PREPARATION AND EVALUATION OF OXYTETRACYCLINE HYDROCHLORIDE MICROBEADS FOR DELAYED RELEASE

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ABSTRACT

Oxytetracycline HCl microbeads were prepared with sodium alginate and pectin using ionic gelation method and evaluated for morphology, flow properties, drug content and *in vitro* drug release study. SEM confirmed spherical structure of microbeads with rough and porous surfaces and microbeads possessed average particle size range of 639.86 to 685.74 µm. *In vitro* drug release study was carried out in simulated gastric fluid (SGF) for first 2 h and simulated intestinal fluid (SIF) for next 6 h. Selected formulation was coated using enteric polymer cellulose acetate phthalate to minimize burst drug release along with delayed drug release in intestinal medium.

Keywords: Delayed release, *in vitro* dissolution, microbeads, micromeritic studies, oxytetracycline hydrochloride.

INTRODUCTION

Oxytetracycline HCl (OTC HCl) is a broad spectrum bacteriostatic antibiotic commonly used for systemic therapy as well as locally for gastric or intestinal infections. Newly discovered, additional mechanisms of action include antioxidant, anti-inflammatory and immunosuppressive activity of OTC HCl. It is presently considered as therapy of choice in papulopustulous acne, rosacea and perioral dermatitis as well as primary and secondary skin infections (Olszewska, 2006). Conventional oral formulations of OTC HCl lead to gastrointestinal irritative effects such as stomach upset, epigastric burning, nausea and vomiting due to high solubility of this drug in the gastric fluid (Sweetman, 2002). These adverse effects create a potential need to modify its release characteristics in stomach environment and to prolong its release in the small intestine in order to reduce dosing frequency and to improve patient compliance. Microbeads are small, solid and free flowing particulate carriers containing dispersed drug particles either in solution or crystalline form that allow a sustained release or multiple release profiles of treatment with various active agents without major side effects (Aulton, 2002). Additionally, the beads maintain functionality under physiological conditions, can incorporate drug to deliver locally at high concentration ensuring that therapeutic levels are reached at the target site while reducing the side effects by keeping systemic concentration low. The microbeads are produced from several polymers such as cationic polymers e.g. chitosan, anionic polymers e.g. sodium alginate, and binding components e.g. gelatin, chondroitin sulfate, avidin in predetermined ratio (Costa *et al.*, 2001) Considering the advantages of microbeads, the present investigation was undertaken to prepare and evaluate OTC HCl loaded calcium alginate, calcium alginate-pectinate and enteric coated microbeads with an objective to reduce solubility in gastric medium and prolong the drug release in small intestinal environment and to improve the patient compliance.

EXPERIMENTAL

Materials and methods

Oxytetracycline HCl was a generous gift from Siemens Laboratory, Gurgaon, India. All other chemicals were purchased from commercial sources. All-glass double distilled water was used throughout the studies.

Preparation of microbeads

Calcium alginate microbeads were prepared by ionic gelation method (George and Abraham, 2006). A 2 % w/v of drug was added to a 1.75 % w/v aqueous solution of sodium alginate (F1). This solution was dropped manually through a needle size no. 26G from a hypodermic syringe in to a 2 % w/v solution of $CaCl_2$. The gel microbeads formed were allowed to harden in gelling bath for 30 min. After washing with bidistilled water, the beads were air dried at room temp and stored in vacuum desiccator until constant weight was achieved. Calcium alginate-pectinate microbeads were prepared using method as described above, with the exception that 2 % w/v of drug was added to an aqueous solution comprising 1.75 % w/v sodium alginate and 0.3, 0.5 and 1.0 % w/v of pectin in F2, F3 and F4 respectively. CAP-coated microbeads were

