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FORMULATION AND CHARACTERIZATION OF DOUBLE WALLED MICROSPHERES FOR TREATMENT OF AMOEBIASIS AND GIARDIASIS

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Abstract

Entamoeba histolytica infects both small intestine and colon causing giardiasis and amoebiasis respectively. Currently available nitroimidazoles like metronidazole get completely absorbed from upper part of GIT and only negligible part of metronidazole reaches to the small intestine and colon; hence metronidazole is less effective as luminicidal agent. To overcome this problem, double walled microspheres were formulated such that metronidazole should be released in small intestine and colon in effective concentration. Firstly alginate core was formulated and then it was coated with Eudragit L 100. The entrapment efficiency, size and percentage yield of both alginate core and Eudragit shell was calculated. The release kinetics were performed at four different pH conditions like pH 1.2, pH 5.8, pH 6.8 and pH 7.4 by considering GIT conditions and GIT transit time. The drug release study showed that metronidazole was released in both small intestine and colon in effective concentration.

Keywords Metronidazole, Double walled microspheres, Amoebiasis, Giardiasis.

Introduction

Amoebiasis and giardiasis are primarily infections of the upper and lower gastrointestinal tracts, respectively. These are the most common disease of small intestine and the colon which are caused by pathogenic parasite *Entamoeba histolytica*. These *Entamoeba histolytica* trophozoites are found in scrapings taken from ulcerated or inflamed tissue and are seen as large cells with ingested RBCs in H&E-stained colonic biopsies [1]. Since amoebiasis and giardiasis are the diseases of small intestine and colon, the amoebicidal drugs should reach in the small intestine and colon in desired concentrations but due to various factors like pH, dissolution, disintegration, complete absorption in upper part of GIT, hamper the drug reaching to the small intestine and colonic site. Nitroimidazoles are the drugs of choice for the treatment of amoebiasis and giardiasis but they get completely absorbed from the upper part of GIT due to which very low concentration of metronidazole reaches to small intestine and colon which renders it as ineffective in the treatment of above diseases. To achieve successful drug therapy, the drug needs to be protected from absorption and environment of upper part of GIT. Hence the drug delivery technology should be developed which will deliver drug in both small intestine and colon in effective concentration.

Traditional microsphere drug delivery systems using a single polymer have several inherent flaws such as high initial burst [2], low encapsulation efficiency for highly water soluble drugs [3], inability to lend themselves to pulsatile or zero order release and lack of sustained release for periods suitable for periodic therapy [4]. Composite double-walled microspheres adapted for the encapsulation of a highly water-soluble, have the ability to circumvent some of these limitations [4-6]. The limitation of microspheres made of a single polymer encapsulating drugs includes an initial burst caused by the release of the drug trapped on the surface during the encapsulation process and a progressively slower release rate. Therefore, microspheres made with a two-layered structure may have certain advantages over their counterparts made from single polymers [4, 7, 8].

In present study, formulated double walled microspheres consist of two layers of alginate core and Eudragit L 100 shell. Eudragit L 100 dissolves above pH 6.0 thereby protecting the drug from releasing from alginate core before reaching the colonic region. Once the enteric coating of Eudragit L 100 dissolved, the drug release was controlled by alginate core in the colonic region. Hence double walled microspheres is formulated in such a way that metronidazole is released at targeted sites

namely small intestine and colon which will help in the treatment of both amoebiasis and giardiasis.

Experimental

Materials

Metronidazole was a gift sample from Sun pharma ltd. Eudragit L 100 was supplied from Alembic limited, Vadodara. Sodium alginate, Span-80, Methanol, Dichloromethane, Calcium chloride, Isopropanol, n-Hexane were purchased from Central Drug House, New Delhi. Span-60 was purchased from Loba Chemicals Private Limited, Mumbai. All other chemicals used were of analytical grade.

Method

Preparation of double walled microspheres

The double walled microspheres were prepared by two step process. In first step the core alginate microspheres were formulated [9]. Then alginate core microspheres were coated with Eudragit L 100 by allowing evaporation solvent.

