI. The microspheres formed were filtered and washed repeatedly with *n*-hexane to remove any excess surfactant, crosslinking agent and further washed repeatedly with distilled water. These microspheres were dried under vacuum at 40 °C for 12 h and stored in a desiccator before the analytical testing. Placebo microspheres were prepared in a similar manner to be used as control in characterization studies. In all, eight formulations were prepared by varying the drug:polymer ratio and extent of crosslinking. The assigned formulation codes are given in Table 1.

2.2.2. Particle size measurements

Particle size was measured by laser light scattering technique (Mastersizer, 2000, Malvern, UK). The sizes of the completely dried microspheres of different formulations were measured by dry sample technique using a dry sample adapter. The completely dried particles were placed on the sample tray with an inbuilt vacuum and compressed air system was used to suspend the particles. The laser obscuration range was maintained between 1% and 2%. The volume-mean diameter (V_d) was recorded. After measurement of particle size of each sample, the dry sample adopter was cleaned thoroughly to avoid cross contamination. Each batch was analyzed in triplicate, but the average values were considered in data analysis.

2.2.3. Drug content and entrapment efficiency

Estimation of AMP content was done according to the method adopted earlier (Agnihotri & Aminabhavi, 2004). Microspheres of known weights were soaked in 50 mL of pH 7.4 phosphate buffer for 30 min and sonicated using a probe sonicator (UP 400s, Dr. Hielscher, GmbH, Germany) for 10 min to break the microspheres and facilitate extraction of drug. The solution was centrifuged using a tabletop centrifuge (Jouan, MR 23i, France) to remove the polymeric debris. The clear supernatent solution was analyzed for AMP content by UV spectrophotometer (Secomam, Anthelie, France) at the λ_{max} value of 203 nm. The % drug loading and % encapsulation efficiency were calculated, respectively, using Eqs. (1) and (2):

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

$$\%\ \text{Entrapment efficiency} = \left(\frac{\%\ \text{Drug loading}}{\%\ \text{Theoretical loading}}\right) \times 100 \quad (2)$$

2.2.4. Fourier transform infrared spectral (FT-IR) studies

FT-IR spectra were taken on Nicolet (Model Thermo 5700, Milwaukee, WI, USA) to confirm crosslinking and to investigate chemical interactions between drug and polymer matrix. Samples were crushed with KBr to get pellets by applying a pressure of 300 kg/cm^2. FT-IR spectra of starch, placebo microspheres, pristine AMP and AMP-loaded microspheres were scanned in the range between 500 and 4000 cm^{-1}.

2.2.5. Differential scanning calorimetric (DSC) analysis

DSC (Rheometric Scientific, Surrey, UK) was performed on plain starch, placebo microspheres, pristine AMP and drug-loaded microspheres. Samples were heated from ambient to 400 °C at the heating rate of 10 °C/min in a nitrogen atmosphere (flow rate, 10 mL/min).

2.2.6. Scanning electron microscopic (SEM) studies

SEM (Jeol, JSM-840A, Japan) photographs were obtained to examine the shape and surface morphology of starch microspheres as well as the cross-section of the tableted microspheres. Microspheres were dusted onto

Table 1
Results of % encapsulation efficiency and mean size of starch microspheres

Formulation code	AMP (g)	STH (g)	Crosslinking agent (EPI in mL)	% Encapsulation efficiency	Mean particle size (μm)	Water uptake (%, w/w)
F1	3.8	3.8	4.0	70.16 ± 0.93	158 ± 1.52	586
F2	1.9	3.8	4.0	65.24 ± 0.60	140 ± 2.08	529
F3	1.26	3.8	4.0	62.18 ± 0.98	131 ± 1.51	492
F4	0.95	3.8	4.0	60.47 ± 0.68	119 ± 1.52	421
F5	3.8	3.8	8.0	63.48 ± 0.66	134 ± 2.51	522
F6	1.9	3.8	8.0	60.98 ± 0.50	115 ± 1.52	479
F7	1.26	3.8	8.0	57.26 ± 0.27	108 ± 2.08	451
F8	0.95	3.8	8.0	54.17 ± 0.52	96 ± 1.73	380

double-sided tape on an copper stub, which were coated with gold by a sputter coater (Jeol coater). Tablets were broken into half pieces and the fractured parts of the tablet were sputtered with gold to make them conducting and placed on a copper stub. These samples were imaged using a 25 kV electron beam.

2.2.7. Tableting of microparticles

Ampicillin-loaded microspheres were tableted using directly compressible microcrystalline cellulose as diluent. Poly(vinyl pyrrolidone) K-30 was used as a dry binder, while magnesium stearate was used as a lubricant. Each tablet contained 200 mg AMP-loaded microspheres, 184 mg microcrystalline cellulose, 12 mg poly(vinyl) pyrrolidone and 4 mg magnesium stearate. Tablets were compressed using an IR hydraulic pellet maker (Riken Seiki Co. Ltd., Japan) applying a pressure of 300 kgf cm^{-2} for 15 s of dwell time uniaxially. Exactly weighed quantity of the powder mixture was filled into a die of 12.8 mm diameter using a little pressure and then, hydraulic pressure was applied to form the tablet. Tablets containing AMP-loaded microspheres crosslinked with 4 and 8 mL of EPI (formulations F1, F2, F5 and F6) were assigned the formulation codes T1, T2, T5 and T6, respectively.

