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Development, characterization and evaluation of mucoadhesive microspheres of amoxicillin trihydrate

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Abstract

Mucoadhesive microspheres include microparticles and microcapsules (having a core of the drug) of 1-1000µm in diameter and consisting either entirely of a mucoadhesive polymer or having an outer coating of it, respectively. Amoxicillin microspheres were formulated by using solvent evaporation technique. Using Eudragit RS 100 as matrix polymer. Evaluation of the Prepared Mucoadhesive Microspheres done for % yield, Particle size analysis, Particle size distribution Angle of repose, Determination of drug content, Shape and surface characterization, drug entrapment and finally cumulative drug release from microspheres.

Keywords: Mucoadhesive microspheres, Amoxicillin Trihydrate, Eudragit, Angle of repose, characterizationKeywords:

Introduction

It is the most extensively used method of micro encapsulation .[1]Mucoadhesive microspheres include microparticles and microcapsules (having a core of the drug) of 1-1000µm in diameter and consisting either entirely of a mucoadhesive polymer or having an outer coating of it, respectively.[2] Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery; but coupling of mucoadhesive properties to microspheres has additional advantages. Mucoadhesive and biodegradable polymers undergo selective uptake by the M cells of payer patches in gastrointestinal (GI) mucosa [3] this uptake mechanism has been used for the delivery of protein and peptide drugs, antigens.

Material and methods

Amoxicillin Trihydrate and Eudragit RS 100 from Ranbaxy Pvt. Ltd Guargoan, Hydroxy propyl methyl cellulose (K4M) form Orchid Lab, Chennai, and Span 80 from Loba Chemicals. Pvt. Ltd. Mumbai, Light Liquid Paraffin, Acetone AR, Concentrated HCl LR, Potassium dihydrogen phosphate AR, Sodium Hydroxide LR from Nice Chemicals Pvt. Ltd. Cochin, Whatman filter paper from Research labs fine chemical Mumbai, n-Hexane from Cheaper, Chennai.

Instruments: Mechanical Stirrer(Elektrocraft Pvt. Ltd., Mumbai), UV/VIS spectrophotometer(Elico SL-164, India), Single pan Digital balance(Shimadzu), Hot Air Oven(Genuine Lab), Magnetic stirrer(Remi Stirrer), Optical Microscope(Acculab), Digital pH meter(Hanna Instrument), FTIR spectrophotometer(Shimadzu), Scanning Electron Microscope(ZEOL JSM -5610).

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Compatibility studies Fourier Transform Infrared Spectroscopy (FTIR): One of the requirement for the selection of suitable excipients or carrier for pharmaceutical formulation is its compatibility. Therefore in the present work a study was carried out using FTIR using SHIMADZU-FTIR 410 model. The pellets were prepared at high compaction pressure by using KBr and the ratio of sample to KBr is 1: 1000. The pellets prepared were examined and the spectra of drug and other ingredients in the formulation were compared with that of original spectra.
Differential Scanning Calorimetry (DSC): The DSC analysis was carried out to identify the compatibility between the drug and excipients. The DSC analysis of pure drug, 1:1 physical mixture of drug excipient were carried out using mettler Toledo DSC 821, Switzerland. Samples (2-8 mg) were accurately weighed and heated in sealed aluminium pans at a rate of 10^{o}/ min between 0-300 ^{O}C temp ranges under nitrogen atmosphere.
Preparation of Mucoadhesive Microspheres by Solvent Evaporation Technique: Amoxicillin microspheres were formulated by using solvent evaporation technique. Using Eudragit RS 100 as matrix polymer. Eudragit was dissolved in 12 ml of acetone. The mucoadhesive polymer (Hydroxypropyl methylcellulose K_4M) and drug was dispersed with the polymer solution (Mucoadhesive polymer and drug previously passed in sieve no 240). The dispersed content was placed drop wise in 100 ml light liquid paraffin containing span80 maintained at 40^{o}C while stirring at 750 ±50 rpm. The solvent, acetone was then removed by continuous stirring at room temperature for three hours to produce spherical microspheres. The micro sphere were than separated from liquid paraffin by filtration through Whatmann filter paper No-44, the microspheres were collected and washed three times with n-hexane and dried by using vacuum filtration. The product was air-dried to obtain microspheres.

