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ORIGINAL ARTICLE

Preparation of *Sterculia foetida*-pullulan-Based Semi-interpenetrating Polymer Network Gastroretentive Microspheres of Amoxicillin Trihydrate and Optimization by Response Surface Methodology

Sterculia foetida-pullulan Esaslı Yarı İç İçe Geçmeli Polimer Ağı Gastroretentif Amoksisilin Trihidrat Mikrosferlerinin Hazırlanması ve Yanıt Yüzey Metodolojisi ile Optimizasyonu

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ABSTRACT

Objectives: In this study, a novel *Sterculia foetida* and pullulan-based semi-interpenetrating polymer network gastroretentive microsphere formulation was prepared using the emulsion crosslinking method and optimized by central composite design.

Materials and Methods: The effects of the amounts of glutaraldehyde, *S. foetida*, and pullulan on the percent drug entrapment efficiency (EE), percent mucoadhesion at 12 h, and percent *in vitro* drug release at 12 h were optimized. The microspheres were characterized using scanning electron microscopy, fourier transform infrared spectroscopy, and differential scanning calorimetry.

Results: The formulation containing 4% v/v glutaraldehyde, 8.28% w/v pullulan, and 2.14% w/v *S. foetida* had 88.75±1.18% EE, 80.43±1.2% drug release at 12 h, and 81.73±1.50% mucoadhesion at 12 h, which was considered optimum and was used in an *in vivo* radiographic study.

Conclusion: Semi-interpenetrating polymer network microspheres loaded with amoxicillin trihydrate were successfully prepared using *S. foetida* and pullulan. The prolonged retention of microspheres in the stomach with sustained drug release could effectively act against *Helicobacter pylori* reservoirs in the stomach and improve the therapeutic effect of amoxicillin trihydrate against *H. pylori*.

Key words: *Sterculia foetida*, pullulan, semi-interpenetrating polymeric network, central composite design, *in vivo* radiographic study

ÖZ

Amaç: Bu araştırmada, yeni bir *Sterculia foetida* ve pullulan bazlı yarı iç polimer polimer ağ gastroretentif mikrosfer formülasyonu, emülsiyon çapraz bağlama yöntemiyle hazırlanmış ve merkezi kompozit tasarım ile optimize edilmiştir.

Gereç ve Yöntemler: Glutaraldehit, *S. foetida* ve pullulan miktarlarının ilaç tutma etkinliği yüzdesi (DEE), 12 saatte yüzde mukoadezyon ve 12 saatte *in vitro* ilaç salım yüzdesi üzerindeki etkileri optimize edilmiştir. Mikro küreler ayrıca taramalı elektron mikroskobu, fourier dönüşümlü kızılötesi spektroskopisi ve diferansiyel tarama kalorimetrisi ile de tanımlanmıştır.

Bulgular: %4 h/h gluteraldehit, %8,28 a/h pullulan ve %2,14 a/h *S. foetida* içeren formülasyon, %88,75±1,18 EE sahip olarak bulunmuştur. On iki saat içinde %80,43±1,2 ilaç salımı ve 12 saat içinde %81,73±1,50 mukoza yapışması vermiştir ki bu optimum olarak kabul edilmiş ve *in vivo* radyografik çalışma için kullanılmıştır.

Sonuç: Çalışmadan amoksisilin trihidrat yüklü yüklü iç içe geçen polimer ağ mikrosferlerinin *S. foetida* ve pullulan zamkı kullanılarak başarıyla hazırlandığı sonucuna varılmıştır. Mide içerisinde sürekli ilaç salımı ile mikrokürelerin uzun süre tutulması, midedeki *Helicobacter pylori* rezervuarına etkili bir şekilde davranabilir ve amoksisilin trihidratın *H. pylori*'ye karşı terapötik etkinliğini artırabilir.