2.2.8. Physical characterization of tablets

The tablets were evaluated with respect to different physical parameters such as weight variation, thickness and hardness. In all, 10 tablets from each formulation were determined using an electronic balance (Mettler, Model AE 240, Griefensee, Switzerland) to verify the uniformity and conformity of the tablets within each formulation. The mean weight was expressed in mg. For each formulation, the hardness of tablets was examined using a Monsanto type hardness tester to measure the crushing strength of the tablets. The mean hardness was calculated and expressed as kg. Thickness of the tablets was measured using a vernier caliperse. Ten tablets of each formulation were used and the mean thickness was expressed in mm. Disintegration test was performed in distilled water maintained at 37 °C using a USP disintegration tester (Electrolab, Mumbai, India).

2.2.9. Drug content in tablets

Each tablet was crushed into powder in an agate mortar and the powder was soaked in 50 mL of PBS of pH 7.4 for 30 min and sonicated for 5 min. The whole solution was filtered to remove the undissolved debris. The clear supernatent solution was made up to volume, suitably diluted and estimated for AMP content at λ_{max} value of 203 nm using UV–visible spectrophotometer (Model Anthelie, Secomam, France).

2.2.10. In-vitro drug release of microspheres and tableted microspheres

Dissolution experiments were performed using a fully automated dissolution tester coupled with the UV system (Logan Instruments corp., Model D800, NJ, USA) equipped with six baskets at a stirring speed of 100 rpm maintained at constant temperature of 37 °C. Drug release studies for starch microspheres and tableted microspheres were performed in 500 mL of 0.1 N HCl initially for 2 h, followed by 500 mL of pH 7.4 phosphate buffer. The instrument automatically measures the concentration of drug released at particular time intervals through an online UV spectrophotometer coupled with flow-through cells attached to the instrument, which puts the solution back into the dissolution bowl. AMP concentration was determined spectrophotometrically at the λ_{max} value of 203 nm. These studies were performed in triplicate for each sample and average values were considered for data analysis.

2.2.11. Swelling studies

Equilibrium swelling of the AMP-loaded crosslinked microspheres were studied in a simulated gastric environment using 0.1 N HCl. Microspheres were allowed to swell completely for about 24 h to attain equilibrium in an incubator (Stuart Orbital Incubator, Model S150, Staffordshire, UK) maintained at 37 °C. Adhered liquid droplets on the surface of particles were removed by blotting with soft tissue papers and the swollen microspheres were weighed on an electronic balance (Mettler, Model AE 240, Griefensee, Switzerland) to an accuracy of ±0.01 mg. Microspheres were dried in an oven at 50 °C for 5 h until there was no change in dry weight of the samples. From the equilibrium wt%, weight of swollen sample (W_1) and water uptake capacity, Q was calculated by measuring dry weight, W_2 using the equation:

$$Q = \left(\frac{\text{Weight of swollen particles } (W_1) - \text{Weight of dry particles } (W_2)}{\text{Weight of dry particles } (W_2)} \right) \times 100 \quad (3)$$

2.2.12. Statistical analyses

Statistical analyses were done by SPSS statistical package. Analysis of variance followed by the least significant difference (LSD) procedure, which was used for the comparison of drug release rates from different formulations by considering $p < 0.05$ as significant value.

3. Results and discussion

3.1. Preparation and characterization of microspheres

Numerous chemical modifications of starch have been described previously, since there is a need to prevent or control the enzymatic degradation of starch to improve its certain physico-chemical properties. Chemical modifications of starch have been studied before (Sïmkovic, Laszlo, & Thompson, 1996) for their pharmaceutical applications (Bjoèrk & Edman, 1990). The present study describes a technique that enabled production of microspheres by crosslinking starch with epichlorohydrin (EPI), which was

found to be an efficient divalent crosslinking agent for starch (Kartha & Srivastava, 1985). In the present work, water-in-oil emulsion technique was used with sodium hydroxide solution of starch as the dispersed phase and a mixture of organic solvent and light paraffin containing the surfactant as a continuous phase. According to Shinoda and Takeda (1970), addition of sodium hydroxide to aqueous phase would result in a decrease of affinity of water to the hydrophilic head of Span® 80, leading to a modification of surface tension at water-organic phase interface. Efficient crosslinking of starch by EPI required a concentration of sodium hydroxide higher than 1 mol/L. It was found that no stable emulsions were obtained for compositions corresponding to the ones required by the crosslinking process.

Particle size and size distribution were analyzed by dynamic laser light diffraction technique using Mastersizer-2000, Malvern, UK. Volume-mean diameter of the microspheres produced by taking different drug/polymer ratios are summarized in Table 1. On a population basis, the particle size distribution was found to be unimodal. Particle size analysis of STH microspheres containing AMP showed that the mean microsphere diameter was affected by drug/polymer ratio and amount of crosslinking agent in all the formulations. A reduction in microsphere size was observed with increasing polymer ratio and decreasing drug amount. As the drug amount was increased and the polymer ratio decreased, a more viscous internal phase occurred. During the emulsification process, the internal phase was hardly dispersed in the outer phase and larger microspheres were produced. When the amount of drug was decreased as the polymer ratio increased (drug/polymer ratio 1/2, 1/3, 1/4), the size of microspheres decreased due to reduced viscosity of the internal phase. These findings are similar to those reported previously (Kim, Kim, & Oh, 1994). Extent of crosslinking had an effect on particle size. For instance, for microspheres containing 1/2 drug/polymer ratio, with increasing crosslinking by EPI i.e., 4 and 8 mL EPI, particle size decreased from 140 to 115 μm and similar trend is observed for formulations F1, F3, F4, F5, F7 and F8. This is attributed to the fact that with an increase in the amount of EPI, shrinkage of particles might have occurred leading to the formation of smaller particles (Vilivalam et al., 2000).