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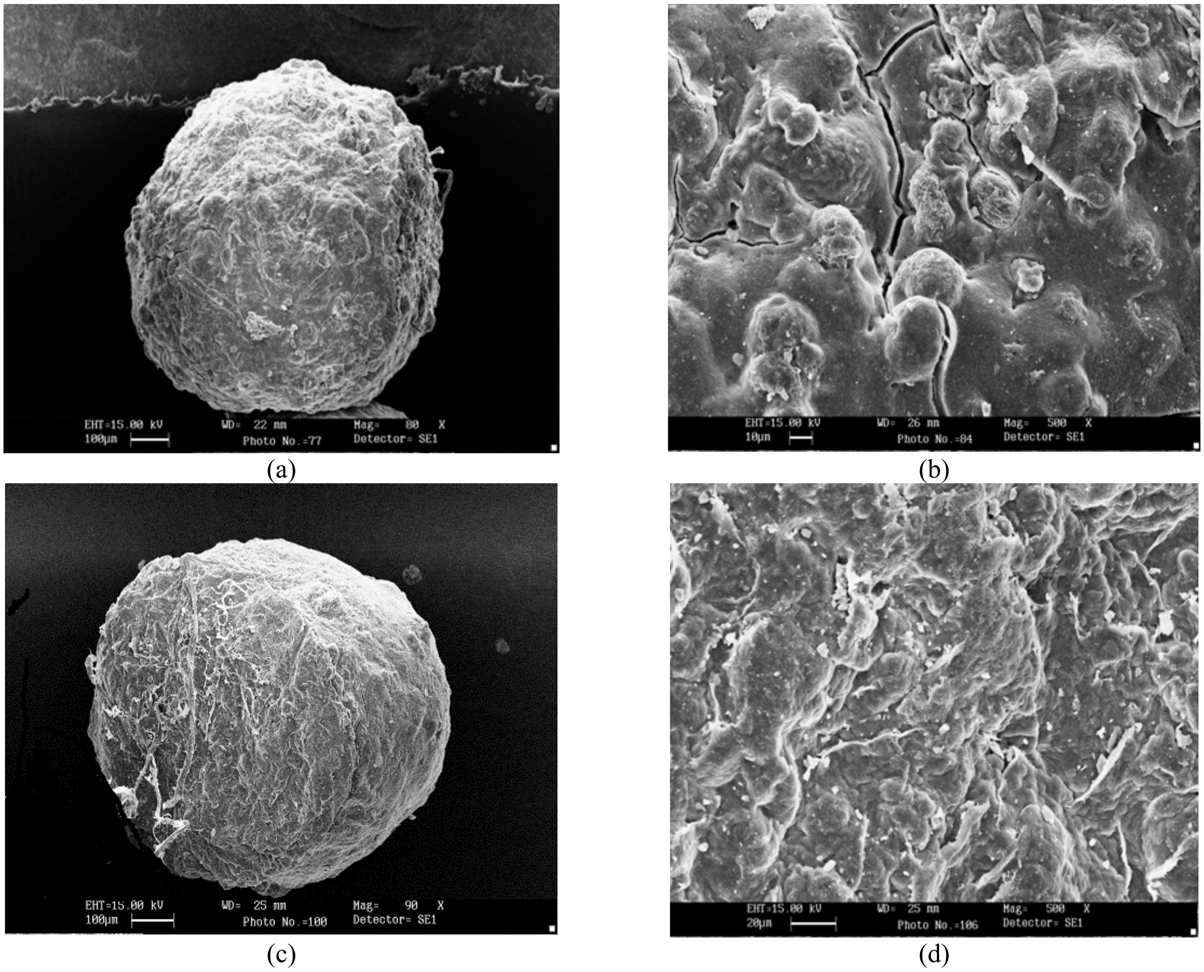

(a) (b)

(c) (d)

Fig. 1: SEM images of formulations. (a): F1 at 80 X magnification (b): F1 at 500 X magnification, (c): F4 at 90 X magnification and (d): F4 at 500 X magnification.

prepared by the procedure as described earlier (Liu *et al*., 1997). Selected batch of microbeads (F4) were transferred in to 0.5 %w/v and 1.0 %w/v solution of CAP in acetone for F5 and F6 respectively and remained for 15 min under gentle magnetic stirring, filtered and dried in air at room temperature (table 1).

Evaluation of microbeads

Morphology

The external and internal morphology of microbeads were studied by scanning electron microscopy. The microbeads were coated with gold palladium under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The coated samples were then observed with a scanning electron microscope.