Anahtar kelimeler: *Sterculia foetida*, pullulan, yarı iç içe geçen polimerik ağ, merkezi kompozit tasarım, *in vivo* radyografik inceleme

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INTRODUCTION

Several disease conditions necessitate the development of controlled and targeted drug delivery systems. Pharmaceutical technology is continuously expanding to provide sustainable, continuous, and therapy-optimal drug levels in the circulation. Drugs whose absorption site is the upper gastrointestinal tract or those that have stability issues can benefit when formulated as mucoadhesive microspheres.[1] Mucoadhesive microspheres can adhere in a specific absorptive part of the gastrointestinal region, thereby improving the drug absorption rate and enhancing bioavailability. Additionally, the microsphere size allows them to remain entrapped in the stomach mucosal lining for an extended duration.

Due to their gastroretention, mucoadhesive microspheres may build up a high drug concentration in the stomach mucosa, which may help eradicate peptic ulcers caused by *Helicobacter pylori* infection.[2] Warren and Marshall indicated that *H. pylori* infection predominantly affects the gastrointestinal tract, causing stomach inflammation, duodenal ulcers, gastric ulcers, and gastric carcinoma. It is reported that *H. pylori* is responsible for 90-100% of duodenal ulcers and 60-90% of gastric ulcers in patients. A literature survey showed that *H. pylori* infection increases the likelihood of developing peptic ulcers, and 10-15% of individuals with *H. pylori* infection have high chances of developing peptic ulcers in their lifetime.[3]

Amoxicillin trihydrate is a semisynthetic antibiotic widely considered effective in the treatment of peptic ulcers caused by *H. pylori*. It is a major component of standard therapy and is used in combination with other antibiotics and antacids. Clinically, it is effective, but drug-related problems and increased antibiotic resistance have decreased the success rate of eradicating *H. pylori* with conventional therapies. Amoxicillin, an acid-soluble drug, dissolves immediately in the stomach and is rapidly absorbed; therefore, it is difficult to retain it in the stomach for long durations unless some rate-controlling means are employed to counter *H. pylori*. Once *H. pylori* is colonized in an acidic stomach environment, it penetrates the stomach's deeper mucus membrane, which is a bacterium niche, owing to its good flagellar motility. Furthermore, *H. pylori* can attach to lipids and carbohydrates present in the membrane, helping it adhere to the epithelial cells. Several studies and reviews recommended that local drug delivery works more effectively than systemically administered antibiotics to eradicate *H. pylori*. Therefore, for enhanced therapeutic efficacy, the formulation should release the drug near the stomach lining. Mucoadhesive microspheres can adhere to the gastric mucosa and release the drug constantly for longer periods to the infected cells. Therefore, they can be proven to be very effective against *H. pylori*.[4,5]

Natural hydrophilic polymers possess structural flexibility; thus, they can be applied in the preparation of oral controlled-release formulations that provide the required drug release profile.[6] *Sterculia foetida* is a medicinally important polysaccharide obtained in the form of exudates from *Sterculia urens*, a small-to-medium-sized bushy tree that belongs to the Sterculiaceae family. It fixes itself with water molecules, although not in a completely soluble form. It can swell, increasing its total volume in relation to dry mass, which is 60 times more than the original volume. It possesses a rhamnogalacturonan-type partially acetylated ramified structure, and its molecular weight is $16x10^3$ kDa. Chemically, it is composed of 55-60% unbiased monosaccharide units (galactose and rhamnose), 8% acetyl groups, and 37-0% acid residues (galacturonic and glucuronic acids). The core part of its structure is α-D-galacturonic acid and L-rhamnose units.[7] *S. foetida* has several potential commercial applications, primarily in the food and pharmaceutical industries. It is edible, odorless, and flavorless; thus, it is used as a food additive. As a result of its bioadhesive, non-toxic, non-immunogenic, non-mutagenic, and non-carcinogenic properties,[8,9] it is considered promising for the development of drug-loaded microspheres. Moreover, *S. foetida* exhibits swelling properties and can modify drug release from the polymeric matrix.[10] In a previous study, *S. foetida* was blended with pectin to produce a sustained-release carrier having floating ability and increasing gastric residence.[11]