Metronidazole was dispersed in 5% sodium alginate solution. The resulting solution was emulsified for 1 hour in liquid paraffin by using 1% span 60 solution as emulsifying agent with the help of mechanical stirrer.

The 5% calcium chloride solution in isopropanol was prepared and added drop wise to the above emulsion at the rate of 2 ml/min with the help of syringe. The emulsion was stirred for 10 more minutes in order to allow the polymer to crosslink. The formed microspheres were collected and air dried for 24 hours.

The previously formulated microspheres, metronidazole and Eudagrit L 100 were dispersed in the organic phase (methanol: dichloromethane, 1:4). The resulting organic phase solution was emulsified in liquid paraffin using 1% Span 80 solution as emulsifying agent. After complete evaporation of the organic solution the double walled microspheres were collected by vacuum filtration and washed with 3-4 times with n-hexane and freeze dried for 24 hours.

Characterization

As double walled microspheres consist of two layers, it is necessary to characterize both alginate core and Eudragit L 100 shell.

Particle size analysis

The Alginate core microspheres were examined by optical microscope. The freshly prepared suspension of microspheres was examined on an optical microscope and size of the microspheres was measured by using a pre-calibrated ocular micrometer and stage micrometer. Around 100 particles of each formulation were counted and observed.

Determination of drug entrapment efficiency

The drug content was calculated as per method by Udupa, N. et al., 1998. 25 mg of dried alginate core microspheres were weighed accurately and drug was extracted from microspheres by digesting (for 36 hours) with 10 ml of phosphate buffer saline (PBS pH-7.4). During this period the suspension was agitated.

After 36 hours the suspension was centrifuged at 3000 rpm for about 3 minutes. The supernatant obtained was assayed spectrophotometrically for drug contents. The observations are shown in table 1. For double walled microspheres, 25 mg of it was dissolved in methanol, as Eudragit L 100 is soluble in methanol. The suspension was centrifuged at 3000 rpm for about 3 minutes. The supernatant obtained was assayed spectrophotometrically for drug contents. The observations are shown in table 2.

Determination of percentage yield

Formula was same for calculating alginate core and double walled microspheres. After frieze drying of microspheres, the microsphere obtained were collected and weighed accurately. The yield percent of microspheres were calculated according to formula described by Shabaraya, et al., 2003, which is given below. The yield of microspheres obtained after using different variables are given in table 1 and 2.

$$\%\ \text{yield of microspheres} = \frac{\text{Total weight of Alginate microspheres}}{\text{Total weight of } \mathbf{drug} + \text{Total weight of } \mathbf{polymer}} \times 100$$

Drug release studies

As double walled microspheres will pass through GIT, it will release drug starting from small intestine to the colon hence drug release study was performed by considering the transit time of GIT. As double walled microspheres consist of two layers so it was necessary to calculate drug release profile of drug in individual layer. Firstly release rate of metronidazole in alginate microspheres was determined by coating it with blank Eudragit L 100 layer (containing no drug) so that drug in outer layer will not interfere with release rate of alginate core microspheres. For release behavior of outer Eudragit L100 layer, the inner alginate blank core was used and coating was done with Eudragit L100 containing metronidazole hence proper release of outer layer can be observed.

In vitro drug release of metronidazole from the various microspheres was performed in different mediums like SGF (pH1.2), phosphate buffer (pH5.8), phosphate buffer (pH6.8) and PBS (pH 7.4). These studies show the effect of different fluid environment of the GIT on the drug release pattern of metronidazole from the prepared microspheres. The release pattern is shown in fig 1 and fig 2.

Results

Different formulations of each of alginate core microspheres and double walled microspheres were prepared. In case of alginate core microspheres, six formulations of drug to polymer ratios 1:1, 1:2, 1:3, 1:4, 2:1 and 3;1 were prepared. Among them, formulation with drug to polymer ratio 1:4 showed maximum percentage yield and entrapment efficiency of

76 and 82 respectively as shown in table 1.