Encapsulation efficiency of microspheres was affected by the drug/polymer ratio. As the amount of polymer increased, encapsulation efficiency decreased; this is due to the fact that higher amount of polymer would produce small size droplets with increased surface area, such that diffusion of drug from such microspheres will be fast, resulting in the loss of drug with a consequent lowering in encapsulation efficiency. A similar finding was reported before by Mateovic, Kriznar, Bogataj, and Mrhar (2002). The effect of extent of crosslinking on the entrapment efficiency data of the microspheres are presented in Table 1. With increasing crosslinking, % encapsulation efficiency decreased. For instance, for microspheres crosslinked with 4 and 8 mL of EPI (F1 to F4 and F5 to F8), entrapment efficiencies are, respectively, 70.16, 60.47% and 63.48, 54.17%. This is due to higher crosslinking, since microspheres are more rigid and the free volume space within the matrix would decrease, resulting in reduced encapsulation efficiency.

3.2. Spectral studies

FT-IR was used to confirm the crosslinking of STH. Fig. 1 compares FT-IR spectra of (a) plain STH and (b) placebo microspheres. In case of STH, broad bands observed at 3423 and 2925 cm^{-1} are due to O—H stretching vibrations (Kweon, Cha, Park, & Lim, 2000), whereas a band at 1240 cm^{-1} is due to O—H bending vibration. The bands in the region 1462–1373 cm^{-1} are attributed to C—H stretching vibrations, while the one observed at 2912 cm^{-1} is due to C—H stretching vibrations (Park & Im, 2000). The bands at 1165 and 1084 cm^{-1} are due to C—O stretching vibrations. In case of placebo microspheres, a broad band at 3421 cm^{-1}, with a smaller intensity compared to STH matrices, is due to the presence of some uncrosslinked hydroxyl groups of STH. Intense bands that appeared at 3009 and 2922 cm^{-1} are due to aliphatic C—H stretching vibrations. The bands at 1449 and 1423 cm^{-1} are assigned to C—H bending vibrations. New bands appearing at 1115 and 1086 cm^{-1} are due to the presence of C—O—C stretching vibrations that are possibly formed due to the reaction of hydroxyl groups of STH with EPI. In all, FT-IR confirms the crosslinking reaction.

FT-IR spectral data were also used to confirm the chemical stability of AMP in STH microspheres. For instance, FT-IR spectra of (a) pristine AMP, (b) placebo microspheres and (c) AMP-loaded microspheres are displayed

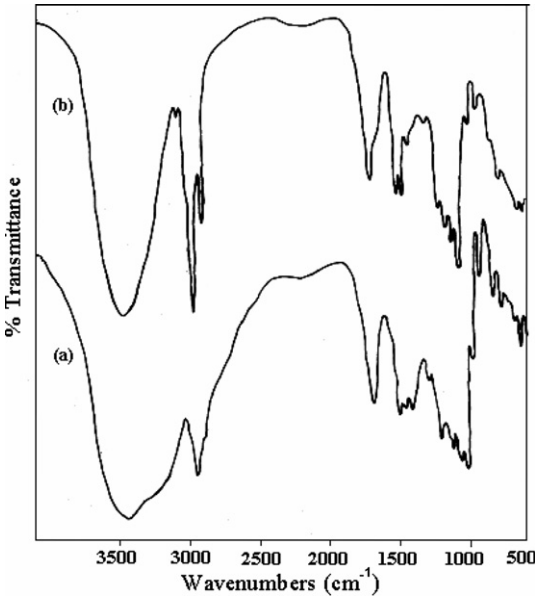

Fig. 1. FT-IR spectra of (a) STH, (b) crosslinked STH.

in Fig. 2. Pristine AMP has the characteristic bands of its different functional groups. The band at 3446 cm^{-1} is due to the N—H stretching vibrations, while those at 3050 and 2969 cm^{-1} are due to the aromatic C—H stretching vibrations. The band at 1606 cm^{-1} is due to C=O stretching vibrations of the secondary amide group. The carbonyl (C=O) stretching and carboxylate anion stretching vibrations are observed at 1773 and 1668 cm^{-1}, respectively. Bands at 1458 and 1376 cm^{-1} are due to the C—H bending vibrations. The bands at 1074 and 766 cm^{-1} are attributed to C—O stretching and aromatic C—H bending vibrations, respectively. Spectra of the AMP-loaded microspheres are not characteristically different from the spectra of placebo microspheres. In the complex matrix of crosslinked STH containing drug, in addition to characteristic bands of the crosslinked STH, some additional bands have appeared due to the presence of AMP, but some bands of AMP are not prominent in the drug-loaded microspheres due to the identical stretching of the placebo microspheres as well as drug-loaded microspheres. The peaks at 3423, 2923, 2854, 1760, 1648, 1600, 1465, 1082 and 766 cm^{-1} for AMP also appeared in the AMP-loaded microspheres, indicating the chemical stability of AMP in the matrix, which further suggests that AMP did not undergo chemical changes during the production of microspheres.