Currently, for the pharmaceutical industry, *S. foetida* is a promising plant polysaccharide. It exhibits acid stability due to acetyl groups in its structure; it also possesses good swelling capacity and good mucoadhesive and biodegradable properties. Various studies have reported the use of *S. foetida* in the treatment of ulcers, irritable bowel syndrome, persistent colonic diseases, and improving glucose metabolism without adverse effects on mineral balance. Furthermore, many researchers have formulated interpenetrating polymer network (IPN) microspheres using polysaccharides and have reported their use for sustained drug delivery.[12]

Recently, increasing attention has been given to polysaccharides obtained from microorganisms and yeast. Pullulan is a neutral homopolysaccharide of glucose composed of repeating maltotriose residues connected by α-1.6 glycosidic bonds and some interspersed maltotetraose units produced by *Aureobasidium pullulans*. Its additional advantages include its biocompatibility, non-toxicity, non-immunogenicity, and non-carcinogenicity. Pullulan is also used as a binder, thickener, lubricant, and gelling agent. Because of its unique linkage pattern, it can capture biomolecules and has exceptional oxygen barrier properties, which increase the stability of these molecules with increased shelf-life. Pullulan is highly soluble in water; thus, it can be used as a drug carrier; however, it involves uncontrolled hydration and drug release-related issues, restricting its applications. The hydrophilicity of pullulan can be reduced by crosslinking it with an anionic polymer such as *S. foetida*. In addition, pullulan can be easily crosslinked using glutaraldehyde, and adding *S. foetida* increases its mechanical strength.[13]

The number of naturally occurring polysaccharides through the formation of IPNs improves mechanical strength and provides the desired release characteristics of the entrapped drug. Several IPNs have been reported thus far and include interactions between *Delonix regia* gum and sodium alginate,[14]

calcium pectinate and tamarind seed gum,[15] carrageenan-alginate,[16] locust bean gum and alginate,[17] guar gum and chitosan,[18] and carboxymethyl xanthan and alginate.[19] However, no attempt has been made to formulate semi-IPN microspheres composed of pullulan with *S. foetida*. Therefore, in this study, *S. foetida*-pullulan-based semi-IPN microspheres were formulated for stomach-specific delivery of amoxicillin trihydrate that can provide good mechanical strength, prevent uncontrolled water uptake, and remain in the stomach to provide drug release over long periods for activity against *H. pylori.* It is proposed that *S. foetida* has antiulcer properties, which could act synergistically against *H. pylori* with amoxicillin trihydrate.

MATERIALS AND METHODS

Experimental part

Materials

Amoxicillin trihydrate was gifted by Ranbaxy Laboratories (Goregaon East, Mumbai, India). Pullulan, having a molecular weight of 200,000 Da, was procured from Kumar Organic Products Limited (Bangalore, India). *S. foetida* was gifted by Research Lab Fine Chem Industries (Mumbai, India); light liquid paraffin (LLP) was supplied by Hi-Media Laboratories Private Limited (Mumbai, India). Pioneer In-Organics (Delhi, India) supplied Span 80. Glutaraldehyde (25%, v/v) was procured from Merck Limited (Mumbai, India). Dichloromethane was obtained from Loba Chem. Private Limited (Mumbai, India). All chemicals used were of analytical grade.

Preparation of S. foetida–pullulan semi-IPN microspheres

The water-in-oil emulsification-crosslinking method was used to prepare *S. foetida*-pullulan-based semi-IPN microspheres containing amoxicillin trihydrate (ASP-MPs).[20] *S. foetida* and pullulan were hydrated in distilled water. First, the two polymeric solutions were homogenized together to obtain a uniform solution. The drug was incorporated into this polymeric solution, and the solution was uniformly mixed using a magnetic stirrer until a uniform drug-polymer mixture was obtained. Next, the drug-containing polymeric mixture with 1% w/v Span 80 was added gradually to LLP with constant stirring at 2.000 rpm for 10 min. After the formation of a a milky white emulsion, glutaraldehyde containing 0.5 mL of 1 N HCl was added gradually, and the resulting emulsion was stirred continuously for 2 h to obtain solid microspheres. The microspheres were then filtered and washed with dichloromethane to remove excess LLP and washed several times with water to remove unreacted glutaraldehyde. The resulting microspheres were then allowed to dry under vacuum at 40°C, and finally, they were stored in desiccators until further use.

