

Amoxicillin Trihydrate loaded Gastroretentive Biodegradable Microspheres: Development and Characterization

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ABSTRACT

The purpose of this research was to formulate and systematically evaluate in-vitro performances of mucoadhesive amoxicillin microsphere for potential use of treating gastric and duodenal ulcers, which were associated with *Helicobacter pylori*. Amoxicillin mucoadhesive microspheres containing poly-ε-caprolactone as mucoadhesive polymer were prepared by solvent evaporation technique with different concentration of PCL polymer. The formulation factors like: drug: polymer ratio, concentration of emulsifier, aqueous: oil phase ratio, viscosity of aqueous phase, stirring speed and stirring time on particle size, drug encapsulation, drug efficiency, process yield and drug release behavior was studied. Microspheres were discrete, spherical, free flowing, high percentage drug entrapment efficiency and mucoadhesive property. Particle size was determined by optical micrometer and average particle size was found in the range of 80-122 micrometer for all batches. The optimized batch exhibited a high drug entrapment efficiency of 80 %, swelling index of 0.723 after 3 hours and percentage mucoadhesion after 3 hour was 75 %. The drug was also sustained for more than 12 hours. In conclusion, the prolonged gastrointestinal residence time and enhanced amoxicillin stability resulting from the mucoadhesive microsphere of amoxicillin must make contribution to complete eradication of *H.pylori*.

Keywords: Gastroretentive Microspheres, Solvent evaporation, Entrapment efficiency.

INTRODUCTION

Amoxicillin Trihydrate (AT) is an orally Anti Microbial drug/Anti-Ulcer drug belonging to the penicillin family. It is widely prescribed for the treatment of infections caused by certain bacteria, including: infections of the ear, nose, throat, genitourinary tract, lower respiratory tract, Genital, urethral infections in male/females and stomach or duodenal ulcer. AT undergoes hepatic metabolism accounts for less than 30% of the biotransformation of its oral dose being excreted unchanged in urine. Bioavailability of AT is 83%. Metabolized in the liver and at least six metabolites have been identified. It has an elimination half life of 1-1.5 h and has an absorption zone from the upper intestinal tract. Efficacy of the administered dose may get diminished due to short gastric retention in stomach. ^(1,2) AT requires multiple daily drug dosage in order to maintain effective antimicrobial in gastric mucous layer or epithelial cell surfaces where *H. pylori* exist. Because of this and rather high frequency of administration, it is necessary to develop mucoadhesive dosage forms to improve the absorption and systemic bioavailability. ⁽³⁾

Mucoadhesive controlled release Microspheres were developed in early 1990

and have since gained considerable attention due to their ability to adhere to the mucus layer and release the loaded drug in a sustained manner. Mucoadhesive Microspheres are advantageous pharmaceutical excipients being low cost, natural and biodegradable products with physiological, non-toxic properties ^(4,5). Microspheres prepared with mucoadhesive and biodegradable polymers undergo selective uptake by the M cells of payer patches in gastrointestinal (GI) mucosa. ⁽³⁾ Polymers need to be inert, nonabsorbable, stable, and easy to process. A requirement for any adhesive polymer is quantifiable and reproducible adhesion to tissues and/or supramucosal gels. ⁶

MATERIALS AND METHODS

MATERIALS

Amoxicillin Trihydrate (AT) was supplied as a gift by Panacea Biotec Limited, Derabassi, India. Poly-ε-caprolactone was supplied by Evonik Industries AG, Mumbai, India. Various chemicals including Polyvinylalcohol (PVA) (Mw = 42 000 daltons) and Dichloromethane (DCM), Span 60 and petroleum ether were obtained from Loba Chemical Private Limited,

Mumbai India. All other chemical reagents were of analytical grade and were used without any further purification. Distilled water was used for all of the experiments.

METHODS

Preparation of microparticles

A solution of AT (5 ml containing 120 mg) in distilled water (internal aqueous phase) was emulsified with a (360 mg) solution of PCL (10 ml) in Dichloromethane (DCM; oil phase) at high speed on a magnetic stirrer till it appeared as a clear solution with the aid of magnetic bead with approximately 10 minutes of stirring at room temperature. The resulting water-in-oil (w/o) emulsion was then emulsified at room temperature into a small volume (45 ml) of 0.5%, w/v PVA solution using 0.1% w/v span 60 emulsifier by vortex-mixing. The emulsion was poured into a larger volume (155 ml) of an ice-cooled (5°C) aqueous phase (PVA 0.5%, w/v) and stirred with a mechanical stirrer (4X4 cm) (United Electrical Industries, Varanasi, India) at a stirring rate of 1000 rpm for 3 h to allow the evaporation of the organic solvent. The hardened microspheres were separated from the aqueous phase by filtration, rinsed with 40 ml of n-hexane, the washings were checked for the absence of organic solvents spectrophotometrically (Systronics, Mumbai, India) and successive washings continued till this was achieved, and vacuum dried overnight at room temperature.⁷ In these studies the effect of the following formulation variable on the microsphere size, surface morphology, drug loading and encapsulation efficiency were investigated: All microsphere formulations were prepared in triplicate.