Micromeritic properties

Average particle size of beads was determined by sieving method using automatic sieve shaker (HICON, India). Flowability of beads was determined by dynamic angle of repose using lab fabricated rotating cylinder. The beads were subjected to bulk density determination using tap density tester (HICON, India) (Mishra *et al*., 2003).

Determination of entrapment efficiency

10 mg of the crushed microbeads were dissolved in 10 ml of methanol, vortexed for 5 min and filtered though whatmann filter paper no. 4. The filtered samples were suitably diluted and analyzed spectrophotometrically at 359 nm against suitably constructed calibration curve (Vijaya *et al*., 2006).

Dissolution studies

The dissolution studies were carried out using USP dissolution rate test apparatus type I at 75 rpm and 37±0.5°C. The beads equivalent to 250 mg of drug were filled in to colorless hard gelatin capsules and placed in basket separately. The dissolution medium was 0.1 N HCl pH 1.2 as simulated gastric fluid (SGF) for the first 2 h, followed by phosphate buffer pH 7.4 as simulated intestinal fluid (SIF) for the next 6 h. 5 ml samples were withdrawn at specified time intervals and was replaced immediately with an equal volume of fresh medium. Samples were suitably diluted and analyzed at 353 nm (Shimadzu 1700). All the tests were carried out in triplicate.

RESULTS

The OTC HCl microbeads were prepared with good yield and entrapment efficiency. The SEM images of beads and pure components are shown (fig. 1). The numerical values of compressibility index in the range of 11.76% to 14.60%, Hausner's ratio less than 1.2 and dynamic angle of repose (20° to 30°) suggested excellent flow properties of all microbeads formulations unlike cohesive pure drug (table 2). The average particle size of pure drug was 18.51 µm and that of microbeads were ranged between 639.86 to 685.74 µm. F4 (calcium alginate-pectinate) exhibited highest drug loading and % entrapment efficiency values of 37.26 ± 0.52 % and 88.51 ± 1.23 % respectively, whereas F1 microbeads (calcium alginate) showed the least value of drug loading and % entrapment efficiency as 32.94 ± 0.20 % and 61.76 ± 0.96 % respectively (table 3). The dissolution studies showed that the pure drug was almost completely dissolved (94.09 ± 2.48 %) in 2 h while the release of drug from calcium alginate microbeads (F1) was found to be 67.24 ± 2.44 % in pH 1.2 within 2 h. After 2 h, the calcium alginate microbeads (F1) disintegrated and lost remaining drug within 3 h in the dissolution medium SIF (pH 7.4). With the addition of pectin with sodium alginate i.e. calcium alginate-pectinate microbeads (F2, F3 and F4), the release of entrapped drug during first 2 h in SGF was significantly reduced. Three different pectin concentrations with sodium alginate were used in order to study the effect of pectin concentration on drug release from microbeads. The F2, F3, and F4 released 60.24 ± 2.87, 56.36 ±1.13, and 51.08 ± 2.04 % of OTC HCl respectively at pH 1.2 within 2 h but after 2 h, only F4 followed the drug release pattern extending up to 8 h (fig. 2). This suggested that at least 1.0 % w/v conc. is required to delay the release up to 8 h. Model independent parameters such as dissolution efficiency after 2 h (DE_{120}) and $t_{50\%}$ of pure drug and formulations are summarized in table 4. Selected formulation F4 was coated with enteric polymer cellulose acetate phthalate (CAP). The $t_{50\%}$ value of coated formulations F5 and F6 was found to be 4 h and 5 h respectively.