Evaluation of the Prepared Mucoadhesive Microspheres

Determination of % yield of microspheres[4] Thoroughly dried microspheres were collected and weighed accurately. The percentage yield was than calculated using formula given

$$\% \text{ yield} = \frac{\text{Mass of micro spheres obtained}}{\text{Total weight of drug and polymer}} \times 100$$

Particle size analysis[5]

Particle size distribution : Particle size determination was done by sieving method. Size distribution plays an important role in determining the release characteristics of the microspheres.

Angle of repose[6] Angle of repose was determined by using funnel method; the accurately weighed spheres were taken in funnel. The height of funnel was adjusted in such as way that the tip of funnel just touches the apex of heap of blends. The blends were allowed to flow through funnel freely on to surface. The diameter of powder cone was measured; angle of repose was calculated by using following equation. Tan $\theta = h/r$, Where h – height of pile, θ – angle of repose
r – Radius of base.

Determination of drug content: Accurately weighed 100 mg microspheres, were crushed in glass mortar and pestle and powder microspheres were suspended in 100 ml of 0.1N HCl. After 12 hours the solution was filtered and the filtrate was analyzed for the drug content using UV-Visible spectrophotometer.

Encapsulation efficiency: Encapsulation efficiency was calculated using the following formula

$$\text{Encapsulation efficiency} = \frac{\text{Estimated drug content}}{\text{Theoretical drug content}} \times 100$$

Swelling studies: A known weight (50 mg) of microspheres was placed in a glass vial containing 10 ml of distilled water at 37 ± 0.5^{0}C in incubator with occasional shaking. The microspheres were periodically removed, blotted with filter paper and their changes in weights were measured during the swelling until equilibrium was attained. Finally, the weight of the swollen microspheres was recorded after a period of 3 hours, and the swelling ratio (SR) was then calculated from the formula. The studies were carried out in triplicate.

$$\text{Swelling Ratio (SR)} = \frac{We - W_O}{W_O}$$

Where, Wo = Initial weight of the dry microspheres, We = weight of the swollen microspheres at equilibrium swelling in the media.

ISSN 0976-7126

In vitro wash-off test[7] The mucoadhesive property of microspheres evaluated by an *In vitro* adhesion testing method known as wash-off method. Freshly excised piece of intestinal mucosa (2 x 2 cm) from goat were mounted on to glass slides (3 x 1 inch) with cyanoacrylate glue. Two glass slides were connected with a suitable support, about 100 microspheres were spread on to each wet rinsed tissue specimen and immediately thereafter the support was hung on to the arm of a USP tablets disintegrating test machine. When the disintegrating test machine was operated, the tissue specimen was given slow, regular up-and-down moment in the test fluid (900 ml of 01N HCL) at 37± 0.5^0C. At the end of 30 min, at the end of one hour, and at the hourly intervals up to 5 hours, the machine was stopped and number of microspheres still adhering to tissue was calculated. The studies were carried out in triplicate.

In vitro dissolution studies[6] Dissolution studies were carried out for all the formulation, employing USP XXIII apparatus (Basket method) at 37± 0.5^0C rotated at constant speed of 50 rpm using 0.1N HCL as the dissolution medium. A sample of microspheres equivalent to 100 mg of amoxicillin trihydrate was used in each test. An aliquot of the sample was periodically with drawn at suitable time interval and the volumes were replaced with fresh dissolution medium in order to maintain the sink condition. The sample was analyzed spectrophotometrically at 272 nm.

Kinetics of drug release[8] In order to understand the mechanism and kinetic of drug release, the drug release data of the *in-vitro* dissolution study were analyzed with various kinetic model like zero order, first order, Higuchi's Papa's and Coefficient of correlation (r) values were calculated for the liner curves by regression analysis of the above plots.

Shape and surface characterization The shape and surface characterization of microspheres were observed under a Scanning Electron Microscope (SEM). The instrument used for this study was ZEOL JSM – 5610 scanning electron microscope. The microspheres were mounted directly on the SEM sample stub, using double-sided sticking tape, and coated with gold film (thickness 200 nm) under reduced pressure (0.001 torr) and photographed.