Experimental design for optimizing the ASP-MP formulation

ASP-MPs were prepared and optimized using three factors and a three-level randomized central composite design to determine the effect of formulation variables on the prepared microspheres. The three independent variables, i.e., the volume of crosslinker glutaraldehyde (A), amount of pullulan (B), and amount of *S. foetida* (C), were varied at three levels (Table 1). The statistical trial design was generated and evaluated using Design-Expert version 12.0.3.0 software (Stat-Ease Inc., USA). Percent drug entrapment efficiency (DEE), cumulative percent drug release for 12 h, and percent mucoadhesion were selected as dependent variables. Design matrices showing the effect of the formulation variables on the studied response variables are shown in Table 2, and response surface plots are shown in Figure 1a-c. Optimization was performed by modeling the effect of independent variables on the responses using a quadratic mathematical model, as shown in equation

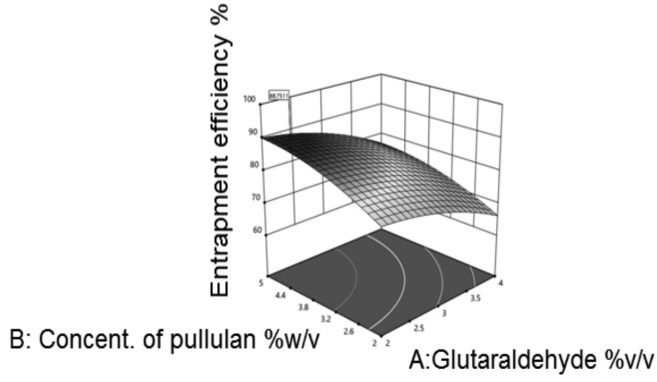


Figure 1a. 3D response surface plot graph showing the effect of pullulan and glutaraldehyde amount on percent DEE

DEE: Drug entrapment efficiency

Table 1. Coded levels of process variables used in the experiments

Independent variable	Level				
	1.68179	-1	0	+1	-1.68179
A- Glutraldehyde (% v/v)	9.3634	4	6	8	2.6364
B- Pullulan (% w/v)	12.0452	4	7	10	1.9546
C- *Sterculia foetida* (% v/v)	4.6818	2	3	4	1.3182
Dependent variables					
Y1	Entrapment efficiency in %	Maximize			
Y2	Drug release in 12 h in %	Maximize			
Y3	Mucoadhesion in %	Maximize			

1, and constraints on responses were maximum EE, maximum drug content, and maximum mucoadhesion.[21,22]

$$Y_o = b_o + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A_2 + b_{22}B_2 + b_{33}C_2$$

equation (1)

where Y is the response; b_0 is the intercept; and A, B, and C are the independent formulation variables.

Characterization of ASP-MPs

Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra of *S. foetida* gum, pullulan, amoxicillin trihydrate, and a physical mixture of *S. foetida* and pullulan with amoxicillin

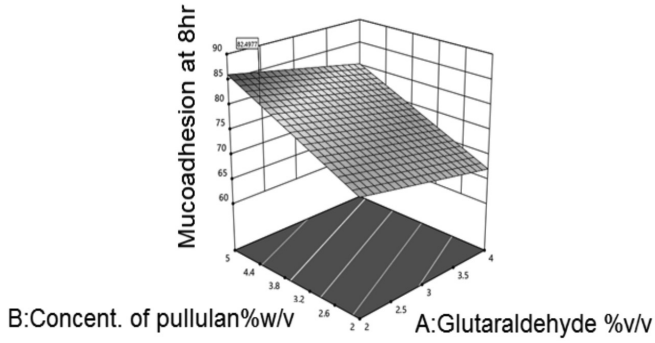


Figure 1b. 3D response surface plot graph showing the effect of pullulan and glutaraldehyde amount on percent mucoadhesion