1. Polymer: drug ratio

This was investigated by variation in the polymer: drug ratio (1:1, 2:1, 3:1, 4:1 and 5:1, w/w).

2. Nature and concentration of emulsion stabilizer in the external aqueous phase

While maintaining a constant volume for the external aqueous phase, microspheres were produced using stabilizer span 60 at various concentration (0.05, 0.1, 0.2%, w/v)

3. Viscosity of external aqueous phase

The viscosity was modified by adding PVA in various concentrations (0.1, 0.5 and 1.0%, w/v) in the distilled water.

4. Volume of external aqueous phase

This was studied by variation in the volume of the second aqueous phase while the PVA concentration was maintained at 0.5%, w/v.

5. Stirring rate

(500, 1000 and 1500 rpm)

6. Duration of agitation during emulsification

(2, 3, 4 h).⁸

RESULT AND DISCUSSION

The solvent evaporation/extraction techniques are commonly used for the microencapsulation to extend the release of drugs. These techniques can be utilized for the encapsulation of either water insoluble or water soluble drug with hydrophobic polymers. In this study, the w/o/w emulsion solvent evaporation method has been selected to entrap both a water insoluble and a water soluble drug in microspheres. First, ATZ was dissolved in water and emulsified in a solution of Poly-ε-caprolactone. In the second step, the primary w/o emulsion was emulsified in the external aqueous phase to form a w/o/w emulsion. The organic or middle phase separated the internal water droplets from each other as well as from the external aqueous phase. After solvent evaporation, the polymer precipitated and the microspheres solidified.

Polymer: drug ratio

AT loaded microsphere were prepared using different polymer: drug ratio (from 1:1, 2:1, 3:1, 4:1, 5:1, w/w) by variation in the weight of polymer dissolved in dichloromethane to investigate the eventual modification of the particle size, drug loading, efficiency of entrapment and process yield. Increasing the weight of polymer in a fixed volume of organic solvent resulted in an increase in mean particle size (from $75.43 \pm 2.65 \mu\text{m}$ to $112.52 \pm 2.17 \mu\text{m}$ for 1:1 to 5:1, i.e. a increase of 49.33%). For 1:1 polymer: drug ratio the particles obtained were spherical in shape but with a rough surface and a mean diameter of $75.43 \pm 2.65 \mu\text{m}$, a process yield of $58.08 \pm 1.88\%$ (w/w), an encapsulation efficiency of $43.48 \pm 2.92\%$ (w/w) and a drug loading of $22.74 \pm 1.54 \%$ (w/w). Further increase in polymer: drug ratio from 2:1 to 4:1 led to the mean diameters of $86.83 \pm 1.98 \mu\text{m}$, $101.87 \pm 1.76 \mu\text{m}$ and $109.44 \pm 1.21 \mu\text{m}$, the process yields of $64.46 \pm 1.81\%$ (w/w), $85.43 \pm 1.94\%$ (w/w) and $72.53 \pm 1.48\%$ (w/w), the encapsulation efficiencies of $59.26 \pm 1.75\%$ (w/w), $76.48 \pm 1.67\%$ (w/w) and $68.62 \pm 2.05\%$ (w/w) and the drug loadings of $19.75 \pm 1.87\%$ (w/w), $18.62 \pm 2.54\%$ (w/w) and $14.98 \pm 1.89\%$ (w/w), respectively. A further increase in polymer: drug ratio, i.e., 5:1 led to production of spherical particles in aggregates with a mean diameters of $112.52 \pm 2.17 \mu\text{m}$, a

process yield of $65.67 \pm 2.87\%$ (w/w), an encapsulation efficiency of $63.87 \pm 2.22\%$ (w/w) and a drug loading of $11.48 \pm 2.73\%$

(w/w). Table I, summarizes the detailed result of effect of polymer: drug ratio. (Table 1).

Table 1: Effect of polymer: drug ratio on microspheres characteristics

Polymer: drug ratio	Mean diameter ^a (μm) \pm S.D.	Drug Loading ^a (%w/w) \pm S.D.	Entrapment efficiency ^{a,c} (% w/w) \pm S.D.	Process yield ^{a,b} (%) \pm S.D.
1:1	75.43 \pm 2.65	22.74 \pm 1.94	43.48 \pm 2.92	58.08 \pm 1.88
2:1	86.83 \pm 1.98	19.75 \pm 1.87	59.26 \pm 1.75	64.46 \pm 1.81
3:1	101.87 \pm 1.76	18.62 \pm 2.54	75.48 \pm 1.67	85.43 \pm 1.94
4:1	109.44 \pm 1.21	14.98 \pm 1.89	68.62 \pm 2.05	72.53 \pm 1.48
5:1	112.52 \pm 2.17	11.48 \pm 2.73	63.87 \pm 2.22	65.67 \pm 2.87

^a Data represent the mean of three independent experiments.