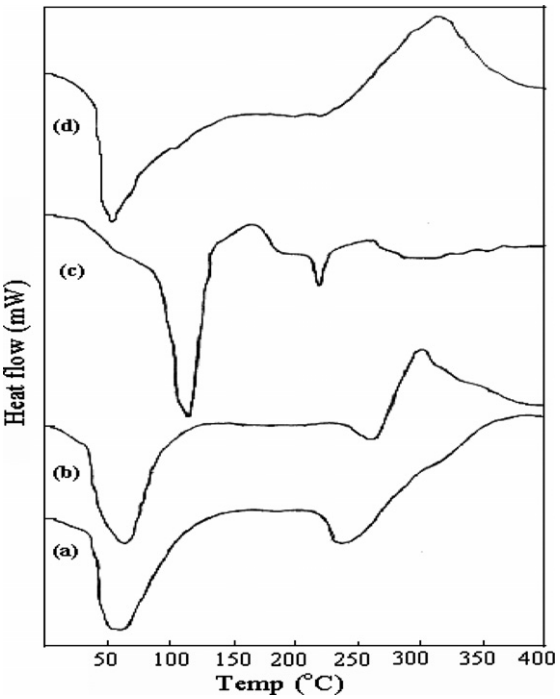

Fig. 3. DSC thermograms of (a) plain STH, (b) placebo microspheres, (c) plain AMP and (d) AMP-loaded microspheres.

3.3. Thermal analysis of the microspheres

DSC thermograms of (a) plain starch, (b) placebo microspheres, (c) pristine AMP and (d) AMP-loaded microspheres are presented in Fig. 3. The polymorphism of AMP and melting temperature (T_m) of the polymer were determined. For plain STH, two endothermic peaks were observed, one with a minimum at 60 °C, which corresponds to melting process and the other at 238 °C due to thermal decomposition. The placebo microspheres display endothermic peak at 68 °C and a broad endotherm ranging from ambient temperature to about 110 °C [see Fig. 3(b)]. This could be due to the gelatinization of the matrix due to the disruption of hydrogen bonds (Hanna, Basta, El-saied, & Abadir, 1998). In addition, the placebo microspheres exhibit a endothermic peak at 259 °C with a shift in endothermic peak toward higher temperatures as compared to plain STH. This could be due to the formation of more crystalline polymer matrix as a result of crosslinking and the formation of rigid structure. This shift in endothermic peak towards higher temperature also supports the crosslinking of STH due to chain entanglements. The thermogram also depicts a small exotherm at 280 °C due to crystallization of the starch. The small crystallites melt at 247 °C and undergo thermal decomposition beyond 284 °C as seen by a broad exotherm [see Fig. 3(b)]. These data are in agreement with published reports (Kweon et al., 2000). Thus, the process at 280–350 °C may be attributed to the degradation of starch components. To understand the polymorphism of the drug in the matrix, DSC of placebo, drug and the drug-loaded microspheres have been analyzed. AMP showed a large peak around 110–150 °C due to dehydration of the crystalline network, followed by a sharp melting peak at 212 °C (Giunchedi,

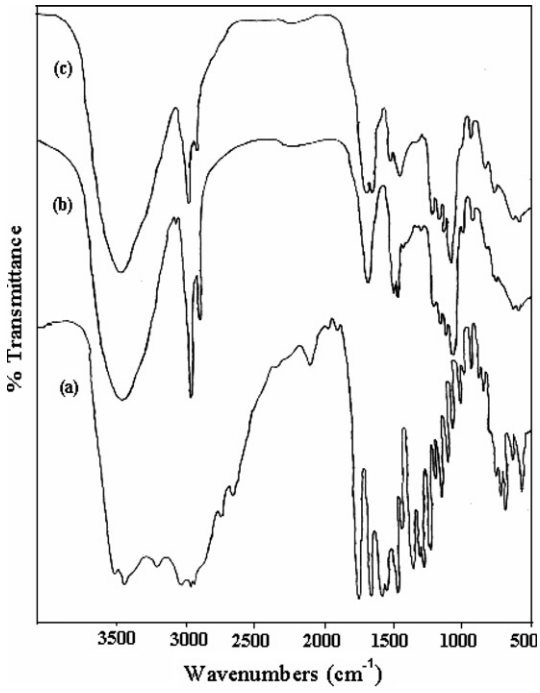

Fig. 2. FT-IR spectra of (a) pristine AMP, (b) placebo microspheres and (c) AMP-loaded microspheres.

Genta, Conti, Muzzarelli, & Conte, 1998), but a change has taken place in the thermal behavior of drug, since drug-loaded microspheres did not show peaks that are typical of AMP. At any rate, DSC data suggests that AMP containing STH polymeric network is completely amorphized.

3.4. Physical characteristics of the tableted microspheres

Ampicillin-loaded microspheres were compressed into tablets using the directly compressible excipients. Tablets were characterized for drug content, hardness, thickness, weight uniformity and disintegration time. These data are presented in Table 2. During the preliminary studies, tablets were produced without poly(vinyl pyrrolidone) K-30, but tablets with sufficient hardness to withstand the conditions of friability could not be produced. On the other hand, tablets with good mechanical strengths were obtained after adding poly(vinyl pyrrolidone) K-30 with a hardness ranging from 4.2 to 5.0 kg/cm^2. Drug content, weight uniformity and thickness of the tablets were well within the acceptable limits. Drug content was analyzed on five different tablets of each formulation individually, but only the mean values are presented in Table 2. Drug content varied between 95.58 and 98.26, while the disintegration time varied between 16 and 18 min. Since our objective is to prolong the disintegration time to prevent the burst release effect, during the disintegration of tablets, it was noticed that individual microspheres were separated without any agglomeration of microparticles. This could be advantageous in CR applications.