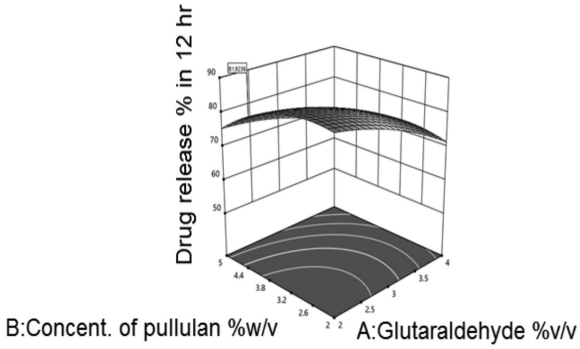


Figure 1c. 3D response surface plot graph showing the effect of pullulan and glutaraldehyde amount on percent drug release

Table 2. Design matrix and measured responses

Actual value of the independent variables						Responses		
Formulations	**Glutraldehyde % V/V (A)**	**Pullula % W/V (B)**	***Sterculia foetida* % W/V (C)**	**% EE**	**Drug content**	**% drug release at 12 hr**	**Mucoadhesion at 12 hr**	**Particle size**
APS-1	4	10	4	88.12±1.16	33.50±2.25	70.23±2.59	87.35±1.32	117.48±1.05
APS-2	4	10	2	84.53±1.18	30.52±2.82	78.56±1.79	83.32±1.87	111.75±0.76
APS-3	6	7	3	82.10±2.08	29.54±2.11	82.24±1.88	78.54±1.93	84.53±1.21
APS-4	8	10	2	72.15±1.70	25.36±1.17	68.86±1.98	80.54±2.28	94.73±1.28
APS-5	4	4	4	79.26±1.06	28.23±1.85	80.47±2.15	75.81±1.98	77.34±0.93
APS-6	4	4	2	73.60±1.67	26.13±1.83	89.41±1.99	68.51±1.77	69.94±1.14
APS-7	8	10	4	76.42±1.66	27.82±2.04	59.68±2.81	82.48±2.72	92.46±1.12
APS-8	9.36	7	3	63.49±1.69	20.43±1.78	64.63±1.23	69.24±2.53	60.70±0.82
APS-9	6	7	3	82.10±2.08	29.54±2.11	82.24±1.88	78.54±1.93	84.53±1.21
APS-10	6	7	3	82.10±2.08	29.54±2.11	82.24±1.88	78.54±1.93	84.53±1.21
APS-11	6	7	3	82.10±2.08	29.54±2.11	82.24±1.88	78.54±1.93	84.53±1.21
APS-12	6	7	4.6818	86.32±1.49	31.93±1.95	62.93±2.18	83.33±2.02	105.67±1.45
APS-13	6	1.9542	3	62.38±1.57	18.74±2.25	75.31±2.15	62.29±1.37	64.15±1.26
APS-14	6	7	1.3182	69.85±1.96	21.45±2.27	80.10±1.73	65.48±2.01	65.99±1.17
APS-15	2.62	7	3	87.69±2.65	32.56±2.21	84.59±2.46	79.63±1.76	96.76±0.72
APS-16	6	7	3	82.10±2.08	29.54±2.11	82.24±1.88	78.54±1.93	84.53±1.21
APS-17	6	7	3	82.10±2.08	29.54±2.11	82.24±1.88	78.54±1.93	84.53±1.21
APS-18	8	4	4	71.23±2.55	23.26±2.11	66.34±2.40	70.26±2.09	65.70±1.36
APS-19	8	4	2	60.37±1.85	16.64±2.06	72.90±2.26	60.78±2.13	57.99±1.53
APS-20	6	12.0453	3	88.24±2.46	34.80±2.20	60.32±2.09	81.16±1.42	121.90±1.38

Volume of aqueous phase was 100 mL, EE: Entrapment efficiency

trihydrate and ASP-MPs were obtained between 4.000 and 400 cm^{-1} using KBr pellets (FTIR-84005, Shimadzu Asia Pacific Pvt. Ltd. Singapore) (Figure 2).