^b Percentage of weight of microparticles recovered with respect to weight of polymer utilized.

^c Percentage of encapsulated drug with respect to the total amount used

Microspheres were produced by w/o/w emulsion solvent evaporation method using polymer: drug ratio: 3:1, aqueous: oil phase ratio of 15:1, stirring speed: 1000 rpm, emulsifier concentration: 0.1%w/v and stirring time: 3 h.

Nature and concentration of emulsion stabilizer in the external aqueous phase

Amongst the stabilizer studied (tween 80, span 20, span 60), span 60 resulted in successful preparation of microspheres. Nevertheless 0.1%w/v span 60 was selected as a stabilizer of choice, since it allowed the preparation of particles in the size range of $99.52 \pm 2.42 \mu\text{m}$ with an interesting drug loading of $21.37 \pm 1.0\%$ (w/w). Except when 0.1% span 60 is used. (Table 2).

Table 2: Effect of Emulsifier concentration on microspheres characteristics

Emulsifier concentration (%w/v)	Mean diameter ^a (μm) \pm S.D.	Drug Loading ^a (%w/w) \pm S.D.	Entrapment efficiency ^{a,c} (% w/w) \pm S.D.	Process yield ^{a,b} (%) \pm S.D.
0.05	112.98 \pm 1.83	18.98 \pm 1.14	78.54 \pm 1.82	71.30 \pm 2.83
0.1	95.56 \pm 2.43	21.37 \pm 1.05	80.18 \pm 1.01	86.30 \pm 2.11
0.2	75.67 \pm 1.83	15.16 \pm 1.93	65.86 \pm 0.96	68.80 \pm 0.58

^a Data represent the mean of three independent experiments.

^b Percentage of weight of microparticles recovered with respect to weight of polymer utilized.

^c Percentage of encapsulated drug with respect to the total amount used.

Microspheres were produced by w/o/w emulsion solvent evaporation method using polymer: drug ratio: 3:1, aqueous: oil phase ratio of 15:1, stirring speed: 1000 rpm, emulsifier concentration: 0.1%w/v and stirring time: 3 h.

Aqueous: oil phase ratio

As external dispersing phase different volumes of PVA aqueous solution (150, 200, 300 ml) were employed, resulting in different ratios between aqueous external and oil internal phases (w/o ratio), namely 10:1, 15:1, 20:1. The polymer: drug ratio was 3:1. The use of the lower w/o ratio (10:1, i.e., 150 ml) led to

formation of irregular microspheres with a mean diameter of $125.91 \pm 0.67 \mu\text{m}$, a process yield of $65.08 \pm 0.85\%$ (w/w), an encapsulation efficiency of $67.72 \pm 1.12\%$ (w/w) and drug loading of $15.68 \pm 1.84\%$ (w/w). The highest w/o ratio (20:1, i.e., 300 ml) led to a aggregates of particles after isolation. Conversely particles produced by a 15:1 w/o ratio (200 ml) enabled the production of spherical microspheres with a mean diameter of $110.67 \pm 1.65 \mu\text{m}$, a process yield of $86.34 \pm 1.97\%$ (w/w), the encapsulation efficiency of $75.53 \pm 1.72\%$ (w/w) and drug loading of $19.63 \pm 0.08\%$ (w/w). (Table 3)

Table 3: Effect of aqueous: oil phase ratio on microsphere characteristics

Aqueous:oil phase ratio	Mean diameter ^a (μm) \pm S.D.	Drug Loading ^a (%w/w) \pm S.D.	Entrapment efficiency ^{a,c} (% w/w) \pm S.D.	Process yield ^{a,b} (%) \pm S.D.
10:1	125.91 \pm 1.84	15.68 \pm 1.84	63.72 \pm 1.12	65.08 \pm 0.85
15:1	110.67 \pm 1.65	19.63 \pm 0.88	78.56 \pm 1.72	86.34 \pm 1.97
20:1	85.68 \pm 2.08	17.12 \pm 1.11	64.78 \pm 2.15	68.754 \pm 2.88

^a Data represent the mean of three independent experiments.

^b Percentage of weight of microparticles recovered with respect to weight of polymer utilized.

^c Percentage of encapsulated drug with respect to the total amount used.

