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Evaluation of Ceftriaxone Releasing from Microspheres Based on Starch Against *Salmonella* spp.

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Abstract: In this study, ceftriaxone-loaded microspheres were prepared by an inverse emulsion polymerization method using starch as raw material. The effects of cross linking agent, glutaraldehyde and the time of cross linking on antimicrobial behavior of drug loaded-microspheres were investigated against *Salmonella typhi*, *Salmonella paratyphi* A and *Salmonella paratyphi* B. Surface morphological characteristics and size distribution of prepared microspheres were studied by using an optical microscope. Microspheres loaded by ceftriaxone displayed different activities against microorganisms. The maximum diameter of inhibition zone caused by the microspheres was 19 mm and prolonged release pattern for 24 h. Microspheres had spherical shape and the size of cross linked microspheres was larger than uncross linked ones. Their size distributions were between 1 to 40 μm. It is probable, with more study, to increase the efficacy of treatment by this method.

Key words: Ceftriaxone, drug delivery, microspheres, *Salmonella*, starch

INTRODUCTION

Biodegradable polymers are typically used to control the rate of drug releasing from parenteral drug delivery systems. Such polymers hold enormous potential, especially for the delivery of peptides and proteins, which are not orally active (Heller, 1993; Rothen-Weinhold and Gurny, 1997; Breitenbach *et al*., 2000). Natural biodegradable polymers, such as starch have been studied for pharmaceutical applications. Starch based preparations are suitable for a range of biomedical applications (Sturesson and Wikingsson, 2000; Elvira *et al*., 2002; Henrist *et al*., 2001). However, natural starch may not be appropriate in some parenteral controlled drug delivery systems, as many drugs are released too quickly from such unmodified starch-based systems (Pereswetoff-Morath, 1998; Michailova *et al*., 2000). This is due to the substantial swelling and rapid enzymatic degradation of natural starch. Starch is a mixture of amylose and amylopectin, where amylose is a linear polymer of α-D-glucopyranosyl units linked by 1, 4-α-D-glucosidic linkages and amylopectin is a branched polymer of α-D-glucopyranosyl units, containing both 1, 4-α-D-glucosidic linear linkages and 1, 6-α-D-glucosidic linkages at the branch points. α-Amylase is the main enzyme involved in the hydrolysis of the 1, 4-a-d-glucosidic linkages in starch (Clausen and Bernkop-Schnurch, 2001).

In order to improve starch characteristics and use it in pharmaceutical applications, such as controlled drug delivery systems, many studied have been done. For example, Tuovinen *et al*. (2004) evaluated releasing from the acetiled starch microparticles and films. These researchers used calcein, timolol and starch as drugs and biological agent, respectively. The results showed that the acetiled starch microparticles which loaded by drug or biological agent were decreased rate of release rather than untreated microparticles, although the environmental conditions such as pH, enzyme presence can effect on degradation rate of polymer and also these parameters have a great influence on the rate of releasing from treated starch microparticles and films. The objective of the present study was to evaluate the cross linked starch microspheres for delivering ceftriaxone and its antibacterial effect.

MATERIALS AND METHODS

This study was conducted in Shahed University and Amirkabir University of Technology, Tehran, Iran

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Table 1: Characteristics of microspheres preparation

Sample name	Starch (mg)	Drug (mg)	H_2O (mL)	Chloroform (mL)	Cyclohexan (mL)	Glutaradehyde/acetone (mL)	Time of cross linking (h)
N1	500	40	30	8	32	-	-
N2	500	40	30	8	32	40	1
N3	500	40	30	8	32	40	2
N4	500	60	30	8	32	-	-
N5	500	60	30	8	32	40	1
N6	500	60	30	8	32	40	2
N7	500	120	30	8	32	-	-
N8	500	120	30	8	32	40	1
N9	500	120	30	8	32	40	2

- = Without cross linking

(July- November 2006). Soluble starch $(C_6H_{10}O_5)_n$ was purchased from Merck (Germany), having 70-80% amylopectin and 20-30% amylose. Cyclohexane, chloroform and acetone were obtained from Merck (Germany). Glutaradehyde ($C_5H_8O_2$) with the average Mw 100.12 g mol^{-1} was supplied by Merck (Germany) and used as a crosslink agent. Ceftriaxone with maximum absorption wavelength of 260 nm was donated by Exir Pharmaceutical Co. (Iran).

Microspheres were prepared by inverse emulsion-polymerization method. For this purpose, 40, 60 and 120 mg of ceftriaxone were diluted into 30 mL distilled water and then 500 mg of starch was added to each sample. For preparing the organic phase, cyclohexane and chloroform (80:20 ratios) was mixed. The aqueous phase was emulsified in this solution using a homogenizer at 20000 rpm for 2 min at 25°C. After forming the emulsion, a cross linker solution containing glutaraldehyde: acetone (80:20, v:v) was added to emulsion and kept for 1 or 2 h for completing cross linking process at 25°C. Afterward, the drug loaded starch microspheres were isolated by centrifugation, washed twice with distilled water and dried for 12 h by freeze drier (Christ, Alpha 1-2 LD) (Table 1).