Results and discussion

Pre formulation studies for drug and carrier interaction .Angle of repose: The angle of repose is in between 22 to 25^o revealed that the microspheres of all the batches had good flow characteristics and flow rates. **Fourier Transform Infrared Spectroscopy (FTIR):** The identification of drug was done by IR spectroscopy. The IR spectrum of pure drug Amoxicillin trihydrate is shown below which was concordant with the reference spectrum of Amoxicillin trihydrate.150 when the wave number of Amoxicillin trihydrate was compared with the standard peaks, the peaks were similar and by this the purity of drug can be confirmed. The FTIR spectral analysis showed that there was no appearance or disappearance of any characteristic peaks of pure drug amoxicillin trihydrate in the physical mixture of drug and polymer, which confirms the absence of chemical interaction between drug and polymers. The DSC spectral analysis also reveals the same. **Differential Scanning Calorimetry (DSC):** The DSC analysis was carried out to identify the compatibility between the drug and excipients. The DSC analysis of pure drug, 1:1 physical mixture of drug excipient were carried out using mettler Toledo DSC 821, Switzerland. Samples (2-8 mg) were accurately weighed and heated in sealed aluminium pans at a rate of 10^o/ min between 0-300 ^{O} C temp ranges under nitrogen atmosphere. The thermogram of pure drug showed endothermic peak at - 124.180 ^{O} C.The physical mixture of drug and excepient shows peak at 126.517 ^{O} C. **Particle size distribution:** The microsphere prepared by this method was found to be discreet, spherical, free flowing and it was observed by scanning electron microscopy (SEM) **In vitro dissolution studies:** The percentage yield of microspheres of all formulation was in the range of 78.90% to 90.95%. **Shape and surface characterization** The XRD analysis of final formulation shows that the peak intensity of Amoxicillin Trihydrate slightly reduced it indicates that the crystallinity of Amoxicillin Trihydrate slowly reduced in formulation.

In the present work effort have been made to design and evaluate Mucoadhesive microspheres of Amoxicillin trihydrate and the results obtained in the study have been summarized below. A 2^3 full factorial design was performed to study the effect of formulation variables (concentration of polymers) on the release properties by applying optimization technique. The polymer concentration is a major factor affecting the release and mucoadhesion strength of the prepared microspheres. The observed response ($t_{90\%}$) is close agreement with the

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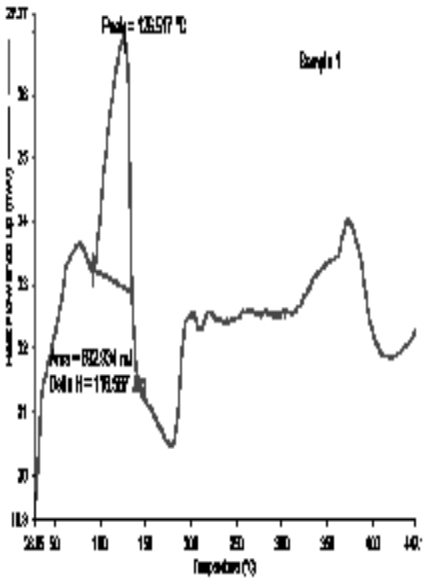
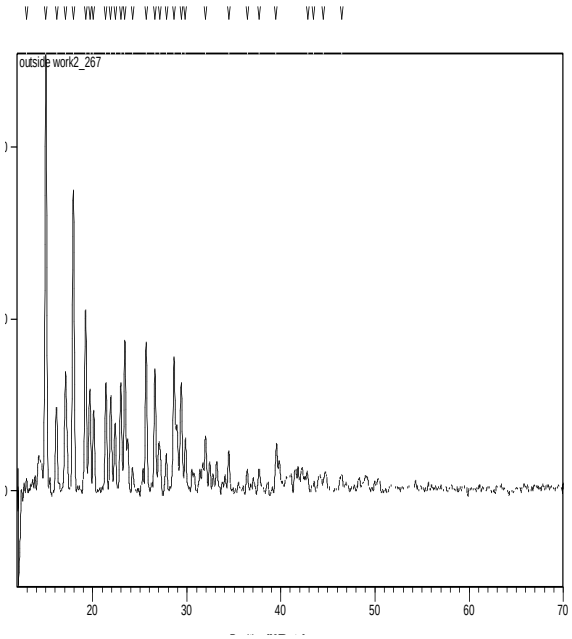
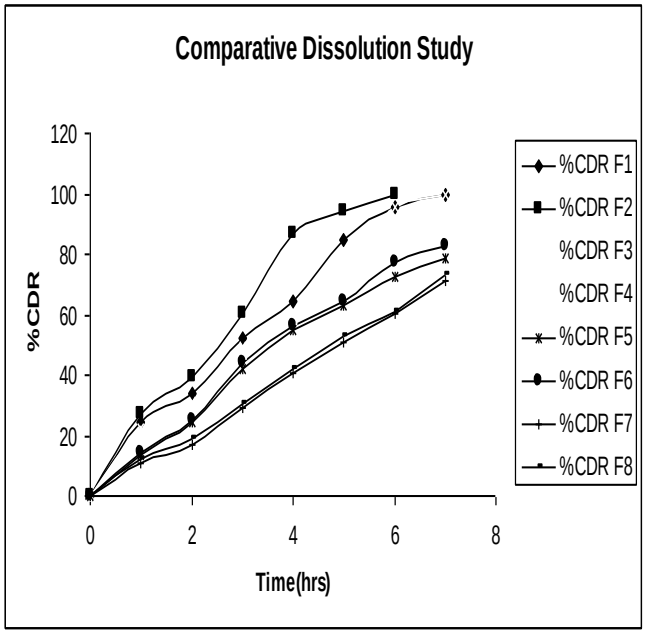
predicted $t_{90\%}$ value there by demonstrating the feasibility of the optimization procedure in developing mucoadhesive microspheres containing Amoxicillin trihydrate.