Microspheres were produced by w/o/w emulsion solvent evaporation method using polymer: drug ratio: 3:1, aqueous: oil phase ratio of 15:1, stirring speed: 1000 rpm, emulsifier concentration: 0.1%w/v and stirring time: 3 h.

Viscosity of the external aqueous phase

Increasing viscosity of the external phase by addition of the increasing concentration of PVA (0.1w/v) led to a slight increase in the particle size (85.58 ± 1.28 µm with 0.5% PVA to 110.47 ± 1.73 µm with 1.0% PVA without alteration on the unimodal size distribution. In fact using 3:1 polymer: drug ratio and 15:1 w/o ratio, it was found that 0.1 % and 1% PVA

concentration led to microsphere with large mean diameters of 85.58 ± 1.28 µm and 158.23 ± 0.89 µm, process yield of 63.34 ± 1.13% (w/w) and 64.57 ± 0.82 % (w/w), encapsulation efficiencies of 71.37 ± 0.65 (w/w) and 66.23 ± 0.78% (w/w) and drug loading of 15.83 ± 2.18% (w/w) and 14.19 ± 1.98% (w/w) respectively, which could be due to a reduction in the stirring efficiency. However it is in agreement with the findings of Arshady⁽⁸¹⁾ et al. The use of 0.5% PVA led to the formation of spherical particles with a mean diameter of 110.47 ± 1.73 µm, a process yield of 85.74 ± 0.48% (w/w), drug loading of 18.46 ± 1.25% (w/w) and an encapsulation efficiency of 75.78 ± 0.56% (w/w). (Table 4).

Table 4: Effect of Viscosity of the external aqueous phase on microspheres characteristics

Viscosity of Aqueous Phase (% w/v)	Mean diameter ^a (µm) ± S.D.	Drug Loading ^a (%w/w) ± S.D.	Entrapment efficiency ^{a,c} (% w/w) ± S.D.	Process yield ^{a,b} (%) ± S.D.
0.1	85.58±1.28	15.83±2.18	71.37±0.65	63.34±1.13
0.5	110.47±1.73	18.46±1.25	75.78±0.56	85.74±0.48
1.0	158.23±0.89	14.19±1.98	66.59±0.52	64.57±0.82

Microspheres were produced by w/o/w emulsion solvent evaporation method using polymer: drug ratio: 3:1, aqueous: oil phase ratio of 15:1, stirring speed: 1000 rpm, emulsifier concentration: 0.1%w/v and stirring time: 3 h and viscosity of external aqueous phase: 0.5 % w/v.

Stirring speed

Microspheres were prepared using 3:1 polymer: drug ratio, 15:1 w/o ratio and 0.5% viscosity of aqueous phase, it was found that a 500 rpm stirring speed produced particles with

rough and irregular surface. On the contrary, a triple stirring speed, namely 1500 rpm, led to the production of spherical microspheres, characterized by 80.48 ± 1.82 µm mean diameter, 62.34 ± 2.77% (w/w) process yield, drug loading 14.31 ± 1.98% (w/w) and 57.89 ± 2.13% (w/w) encapsulation efficiency. The best results in term of process yield was obtained by use of 1000 rpm stirring speed with a 95.86 ± 1.83 µm mean diameter, drug loading 17.13 ± 2.92% (w/w) and the encapsulation efficiency was 72.57 ± 1.24% (w/w)

Table 5: Effect of stirring speed on microspheres characteristics

Stirring speed (rpm)	Mean diameter ^a (µm) ± S.D.	Drug Loading ^a (%w/w) ± S.D.	Entrapment efficiency ^{a,c} (% w/w) ± S.D.	Process yield ^{a,b} (%) ± S.D.
500	110.43±1.34	15.18±1.78	66.71±1.75	65.34±1.85
1000	95.86±1.83	17.13±2.92	72.53±0.74	86.34±1.85
1500	80.45±1.82	14.31±1.98	57.87±2.13	62.34±2.77

^a Data represent the mean of three independent experiments.

^b Percentage of weight of microparticles recovered with respect to weight of polymer utilized.

^c Percentage of encapsulated drug with respect to the total amount used.

Microspheres were produced by w/o/w emulsion solvent evaporation method using polymer: drug ratio: 3:1, aqueous: oil phase ratio of 15:1, stirring speed: 1000 rpm, emulsifier concentration: 0.1%w/v and stirring time: 3 h.

Duration of agitation during emulsification

For a constant speed of 1000 rpm, a polymer: drug ratio of 3:1, a w/o ratio of 15:1 and a 0.5% viscosity of aqueous phase, an increase of the stirring time from 2 to 4 h resulted in reduction in microspheres size (from 122.18 ± 2.12 to 95.46 ± 0.65 µm). These observations could be explained by the increased shear stress generated in the emulsions associated

to the increase in the duration of agitation tending to divide the droplets of the emulsions and finally inducing a decrease in the mean particle size. A 3 h stirring time was chosen

because the entrapment efficiency was higher ($78.48 \pm 1.09\%$, w/w) than after 3 h ($65.18 \pm 1.12\%$, w/w). (Table 6).