Determination of particle size of loaded microparticles with antibiotic, a light microscope (TS100-F, Nikon) was used. Therefore, the diameter of fifty particles of each sample was measured.

Antimicrobial effects of microspheres were evaluated against *Salmonella typhi* NCTC 5761, *Salmonella paratyphi* A NCTC 5702 and *Salmonella paratyphi* B NCTC 8390, by disk-diffusion method (Anhalt and Washington, 1985). Plates of Mueller Hinton agar were inoculated using sterile swabs with the bacterial suspension at 0.5 Mac Farland's scale. The blank disks measuring 6 mm diameter were loaded with 10 μL of sample with concentration of 30 μL mL^{-1} and placed on the surface of the inoculated plate. After incubation at 37°C for 24 h, inhibition zone was measured by vernier clipper. This test was repeated 3 times for all samples.

RESULTS AND DISCUSSION

Light microscopic investigation showed that the particles appeared spherical. The size of cross linked samples, which treated with glutaraldehyde were larger than uncross linked samples and most of them were between 16-20 μm (Table 2). In addition, the amount of drug loading did not affect the particle size distribution. Starch is a common excipient with a long tradition within the pharmaceutical industry where it is used as a disintegrant, a binder or a bulking agent. Starch has been used as a biodegradable polymer in microspheres for nasal delivery of drugs (Shirui *et al*., 2004) or for the delivery of vaccine given orally and intramuscularly (Rydell *et al*., 2005). The main part of these starch particles has been produced by polymerization of acryloylated starch in a water-in-oil emulsion (Rydell *et al*., 2005) or as in the present study, by cross-linking soluble starch (Hamdi *et al*., 2001).

All the samples showed antimicrobial effect against *Salmonella paratyphi* B, in contrast, the response of microspheres had different effects against *Salmonella typhi* and *Salmonella paratyphi* A and some samples did not show any antimicrobial activity (Table 3). Increasing drug load brought about growth inhibition zone which indicates effect of prepared system against the test microorganisms. There were some exceptions in drug loading impression. Samples encountered with *Salmonella paratyphi* B did not reveal the same behavior that could be related to the strain type. Table 3 shows that the samples with 2 h cross linking time exhibit superior antimicrobial behavior with the inhibition zone diameters of 10 to 19 mm. With the exception of *Salmonella paratyphi* B, in other samples, this region did not grow by increasing in drug load and thus there is a reverse activities which is related to the microbial type as implied before.

After 24 h, the extent of inhibition zone in samples with 1 h cross linking was 10-18 mm and just N2 and N5 in

Table 2: Particle size distribution in N1, N2, N6 and N8

Particle size (μm)	Relative abundance (%) N1	N2	N6	N8
1-3	14	0	0	0
4-7	0	0	0	0
8-11	6	8	0	8
12-15	8	6	10	4
16-19	62	58	62	70
20-23	4	4	14	6
24-27	6	12	14	8
28-30	0	12	0	4

Table 3: Average of diameter of inhibition zone (mm±SD)

Sample name	*S. typhi*	*S. paratyphi* A	*S. paratyphi* B
N1	-	-	10±3
N2	-	10±2.5	13±4
N3	14±4	11±3	11±0.35
N4	-	-	10±0.2
N5	-	11±3	12±3
N6	18±5	10±3	14±3.5
N7	14±4	-	10±3
N8	18±4.5	11±2.5	12±2.5
N9	19±4	10±2	14±3

- = Without inhibition zone

the vicinity of *Salmonella typhi* did not have any inhibition zone (Table 3). Inhibition zone diameter in cross linked samples was larger than uncross linked ones.

This study showed that it is possible to load the Ceftriaxon into the starch microcapsules. Drug release from microcapsules continued for 24 h and during this time displayed antimicrobial behavior completely. The largest inhibition zone was of 2 h cross linked samples, so that N9 had the maximum inhibition zone diameter of 19 mm at the vicinity of *Salmonella typhi*. Cross linking of the starch microspheres with glutaraldehyde yielded high efficiency of entrapping bioactive agents or drugs. This is evident by larger inhibition zone diameters in cross linked samples than uncross linked ones. The enhanced activity could be due to the chemical link type adopted in this study. This is supported by an observation that the physical and molecular properties of the starch material in combination with additives, such as polyethylene glycol, bovine serum albumin and buffer, influenced the quality of the microspheres (Elfstrand *et al*., 2007). Evaluations of particles microstructure with optical microscope exhibited the spherical uniform structures. There will be a homogenous drug distribution in the biological environment.

CONCLUSIONS

The results suggest feasibility of ceftriaxone encapsulation within starch microspheres which could deliver bioactive agents to the physiological milieu. One of the fall outs of the present study could be evaluation of starch microsphere intended for subcutaneous injections. The influence of ions on starch microspheres could be investigated. The addition of a variety of buffers to starch microspheres may significantly affect their kinetics. The effect would depend on the type of salt as well as on salt concentration, but also on the temperature of incubation. These parameters have not yet been investigated.

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