Differential scanning calorimetry (DSC)

The thermal and crystalline properties of *S. foetida* gum, pullulan, and a physical mixture of *S. foetida* and pullulan with amoxicillin trihydrate and ASP-MPs were examined using DSC (Mettler Toledo AG, Analytical, Switzerland) at a heating rate of 10°C/min from 0°C to 400°C with continuous nitrogen purge at a rate of 50 mL/min (Figure 3).

X-ray diffraction (XRD)

XRD (Bruker model D8 advance theta/2theta) was conducted to study the crystalline properties of amoxicillin trihydrate upon entrapment in the microspheres. The radiation source was CuKα, at k¼1.5406 Å. The samples were spread over a low background sample holder (amorphous silica) and set in a goniometer. Scanning was performed at a scan speed of 5˚/min with increments at 0.02 and 2Θ from 0˚ to 80˚. The current and voltages were 40 mV and 35 mA, respectively (Figure 4).

Determination of particle size and surface morphology

The required quantity of microspheres was distributed in an insoluble solvent, and the suspension was diluted as needed. One drop of the suspension was placed on a glass slide and examined under a microscope; a digital picture was also taken (DMWBf model, Motic, China). The sample was placed on a piece of adhesive carbon tape set on a brass stub for scanning electron microscopy (SEM). The sample was then gold-coated using a sputtering unit (model: JFC1600, USA) for 10 s at 10 mA. Finally, the gold-coated sample was placed in an SEM chamber (Jeol, JSM 6390LA), and secondary electron images were recorded (Figure 5).

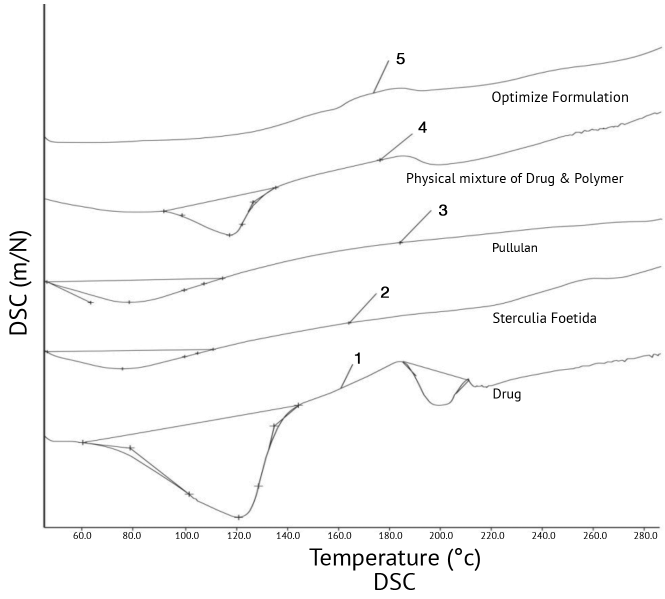


Figure 3. DSC thermogram of 1) pure drug, 2) *Sterculia foetida*, 3) pullulan, 4) physical mixture of drug and polymers, and 5) optimized formulation