Table 6: Effect of stirring time on microparticles characteristics

Stirring time (h)	Mean diameter ^a (μm) \pm S.D.	Drug Loading ^a (%w/w) \pm S.D.	Entrapment efficiency ^{a,c} (% w/w) \pm S.D.	Process yield ^{a,b} (%) \pm S.D.
2	122.18 \pm 2.12	17.37 \pm 2.14	68.47 \pm 0.87	64.37 \pm 1.09
3	104.85 \pm 1.24	19.37 \pm 1.92	78.48 \pm 1.09	86.37 \pm 1.29
4	95.46 \pm 0.65	16.79 \pm 2.11	65.18 \pm 1.12	62.48 \pm 1.82

^a Data represent the mean of three independent experiments.

^b Percentage of weight of microparticles recovered with respect to weight of polymer utilized.

^c Percentage of encapsulated drug with respect to the total amount used.

Microspheres were produced by w/o/w emulsion solvent evaporation method using polymer: drug ratio: 3:1, aqueous: oil phase ratio of 15:1, stirring speed: 1000 rpm, emulsifier concentration: 0.1%w/v and stirring time: 3 h.

At last the "standard conditions" for microspheres production by w/o/w emulsion solvent evaporation technique have been assessed: (a) a polymer: drug ratio of 3:1 (w/w), (b) a dispersing phase constituted of 200 ml of PVA aqueous solution (w/o ratio, 15:1), (c) a viscosity of 0.5% w/v of aqueous phase, (d) a stirring speed of 1000 rpm, (e) a stirring time of 3 h. In these conditions the obtained microspheres were characterized by spherical shape, absence of aggregates, a mean diameter of $101.75 \pm 0.82 \mu\text{m}$, a process yield of $87.65 \pm 1.89\%$ (w/w), drug loading of $18.72 \pm 2.05\%$ (w/w) and an encapsulation efficiency of $74.879 \pm 1.23\%$ (w/w).

Particle size analysis

The particle size and size distribution of the prepared microspheres were measured by laser diffraction in a particle size analyzer (Mastersizer, Malvern Instruments, UK). The

dried powder samples were suspended in deionised water and sonicated for 1 min with an ultra-sound probe before measurement. The obtained homogeneous suspension was determined for the equivalent volume diameter and triplicate measurements were made for each batch of microspheres.

Drug- Polymer Compatibility Infra Red Study

IR study was carried out to check the compatibility between the selected polymer PCL and Amoxicillin Trihydrate. This study was performed to assure that there is complete physical entrapment of the drug into the polymer without any mutual interaction. All the spectra were compared for shifting of major functional peaks and also for the loss of functional peaks, if any. When the spectra were compared it was found out that there was no shifting of functional peaks and no overlapping of characteristic peaks and also there was no appearance of new peaks. No significant change in the IR spectra of Amoxicillin Trihydrate-PCL complexes was observed, except for the broadening of the peaks.

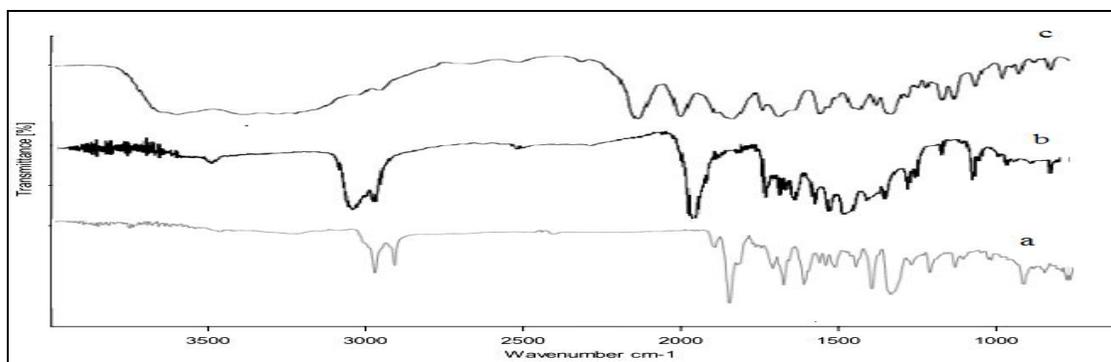


Fig. 1: Overlay of IR spectra ,a) Amoxicillin trihydrate and PCL physical mixture, b) PCL, c) Amoxicillin trihydrate

XRD Study

In order to investigate the physical nature of the encapsulated drug, the powder X-ray diffraction technique was used. Diffraction patterns amoxicillin trihydrate, physical mixture of drug and polymer and drug loaded microsphere formulation were studied. The powder XRD patterns of pure amoxicillin trihydrate 2θ values appeared at $12.20^\circ/15.15^\circ/16.26^\circ/18.1^\circ/19.39^\circ/26.6^\circ/28.76^\circ$

$^\circ$. All these peaks of amoxicillin trihydrate were detectable in both physical mixtures and microsphere formulation, indicating the crystalline state of amoxicillin trihydrate within the samples although the peak intensity of Amoxicillin trihydrate slightly reduced it indicates that the crystallinity of amoxicillin trihydrate slowly reduced in formulation.