In case of double walled microspheres, five formulations of different alginate core microspheres to Eudragit L 100 ratio 1:1, 1:2, 1:3, 1:4 and 1:5 were prepared. Among these five, formulation with core microspheres to Eudragit L 100 ratio 1:5 showed maximum percentage yield and entrapment efficiency of 73 and 81 respectively as shown in table 2.

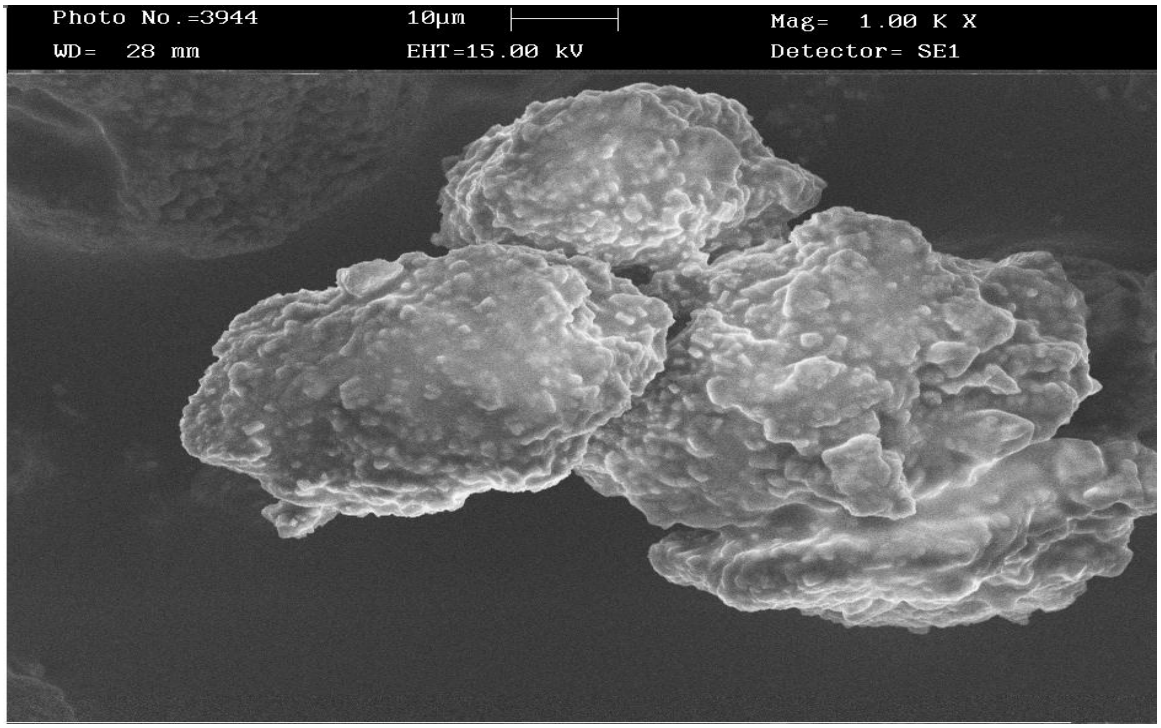
Table 1 Effect of various evaluating parameters on alginate core microspheres

Formulation code	Evaluation parameter	Percentage yield	Drug entrapment
	Drug: polymer ratio		
A_1	1:1	68.85	68.50
A_2	1:2	71.50	72.47
A_3	1:3	76.88	72.81
A_4	1:4	81.90	76.40
A_5	2:1	72.66	70.55
A_6	3:1	77.75	67.15
	Polymer:crosslinking agent ratio		
B_1	1:0.5	52.66	64.13
B_2	1:1	69.50	71.68
B_3	1:1.5	81.20	74.86
B_4	1:2	82.39	63.14
	Concentration of surfactant (%)		
C_1	0.5	70.20	70.21
C_2	1.0	74.80	74.05
C_3	1.5	77.00	75.33
C_4	2.0	81.21	79.05