Table 1: Composition of microbeads formulations

Batch code	Drug (%w/v)	Sodium alginate (%w/v)	Pectin (%w/v)	CAP solution (%w/v)
F1	2	1.75	–	–
F2	2	1.75	0.3	–
F3	2	1.75	0.5	–
F4	2	1.75	1.0	–
F5	2	1.75	1.0	0.5
F6	2	1.75	1.0	1.0

The comparative percentage drug release profile of without (F4) and with (F5 and F6) enteric coated microbeads showed that the drug released was 51.08 % ± 2.04 for F4 whereas 23.86 % ± 0.86 for F5 and 17.79 % ± 0.73 for F_6 in pH 1.2 (SGF) within 2 h. After 2 h, with changes in dissolution medium at pH 7.4, the drug release from F4, F5 and F6 was 76.43 % ±0.72, 71.22 % ± 0.44 and 63.08 % ± 0.60 % respectively in 8 h (fig. 3). *In vitro* drug release data of coated and uncoated microbeads is summarized in tables 4 and 5. While considering higher correlation coefficient (r^2), the release data appeared to fit the first order model better for the coated microbeads.

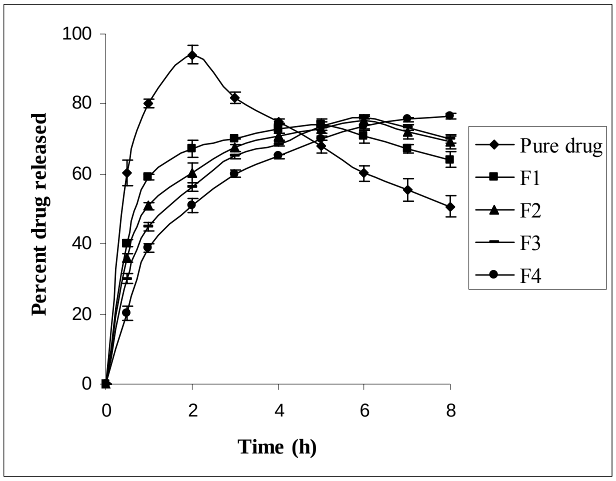

Fig. 2: Comparative dissolution profiles of pure drug and formulations.

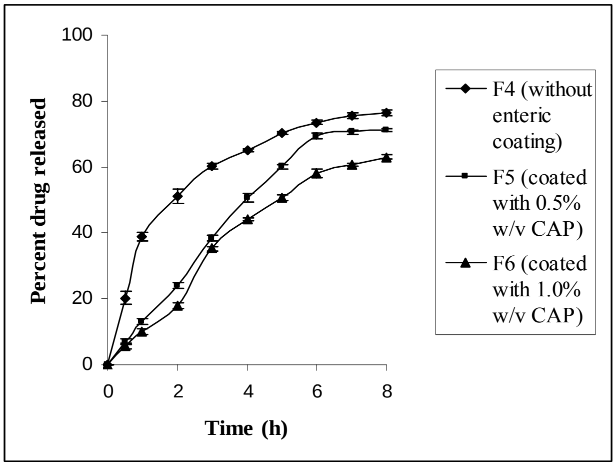

Fig. 3: Comparative dissolution profiles of uncoated microbeads (F4) and coated microbeads (F5 and F6).

DISCUSSION

The gelled microbeads were formed by ionic interaction between the negatively charged carboxyl groups of sodium alginate and the positively charged counter ion such as Ca^{++}. The addition of the divalent ions such as Ca^{++} produced a partial neutralization of carboxylate groups present on the alginate chain, forming insoluble

Table 2: Comparative micromeritic studies of pure drug and formulations

Parameters	Drug	F1	F2	F3	F4	F5	F6
Poured density (g/cm^3)	0.31	0.66	0.68	0.75	0.76	0.77	0.79
Tapped density (g/cm^3)	0.60	0.75	0.78	0.85	0.89	0.90	0.94
Compressibility index (%)	47.62	12.15	12.80	11.76	14.60	14.44	15.96
Hausner's ratio	1.90	1.14	1.15	1.13	1.17	1.17	1.19
Dynamic angle of repose	75°	25°	24°	21°	22°	21°	20°
Average particle size (μm)	18.51	639.86	667.65	673.80	685.77	692.37	698.52

Table 3: Drug content and percentage entrapment efficiency of microbeads

Formulation	Drug content (%)*	Entrapment efficiency (%)
F1	32.94 ± 0.20	61.76 ± 0.96
F2	34.38 ± 0.56	68.76 ± 1.12
F3	35.62 ± 0.78	75.70 ± 1.46
F4	37.26 ± 0.52	88.51 ± 1.23
F5	38.53 ± 0.45	88.74 ± 0.85
F6	38.91 ± 0.33	89.15 ± 1.14