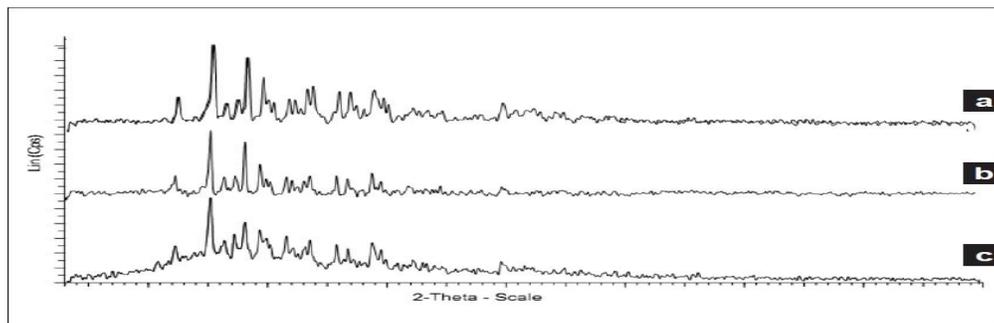


Fig. 2: XRD pattern of pure amoxicillin trihydrate (a), physical mixture (b), and amoxicillin trihydrate-loaded microsphere (c)

In vitro release studies

In vitro dissolution studies were carried out on the microspheres at $37^\circ\text{C} (\pm 0.5^\circ\text{C})$ at 100 rpm with USP XXIV basket apparatus (Lab India, DS 8000, Mumbai, India). An accurately weighed sample of microsphere was suspended in the dissolution media consisting of 900 ml of 0.1 N (pH 1.2) hydrochloric acid without enzymes. Five milliliters of sample solution was withdrawn at predetermined time intervals, filtered through a $0.45 \mu\text{m}$ membrane filter, diluted suitably, and analyzed spectrophotometrically. An equal amount of fresh dissolution medium was replaced

immediately after withdrawal of the test sample. Percentage drug dissolved at different time intervals was calculated using the Lambert-Beer's equation. The dissolution was continued until the microspheres were depleted of drug or for 24 h. Aliquots of dissolution fluid were withdrawn at specified time intervals to assay the released drug spectrophotometrically at 228 nm in both stages of dissolution. Each graphical data point was an average of dissolution data from three samples. Corrections were made for the removal of samples (Figure 2).

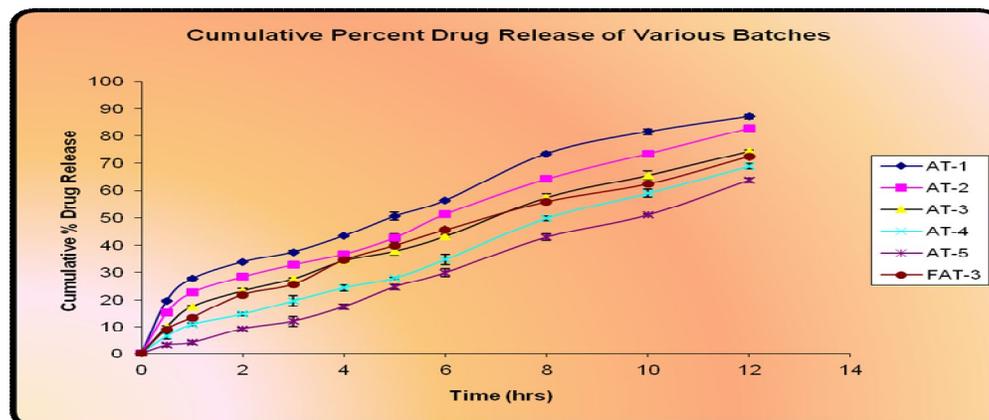


Fig. 2: Release profile of AT from Poly-e-caprolactone microspheres

Drug release pattern from microparticles

In order to investigate the release mechanism of present drug delivery system, the release data of microparticles were fitted to classic drug-release kinetics models. The release rates were analysed by zero-order model, first order model, Higuchi model and Korsmeier

Peppas model, which have been suggested to describe drug-release kinetics from microspheres. Higuchi plot and Peppas plot of final optimized batch of Poly-e-caprolactone microspheres are given in Figure 3a and 3b respectively.