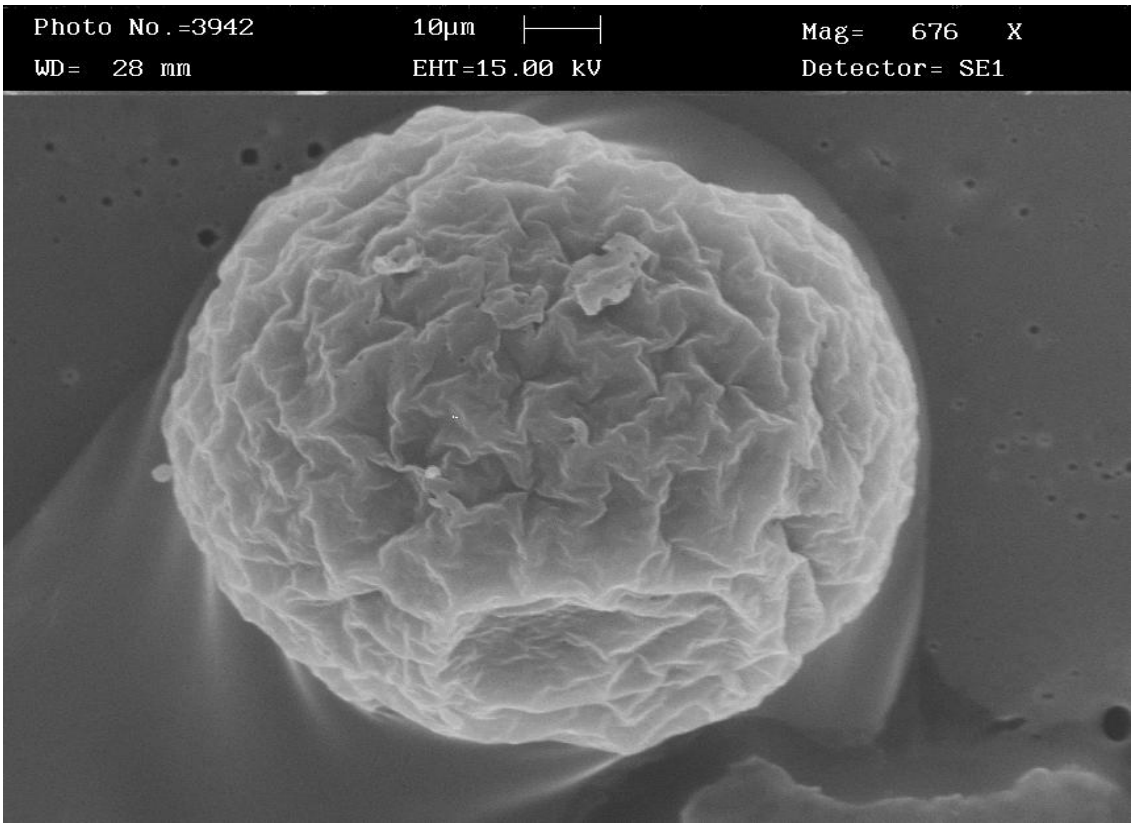
Table 2 Effect of various evaluating parameters on double walled microspheres

Formulation code	Evaluation parameter	Percentage yield	Drug entrapment
	Core microspheres:Eudragit L 100 ratio		
P_1	1:1	48.27	45.65
P_2	1:2	53.11	48.97
P_3	1:3	63.06	54.67
P_4	1:4	69.97	71.16
P_5	1:5	81.23	72.83
	Eudragit L 100:core microspheres ratio		
Q_1	1:0.5	72.32	80.13
Q_2	1:1	73.49	68.27
Q_3	1:1.5	76.44	61.09
Q_4	1:2	80.91	51.99
	Concentration of surfactant (%)		
R_1	0.5	76.45	56.81
R_2	1.0	81.13	71.86
R_3	1.5	80.14	68.12
R_4	2.0	78.09	63.01

Particle size of alginate core microspheres was observed between 22 μm to 54 μm and for double walled microspheres particle size was between 127 μm to 141 μm. The surface morphology and internal texture were determined by scanning electron microscopy as shown in photograph 1 and photograph 2.