* n = 3

gelatineous microbeads. Pectin with low degree of esterification (35%) along with sodium alginate formed gel microbeads by ionic gelation with divalent calcium ions. Gelation occurred due to intermolecular cross-linking between the divalent calcium ions and the negatively charged carboxyl groups of pectin and sodium alginate molecules. Pectin was used in conc. of 0.3, 0.5 and 1.0% W/V, higher conc. of pectin resulted in much higher viscosity of solution which was difficult to process for preparing the microbeads. The SEM images showed surface of calcium alginate microbeads (F1) with cracks, while the calcium alginate-pectinate microbeads (F4), though exhibited rough surface, were devoid of cracks. Absence of cracks may be due to pectin as additional component present in the microbeads. Moreover, the dispersion of OTC HCl as fine crystalline particles on the surface of microbeads was also observed. The OTC HCl microbeads were evaluated for flow properties using percentage compressibility index, Hausner's ratio as well as dynamic angle of repose suggesting excellent flow properties of all microbeads formulations unlike cohesive pure drug. This might be explained as spherical microbeads exhibited low interparticle friction resulting in good flow property.

Microbeads were assayed for drug content and percentage entrapment efficiency was estimated. The F4 (calcium alginate-pectinate) exhibited highest drug loading and % entrapment efficiency values whereas F1 microbeads (calcium alginate) showed the least value of drug loading and % entrapment efficiency which might be attributed to the possible leakage of water soluble drug from calcium alginate microbeads having large gel porosity (Philip, 2004). The % entrapment efficiency was also proportional to concentration of pectin used. An increase in pectin concentration from 0.3 % w/v to 1.0 % w/v led to an increase in % entrapment efficiency, this effect might have occurred due to the increased viscosity of the preparative mixtures which hindered drug migration towards the external phase during microbeads preparation.

Table 4: Dissolution efficiency (DE_{120}) and $t_{50\%}$ of pure drug and microbeads

Formulation	DE_{120} (%)	$T_{50\%}$ (h)
Pure drug	68.64	0.41
F1	48.96	0.84
F2	43.14	0.98
F3	38.50	1.77
F4	32.39	1.95
F5	27.12	4.0
F6	21.50	5.0

The dissolution studies showed that the pure drug was almost completely dissolved (94.09 ± 2.48 %) in 2 h while the release of drug from calcium alginate microbeads (F1) was found to be 67.24 ± 2.44 % in pH 1.2 within 2 h. This fast release of OTC HCl in gastric fluid occurred due to its high solubility in this medium. After 2 h, the calcium alginate microbeads (F1) disintegrated and lost remaining drug within 3 h in the dissolution medium SIF (pH 7.4). According to literature, at pH 7.4, the water of environment penetrates in to the chains of alginate to form hydrogen bridges through their available –OH and COO- groups (Rodriguez *et al.*, 2002).

Table 5: Correlation coefficient (r^2) values for the fit of different kinetic models

Formulation	Zero order model	First order model	Higuchi model	Korsmeyer-Peppas model
F1	0.7873	0.7838	0.8786	0.8429
F2	0.6964	0.8529	0.9593	0.9110
F3	0.7816	0.9209	0.9690	0.9233
F4	0.8358	0.9443	0.9824	0.9293
F5	0.9543	0.9766	0.9736	0.9329
F6	0.9584	0.9840	0.9795	0.9478