Graph: 1



Graph 2:XRD pattern of pure AM



Graph: 3

Formulation Code	*Percentage drug content (% ± SD)
F1	82.22 ± 0.99
F2	76.04 ± 1.08
F3	77.03 ± 1.09
F4	79.56 ± 0.47
F5	81.10 ± 1.00
F6	84.15 ± 0.61
F7	72.03 ± 1.55
F8	81.46 ± 1.11

Table 1: Drug Content of Mucoadhesive Microspheres of Amoxicillin Trihydrate

ISSN 0976-7126

Table 2: Data for Percent Entrapment Efficiency of Formulation of Mucoadhesive Microspheres of Amoxicillin Trihydrate

Formulation Code	Theoretical drug content in%	Practical drug content in%	Entrapment Efficiency in %
F1	24.73	22.05	78.22
F2	54.74	41.08	78.04
F3	20.32	16.06	80.03
F4	43.35	34.06	79.56
F5	18.24	14.61	80.10
F6	40.10	32.94	82.15
F7	14.45	10.40	70.03
F8	40.06	32.63	81.46

Formulation Code	*Angle of repose $\theta=\tan^{-1}(h/r)$ Mean± SD
F1	$22^{O}\ 15' \pm 0.558$
F2	$25^{5O}\ 39' \pm 0.517$
F3	$23^{O}\ 23' \pm 0.901$
F4	$27^{O}\ 51' \pm 0.928$
F5	$22^{O}\ 56' \pm 0.193$
F6	$24^{O}\ 24' \pm 1.058$
F7	$26^{O}\ 27' \pm 0.935$
F8	$28^{O}\ 10' \pm 0.155$

Table 3: Data for Angle of repose of formulation of mucoadhesive microspheres of amoxicillin trihydrate

Fig. 1: Individual Microsphere

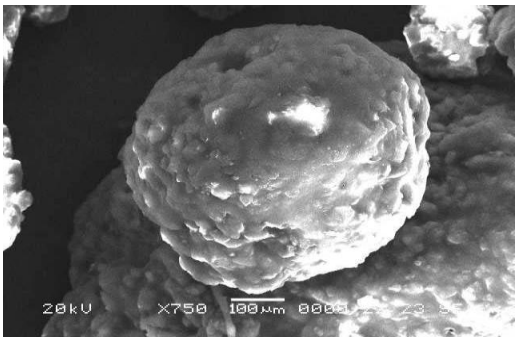


Fig. 2: Surface View of Microsphere

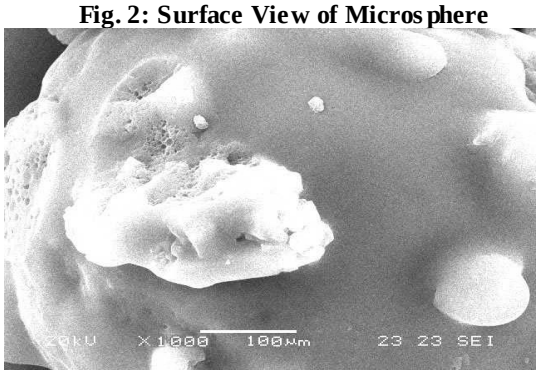


Fig. 3: Groups of Microspheres

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