3.5. Scanning electron microscopic studies

Shape and surface characteristics of the microsphere formulations coded as F2 and F4 are shown in Figs. 4 and 5, respectively. Drug-loaded STH microspheres are spherical and no drug crystals were found on the surface. Microspheres prepared containing higher amount of the polymer (1:4 drug: polymer ratio) exhibited smoother surfaces than those prepared taking a lower amount of the polymer (1:2). Irregular surfaces and larger sizes of the microsphere were observed for those prepared with a lower amount of the polymer. This has greatly affected the morphological characteristics of the microspheres. As the polymer ratio increased, more spherical microspheres with smooth surfaces were obtained as suggested before (Kim et al., 1994).

Shape and surface differences between the two formulations occurred due to the different amount of polymer.

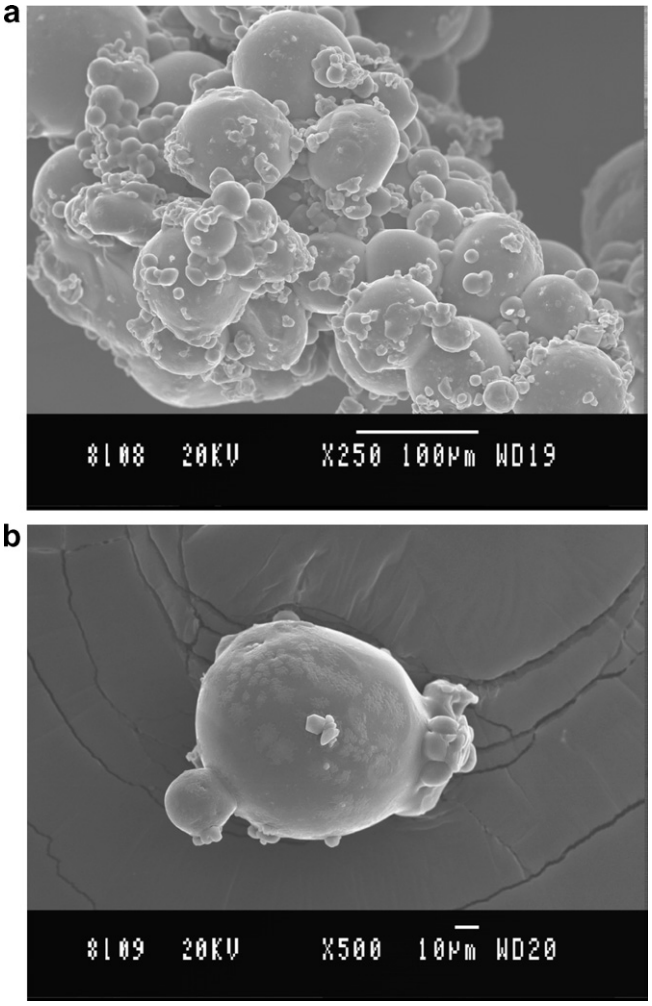

Fig. 4. SEM images of the microspheres: (a) AMP-loaded group of particles of formulation F2 and (b) AMP-loaded single particle of formulation F2.

Span® 80 was completely dissolved in the outer phase of w/o emulsion system during the production of microspheres due to its lipophilic nature. Therefore, homogeneous droplets in the emulsion were solidified to give microspheres having smooth surfaces (Kawata, Nakamura, Goto, & Aoyama, 1986). A cross-sections SEM of the tableted microspheres is shown in Fig. 6(a) and (b), which indicates that in the tablets, microspheres are present as individual particles without getting compact during compression. Since this is an ideal requirement for producing tableted microspheres, the procedure used here may be suitable for tableting.

Table 2
Physical characteristics of the tablet formulations

Formulation code	Weight uniformity (mg)	Hardness (kg)	Thickness (mm)	Disintegration time (min)	Drug content (%)
T1	398 ± 0.57	4.2 ± 0.02	2.34 ± 0.01	18 ± 0.86	96.48 ± 1.26
T2	399 ± 0.50	4.4 ± 0.01	2.40 ± 0.01	17 ± 0.57	97.24 ± 1.18
T5	401 ± 0.57	4.8 ± 0.02	2.33 ± 0.02	16 ± 0.57	95.58 ± 1.27
T6	398 ± 0.57	5.0 ± 0.05	2.32 ± 0.02	17 ± 0.76	98.26 ± 1.14