Photograph 1 SEM micrograph of alginate core microspheres



Photograph 2 SEM micrograph of double walled microspheres

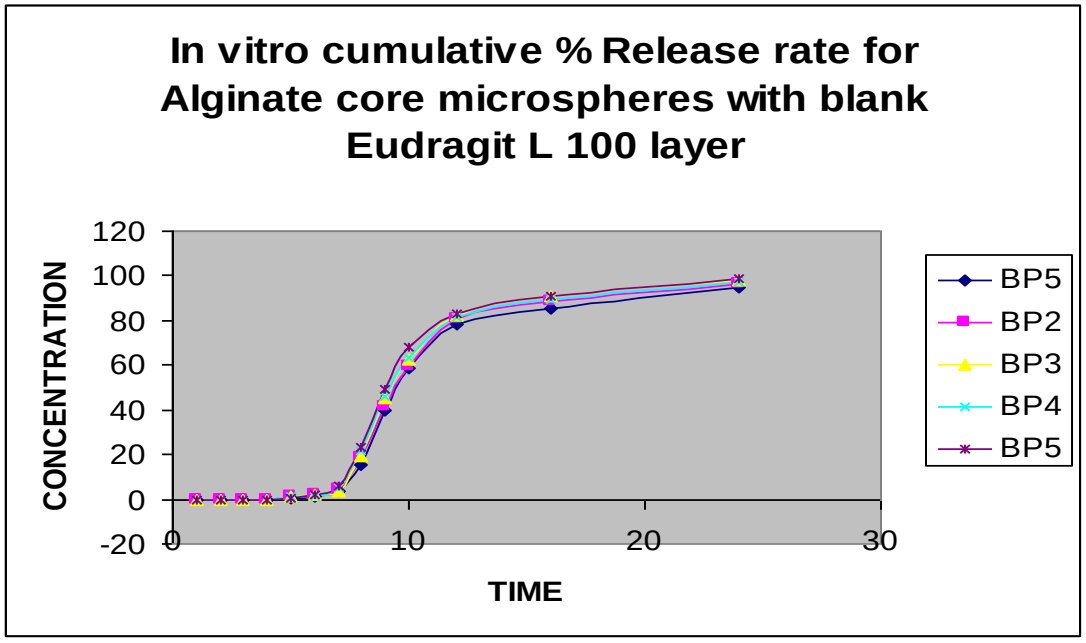


Fig. 1 *In vitro* cumulative % release rate for alginate core microspheres

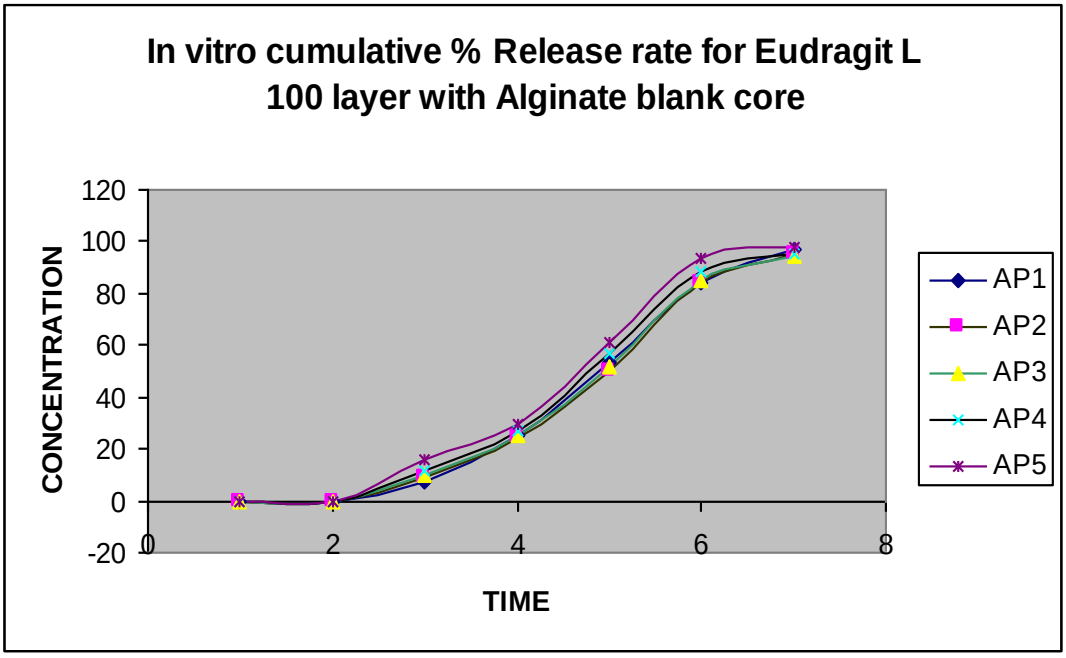


Fig 2 *In vitro* cumulative % drug release rate for outer Eudragit L 100 layer

Discussion

In drug release studies from outer Eudragit L 100 layer no drug release was observed for first 2 hours and then drug was released almost completely in 7 hours. This means the drug release from outer layer takes place in small intestine which is expected to cure giardiasis. From inner alginate layer drug release start from approximately after 5th hour in small quantity but after 7th hour drug will release in

colon as microspheres will pass intestinal transit time.

The effect of coating of Eudragit L 100 on alginate core was studied. It was observed that as the ratio of core mirospheres:Eudragit L 100 increase the release rate of drug increases. At last the drug *In vitro* drug release suggests that metronidazole will be released in both intestine and colon.

The results obtained from all experiments which were performed as a part the project work gives an idea that the developed alginate and Eudragit L 100 double walled microspheres may be successfully useful for release of drug in small intestine and colon for the treatment of amoebiasis and giardiasis.

Conclusion

The present work was the first attempt for double walled microspheres in which same drug (metronidazole) is entrapped in both outer and inner layer of double walled microspheres for delivery of metronidazole in intestine and colon.