As a consequence, the microbeads turn in to a hydrogel and have their diameter increased, favoring the drug diffusion. This suggested that alginate microbeads had probably insufficient cross-linking density to prevent drug molecules to diffuse out. With the addition of pectin with sodium alginate i.e. calcium alginate-pectinate microbeads (F2, F3 and F4), the release of entrapped drug during first 2 h in SGF was significantly reduced. Further, the amount of drug release decreased with increase in pectin concentration. This was expected, since on increasing pectin amount with sodium alginate, interaction between two polymers had increased, forming a closer network, which decreased the diffusion of the drug outwards from the interiors of the microbeads. The release of OTC HCl from microbeads was rapid in pH 1.2 due to the fact that OTC HCl dissolved readily in acidic medium when diffused out through the microbeads matrix. Pure drug showed higher DE_{120} and lesser $t_{50\%}$ i.e. 68.64 % and 0.41 h respectively, while F4 exhibited the lowest dissolution efficiency and highest $t_{50\%}$. i.e. 32.39 % and 1.95 h respectively. Therefore, in order to delay the drug release in pH 1.2, selected formulation F4 was coated with enteric polymer cellulose acetate phthalate (CAP). The $t_{50\%}$ value of coated formulations F5 and F6 was found to be 4 h and 5 h respectively.

Various release kinetic models (Vyas and Khar, 2004) were applied to determine the mechanism of drug release from microbeads and observed that the highest correlation coefficient (r^2) found for Higuchi square root of time profile indicated that the drug release from the microbeads formulations occurred via diffusion mechanism suggesting uniform dispersion of water soluble drug in swellable polymer matrix. Drug release from coated microbeads were retarded till 8 h as compared to uncoated microbeads. This was obvious because of acid insolubility of enteric CAP. F6 exhibited much lesser drug release at pH 1.2 (SGF) within 2 h as compared to F5 formulation due to higher conc. of CAP in coating solution. The release data appeared to fit the first order model better for the coated microbeads as compared to Higuchi matrix model for the uncoated microbeads. This suggested that water soluble drug from polymer matrices is released in a way which is proportional to the amount of drug remaining in its interior, in such a way that the amount of drug released by unit of time diminishes. First order release rate of all the CAP-coated microbeads were calculated and compared to the calculated required first order release rate (0.057 h^{-1}). The data was used for selection of the formulation, which exhibited a first order release rate value closest to the required value. Thus F6 with first order release rate of value 0.058 h^{-1} was selected as best CAP-coated microbeads formulation.

In conclusion, the use of pectin with sodium alginate permits the retardant drug release behavior in gastric conditions and higher drug release at intestinal pH conditions which are of great interest for the delivery of water soluble drugs in to the intestine. The release of drug from microbeads in SGF could also be retarded by coating with polymer such as CAP. Based on these findings, it has been concluded that the CAP-coated microbeads are suitable for delayed release of drug when administered orally. Further more, since these microbeads possessed excellent flow properties, they show sufficient promise for the formulation development into suitable oral solid dosage forms such as tablets or capsules.

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REFERENCES

Aulton ME (2002). Pharmaceutics: The science of dosage form design, 2nd ed., Churchill Livingston, New York, pp. 197-210.

Costa P, Manuel J and Lobo S (2001). Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci.*, **13**: 123-133.

George M and Abraham TE (2006). Poly-ionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan - A Review. *J. Control. Rel.*, **114**: 1-14.

Liu LS, Liu SG, Froix N, Ohno T and Heller J (1997). Controlled release of interleukin-2 for tumour immunotherapy using alginate/chitosan porous microspheres. *J. Control. Rel.*, **43**: 65-74.

Mishra B, Jayanth P and Sankar C (2003). Development of chitosan-alginate microcapsules for colon specific delivery of metronidazole. *Indian Drugs*, **40**: 695-700.

Phillip H (2004). Coacervate microparticles useful for the sustained release administration of therapeutic agents, U.S. Patent No. E-116-2004/0-US-01.

Rodriguez MLG, Holgado MA, Lafuente CS, Rabasco AM and Fini A (2002). Alginate/chitosan particulate systems for sodium diclofenac release. *Int. J. Pharm.*, **232**: 225-234.

Sweetman SC (2002). Martindale: The complete drug reference, The Pharmaceutical Press, 33rd edition, pp.258-260.

Vijaya DR, Razzak M, Tucker I.G. and Medlicott NJ (2006). Encapsulation of vitamin B12 into polylactide(ε-caprolactone) microparticles using w-o-w emulsion system. Indian Drugs, 43: 457-461.

Vyas SP and Khar RK (2004). Targeted and controlled drug delivery, 1st ed., C.B.S. publishers and distributors, New Delhi, pp.417-425.