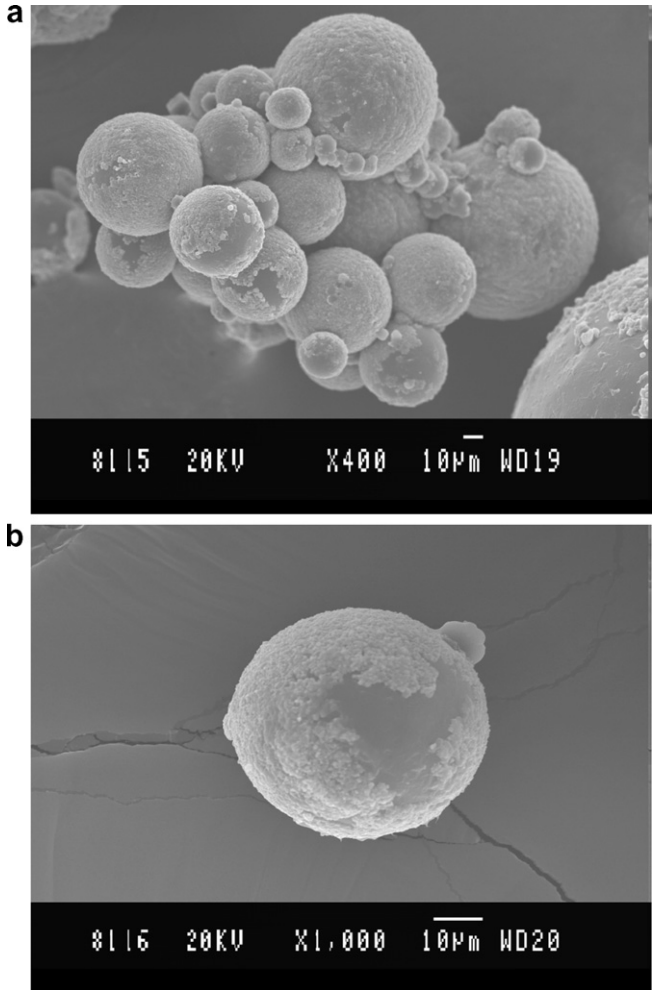

Fig. 5. SEM images of the microspheres: (a) AMP-loaded group of particles of formulation F4 and (b) AMP-loaded single particle of formulation F4.

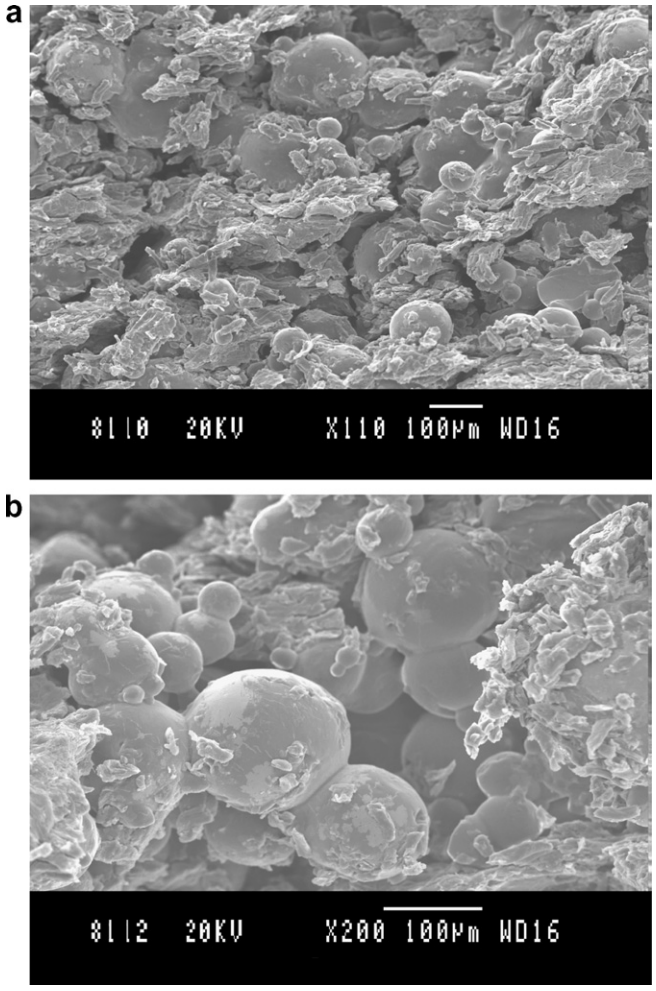

Fig. 6. Scanning electron microscopic photographs of cross-section of tableted STH microspheres loaded with AMP at (a) 100× and (b) 200× magnifications.

3.6. Water uptake studies

Equilibrium water uptake of the crosslinked microspheres exerts an influence on their release rates (Ritger & Peppas, 1987). The % equilibrium water uptake data of the crosslinked STH microspheres presented in Table 1, indicate that as the amount of EPI increases from 4 to 8 mL, equilibrium water uptake decreases significantly from 586% to 380% for formulations F1 to F8. This type of reduction in water uptake capacity is due to the formation of a rigid network structure at a higher concentration of crosslinking agent. Formulations containing higher amounts of STH showed lower swelling rates than those containing small amounts of STH. For instance, F1 (1:1 drug:polymer) exhibits higher swelling than F2, F3 and F4. Thus, there is a insignificant difference in the swelling of formulations F1 to F4 and F5 to F8, which could be attributed to the hydrophilic nature of STH, leading to higher water uptake.

The diffusion coefficient was calculated for water absorption or drug release through the microspheres using:

$$D = \left(\frac{r\theta}{6M_\infty}\right)^2 \pi \tag{4}$$

where θ is slope of the linear portion of the plot of M_t/M_∞ vs. $t^{1/2}$, r is initial radius of the microspheres and M_∞ is maximum (equilibrium) value of sorption. Values of D calculated for drug release included in Table 3, suggest that D decrease systematically with increasing crosslinking as well as with increasing amount of STH in the microspheres. This indicates that with increasing crosslinking, a stiffer polymeric matrix is formed. Also, with increasing amount of STH in the matrix, the rate of swelling of microspheres decreased due to increased entanglement of the crosslinked STH chains.