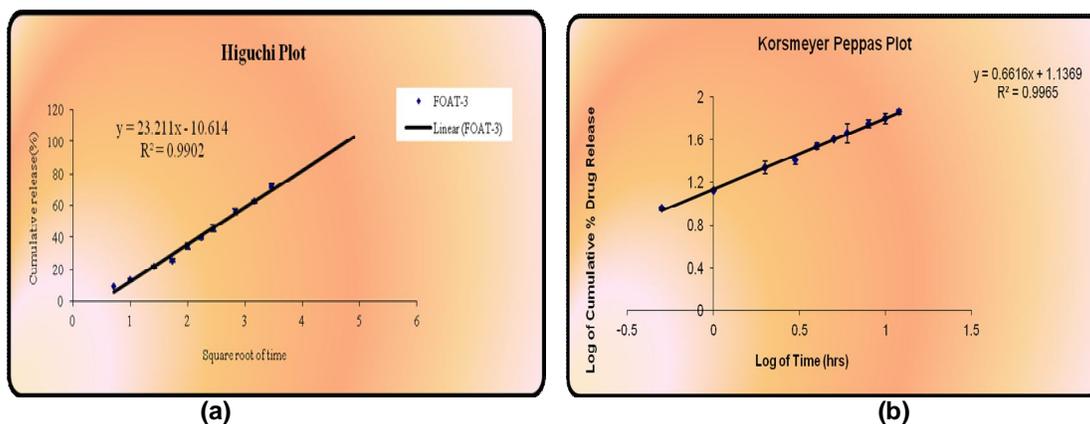


Fig. 3 (a) Higuchi plot (b) Peppas plot of final optimized batch of Poly-e-caprolactone microspheres

Kinetics of drug release

In order to investigate the release mechanism of present drug delivery system, the data obtained from in vitro release of final optimized batch were fitted into equations for the zero-order, first-order, and Higuchi release model and Peppas equation. The interpretation of data was based on the values of the resulting regression coefficients. The in vitro drug release showed the regression coefficient values for Higuchi's model (Figure 3a) ($R^2 = 0.9902$) and Peppas model ($R^2 = 0.9965$) and a value of $n = 0.661$ (Figure 3b) indicating non fickian transport.

Statistical analysis

Statistical data analyses were performed using the Student's t-test and one-way analysis of variance (ANOVA) with $p < 0.05$ as the minimal level of significance.

Swelling Study

Swelling ratio was in the range of 0.2904 to 0.733 for all formulations which indicates those polymers used in concentration are having better capability of swelling. It is observed that as the concentration of polymer has been increased in ratio from 1:3 to 1:5, the percentage swelling was decreased, which may be due to decrease in pore size leading to less penetration of water into the pores and hence reduced swelling.

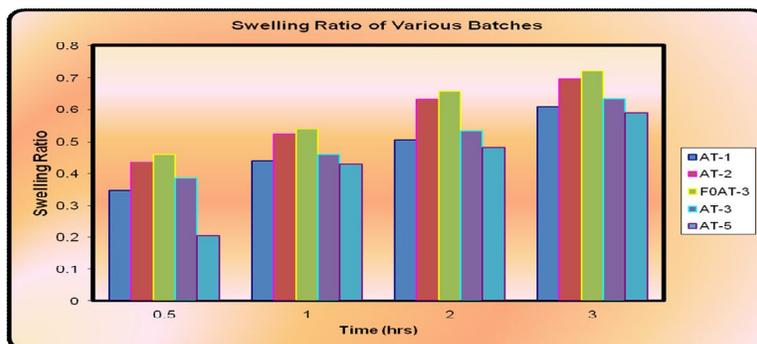


Fig. 4.21: Swelling Index of different batch

Mucoadhesive Study

Microspheres of PCL loaded with Amoxicillin trihydrate exhibited good mucoadhesive properties in the *in vitro* wash off test. At the end of 4hr 71% of the initially placed microspheres were adhered of the optimized batch. The *in-vitro* wash-off test for % mucoadhesion of microspheres increased from 69 to 94 and 47 to 69 at lower and higher levels of drug-to-polymer ratio, 1:1 to 1:5 respectively. As the drug -to-polymer ratio increases, the % mucoadhesion also

increases; because more amounts of polymer results in higher amount of free – COOH groups 17, which are responsible for binding with sialic acid groups in mucus membrane and thus results in increase in mucoadhesive properties of microspheres. *In-vitro* mucoadhesive test showed that amoxicillin mucoadhesive microspheres adhered more strongly to gastric mucous layer and could retain in gastrointestinal tract for an extended period of time.