This study suggested that alginate and Eudragit L 100 double walled microspheres will protect the drug absorption from stomach so that more quantity of metronidazole can be reached to the small intestine and colon. Also the double walled microspheres could serve as an effective carrier system for intestine and colon targeting of metronidazole and will give an effective treatment for longer period of time.

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Conflict of Interest

None

Reference

1. Wright S. Amoebiasis and Giardiasis, Medicine 2005;33(8):47-50.
2. Jalil R, Nixon JR. Microencapsulation using poly(L-lactic acid). IV. Release properties of microcapsules containing phenobarbitone, J. Microencapsul 1990; 7(1):53–66.
3. Bodmeier R, McGinity JW. The preparation and evaluation of drug containing poly (D,L-lactide) microspheres formed by the solvent evaporation, Pharm. Res. 1987;4:465–471.
4. Pekarek KJ, Jacob JS, Mathiowitz E. One-step preparation of double-walled microspheres, Adv. Mater. 1994;6:684–686.
5. Lee HK, Park JH, Kwon KC. Double-walled microparticles for single shot vaccine, J. Cont Rel 1997;44:283–293.

6. Wang FJ, Wang CH. Sustained release of etanidazole from spray dried microspheres prepared by non-halogenated solvents, J. Cont Rel 2002;81:263–280.
7. Langer R, Mathiowitz E. Preparation of multiwall polymeric microcapsules, US Patent 4861627, 1989.
8. Pekarek KJ, Jacob JS, Mathiowitz E. Double-walled polymer microspheres for controlled drug release, Nature 1994;367:258–260.
9. Rahman Z, Kohli K, Khar RK et al. Charectarization of 5- flurouracil micro-spheres for colonic delivery, AAPS Pharmscitech 2006;7(2):E113-E121.

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