3.7. In-vitro release study

In-vitro drug release was performed in 0.1 N HCl followed by phosphate buffer solution pH 7.4 (PBS) to simulate the GIT conditions. The release profiles of AMP microspheres prepared with different drug/polymer ratios

are presented in Fig. 7. As the polymer ratio increased from 1:1 through 1:4, drug release rates decreased dramatically. Increasing amount of polymer in the formulation resulted in a decrease in the dissolution rate as a result of the increase in matrix thickness formed by the polymer, thereby increasing the distance that the drug passed through the surface of the microspheres. Similar arguments were made by Kawata et al. (1986) and Chiao and Price (1994). When microspheres were immersed into dissolution medium, they could swell due to the absorption of water by the matrix forming a gel diffusion layer. This layer would hinder the outward transport of the drug, due to diffusion-CR (Chiao & Price, 1994). However, the release of drug from STH microspheres might occur due to: (1) release from surface of particles, (2) diffusion through swollen rubbery matrix and (3) release due to polymer degradation or erosion. Drug in the microspheres might also act as inert filler and occupies free volume spaces inside the swollen hydrogel; this would create tortuous paths for water molecules to transport freely, but the degree of tortuosity depends upon the volume fraction of the filler (Peppas & Smolen, 1980). In all formulations, of this study, the release rates were extended up to 24 h.

Drug release rates from different formulations were statistically evaluated by ANOVA method. For formulations F1 to F4, F value was 0.725 ($df = 32$, $p > .05$), indicating a insignificant difference in the release rates of AMP from the STH microspheres. Fig. 8 displays the release profiles of microspheres crosslinked with different amounts of EPI. These results exhibit a pronounced effect of matrix crosslinking on the drug release rates for all formulations, but release rates vary depending upon the amount of EPI used in crosslinking. Thus, % cumulative release is higher for microspheres that are crosslinked with 4 mL of EPI (F1 and F2), but the least % release was observed for microspheres crosslinked with 8 mL of EPI (F5 and F6). This is due to the fact that at higher crosslinking, free volume of the matrix will be small, thereby hindering the easy

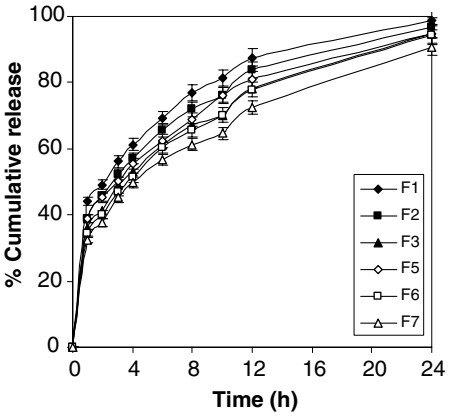

Fig. 8. Effect of crosslinking on drug release profiles.

transport of drug molecules through the matrix. Consequently, this would reduce the rate of swelling and drug release from the matrix. In case of formulations F1 to F3 and F5 to F7, the F value was found 0.492 ($df = 53$, $p > .05$), indicating insignificant differences in the AMP release rates when varying amounts of crosslinking agent were added to form the matrix.

The AMP tableted microspheres were prepared using the excipients as shown in Table 2. Results of % drug release vs. time from the tableted microspheres and neat microspheres are presented, respectively, in Figs. 9 and 10. Release rates of AMP from tableted microspheres are smaller than plain microspheres. Initially, high release rates were observed for microspheres due to the immediate dissolution of the surface adhered AMP particles, but after tableting, initial burst release was slightly less due to the smaller available surface area of the tableted microspheres. Also, coating of the microspheres by hydrophobic excipients like magnesium stearate during the preparation of tablets might be responsible for reducing the burst effect. In case of AMP microspheres, release rates depend upon the extent of crosslinking i.e., release rates decreased with

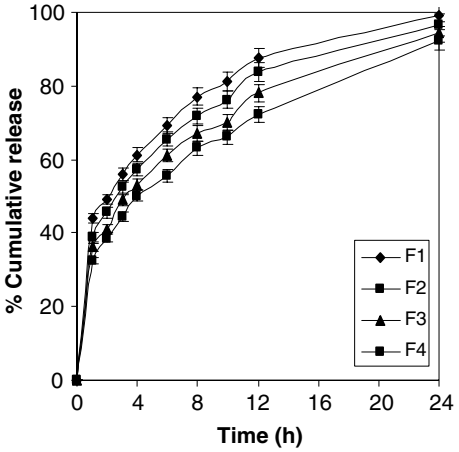

Fig. 7. Effect of drug/polymer ratio on drug release profiles from AMP loaded STH microspheres prepared with Span® 80.

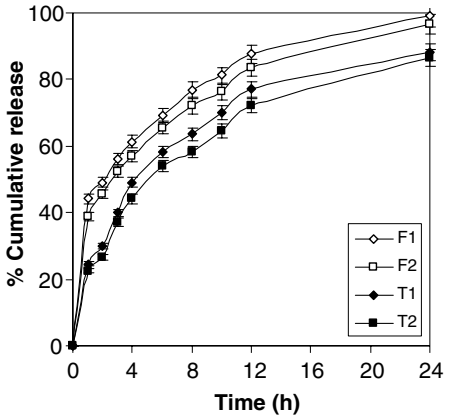

Fig. 9. Comparison of drug release rates of microspheres before and after tableting vs. time for formulations F1, F2, T1 and T2.