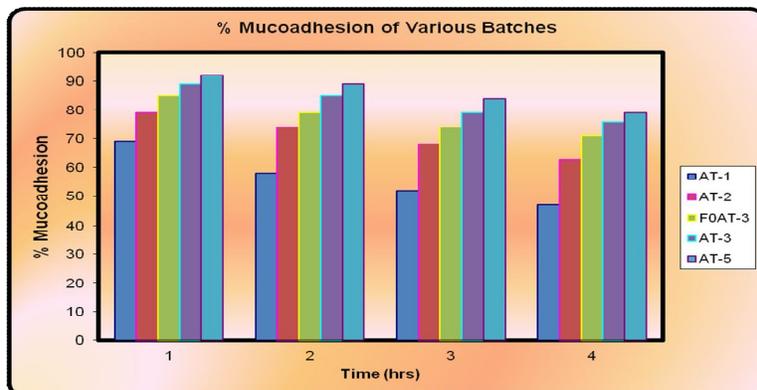


Fig. 4.23: % mucoadhesion of amoxicillin loaded PCL mucoadhesive microspheres after 1, 2, 3 and 4 hrs

Scanning electron microscopic analysis

The prepared microparticles were coated with gold palladium under an air atmosphere for 150 seconds to achieve a 20 nm film (Coater Polaron, 18mA current at 1.4 kV). The coated

sample were then examined using scanning electron microscope (Philips 505, Philips, Holand). The morphology and appearance of microparticles were examined by scanning electron microscopy as shown in figure 4.

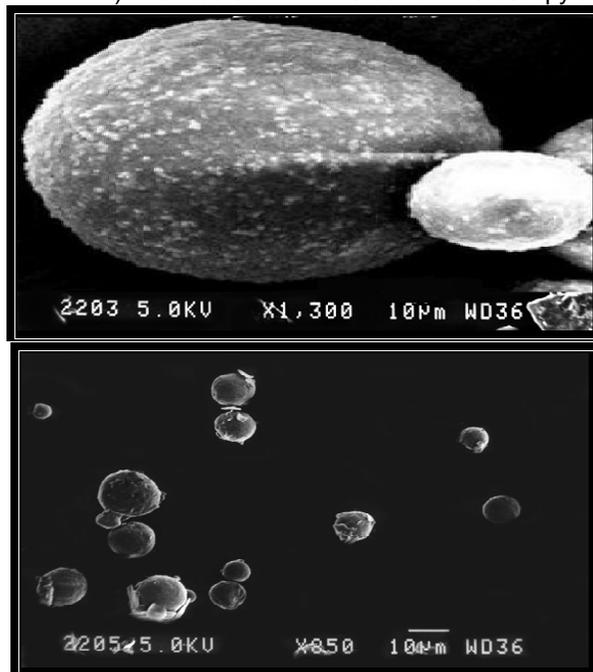


Fig. 4: SEM photographs of final optimized batch of Poly-e-caprolactone microspheres

The spherical shape of microparticles was established by SEM. The surface analysis of empty and of drug loaded microparticles prepared by the w/o/w emulsion solvent evaporation method revealed that the microparticles were spherical and polydispersed with a diameter of $101.75 \pm 0.82 \mu\text{m}$. The surface of these microparticles was found to be smooth with quite a few pock marks. This probably happens due to slow release of DCM during the terminal stage of the evaporation process.

CONCLUSION

AT mucoadhesive microspheres containing Poly-e-caprolactone as mucoadhesive polymer were prepared by W/O/W emulsion solvent evaporation method. These investigations have also provided an understanding of the effects of some process parameters on particle size and shape, and encapsulation efficiency, drug loading and process yield. The investigated system has the potential to remain in treated site for a prolonged periods and capable of maintaining constant concentration of drug through a longer duration of time due to its sustained action. The results of this study indicate that it may be feasible to use PCL mucoadhesive microspheres as a gastric retentive drug delivery system for treatment of Gastric ulcer. The release rate of the antimicrobial agents will be retarded due to the slower dissolution rate of the PCL and mucoadhesion of AT loaded dosage forms, which ultimately improve patient compliance. w/o/w emulsion solvent evaporation method were suitable for the preparation of microspheres in the size range of $101.75 \pm 0.82 \mu\text{m}$, the encapsulation efficiency was $74.879 \pm 1.23\%$ (w/w), drug loading was $18.72 \pm 2.05\%$ (w/w), the process yield was $87.65 \pm 1.89\%$ (w/w) and % mucoadhesion of 80% after 1 hr. Diffusion was found to be non fickian diffusion. It concluded that mucoadhesive microspheres of amoxicillin could sustain the release of the drug for more than 10 hrs and microspheres were successfully prepared by using w/o/w emulsion solvent evaporation method with the selection of appropriate experimental condition. The prepared microspheres proved to be good candidate for site-specific drug release.

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