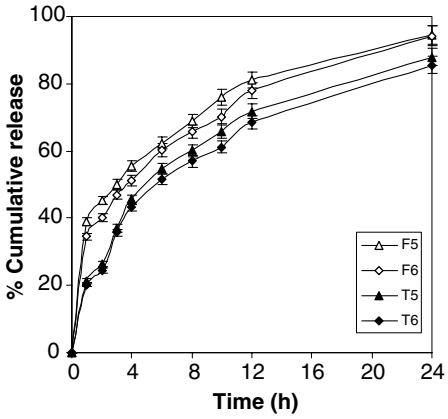

Fig. 10. Comparison of drug release rates of microspheres before and after tableting vs. time for formulations F5, F6, T5 and T6.

increasing crosslinking of the matrix. Release mechanism of the drug from tablet formulations are controlled by the diffusion as well as erosion mechanism. Among the tableted microspheres studied, T5 showed a least drug release due to higher crosslinking. Drug release from microspheres before and after tableting were also evaluated by the ANOVA method. F value was 1.55 ($df = 35$, $p > .05$), indicating no significant difference in the release rates before and after tableting.

3.8. Release kinetics

To determine the mechanism of drug release, the initial portion (i.e., $M_t/M_\infty \leqslant 60\%$) of % drug release vs. time profiles have been fitted to the empirical equation proposed by Ritger and Peppas (1987):

$$\frac{M_t}{M_\infty} = Kt^n \tag{5}$$

where M_t/M_∞ is the fraction of drug released at time t, K is a kinetic rate constant and n is diffusional exponent characterizing the mechanism of drug release. If $n = 0.5$, drug diffuses and releases from the polymer matrix following a Fickian diffusion. For $n > 0.5$, anomalous or non-Fickian type diffusion occurs. If $n = 1$, case II release kinetics is prevalent. Estimated values of n along with the correlation coefficient, r values are presented in Table 3. For all formulations, the values of n ranged between 0.35 and 0.59, indicating that drug release deviates slightly from Fickian trend following anomalous or non-Fickian trends. For formulations containing different amounts of STH, the n values ranged between 0.35 and 0.4, indicating the anomalous release trend. In case of formulations crosslinked with different amounts of EPI, the n values ranged between 0.49 and 0.59, leading to the Fickian diffusion. Fickian diffusion is possible if the time scale of polymer relaxation is either effectively infinite or zero as compared to time required to establish a concentration profile in the polymer (Peppas & Khare, 1993). This signifies the elastic and viscous Fickian diffusion limits. Matrix systems reach equilibrium state of relaxation quite fast with a Fickian diffusion. With the release of surface drug, numerous pores and channels are possibly generated in the matrix structure, which further elevates the rate and extent of AMP release. However, due to the hydrophilic nature of the polymer, when exposed to diffusion media, free volume spaces are generated between the macromolecular chains. After complete solvation of the polymer chains, chain dimensions of the polymer will increase due to polymer relaxation.

Table 3
Results of parameters n and correlation coefficient (r) calculated from Eq. (5) and diffusion coefficients (D) calculated from Eq. (4) in SGF media at 37 °C for various formulations

Formulation code	n	r[a]	$D \times 10^6$ (cm^2/s)
F1	0.40	0.99	4.72
F2	0.38	0.99	4.29
F3	0.36	0.98	3.92
F4	0.35	0.98	3.58
F5	0.59	0.98	3.87
F6	0.58	0.90	3.38
F7	0.59	0.91	3.22
F8	0.49	0.94	3.10

[a] r is correlation coefficient calculated at 95% confidence limit.

In non-Fickian or anomalous transport, both diffusion as well as macromolecular relaxation time scales are similar and both will control the overall rate of penetrant absorption. Non-Fickian release is described by two mechanisms: the coupling of drug diffusion and polymer relaxation. The release mechanism is known to be influenced by (i) nonhomogeneous gel microstructure as well as the existence of polymeric domains within the swollen gel, (ii) rate of fluid ingress into the matrix, (iii) dissociation/erosion and total disentanglement at the dissolution front and (iv) rate of matrix swelling, relaxation as well as molecular diffusion of drug through the swollen gel. In general, the solubility of drug itself governs the rate and extent of diffusional release. For diffusion to occur, the first step is wetting of the drug by water, followed by its dissolution such that the drug is available in its molecular form to diffuse out of the matrix. Hence, the net release rate observed is a cumulative effect of drug's solubility influenced by its structure, molecular weight and other physical parameters.

4. Conclusions

Microspheres have been used widely in the CR of a variety of drugs to enhance their release patterns. In this investigation, starch microspheres were prepared by emulsion crosslinking method. FT-IR and DSC studies confirmed the crosslinking reaction. Ampicillin was successfully entrapped into the starch matrix and was stable in the matrices developed without undergoing any chemical

changes during the microsphere preparation. Thermal studies confirmed the molecular level dispersion of the drug in the polymer. Microspheres were spherical, but their morphologies were affected by the amount of polymer used during formulation. SEM of the cross-section of the tableted microspheres revealed discrete particles. The release of drug from microspheres showed a dependence on the amount of STH, extent of crosslinking of the matrix as well as the amount of drug loading. Drug release followed anomalous or non-Fickian to Fickian trends. Tableted microspheres exhibited good mechanical strength properties and the formulation of tableted microspheres was useful in reducing the initial burst release of the matrix. *In-vitro* release studies indicated that tableted microspheres of this study could be used successfully as CR devices for the release of ampicillin.

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