DSC: Differential scanning calorimetry

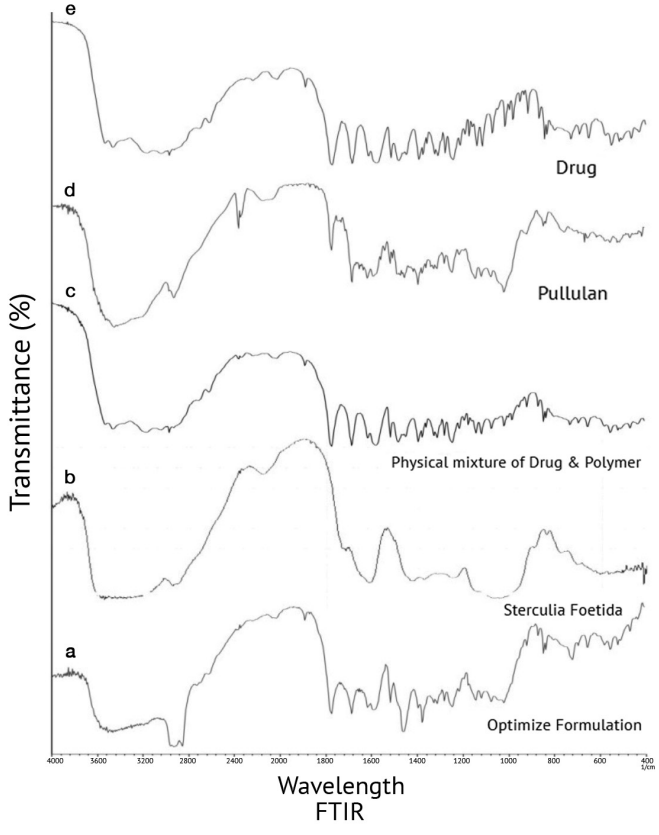


Figure 2. FTIR spectra of a) pure drug, b) pullulan, c) physical mixture of drug and polymers, d) *Sterculia foetida*, and e) optimized formulation

FTIR: Fourier-transform infrared spectroscopy

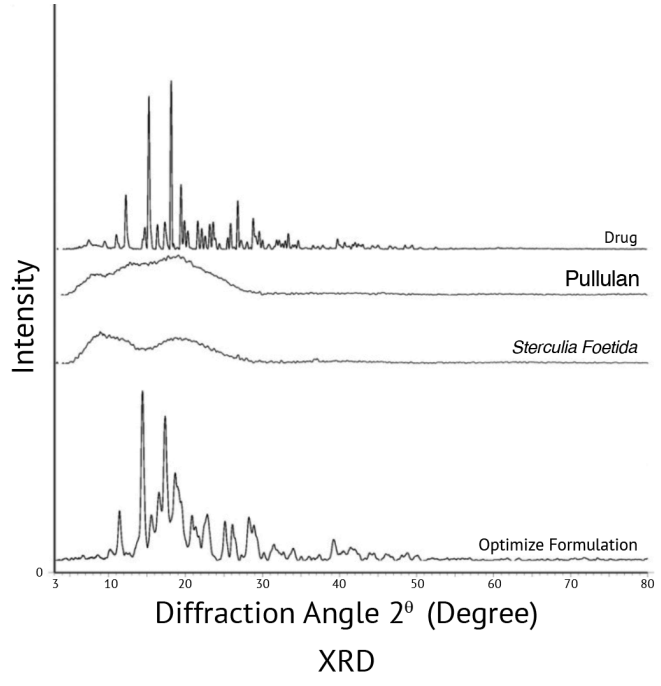


Figure 4. XRD pattern of a) pure drug, b) pullulan, c) *Sterculia foetida*, and d) optimized formulation

XRD: X-ray diffraction

Determination of percent drug loading and percent DEE

Fifty milligrams of microspheres were finely powdered and soaked in 50 mL of simulated gastrointestinal fluid [(SGF), pH 1.2] for 24 h. The resulting suspension was filtered, suitably diluted, and analyzed at 229 nm. Percent drug loading and DEE were then determined according to the following relationship:[23]

$$\% \text{ Drug loading} = \frac{\text{Amount of drug in microspheres}}{\text{Amount of microspheres}} \times 100$$

equation (2)

$$\text{DEE } (\%) = \frac{\text{Actual drug content in microspheres}}{\text{Theoretical drug content in microspheres}} \times 100$$

equation (3)

Evaluatin of mucoadhesive properties in vitro

Goat intestinal mucosa was used to study the mucoadhesive properties of semi-IPN microspheres. First, a dispersion of semi-IPN microspheres was sprayed on goat mucosa mounted on a glass slide. The glass slide was previously incubated in desiccators (90% relative humidity) for 20 min and then placed in the cell. Finally, the cell was attached to the outer assembly at an angle of 45˚. Next, SGF (pH 1.2) was circulated through the cell over the microspheres at a rate of 1 mL/min using a peristaltic pump. The washings were collected after fixed time intervals, and centrifuged and separated microspheres were dried at 40°C.

The weight of the washed microspheres was measured, and mucoadhesion (%) was calculated as follows:[23]

Percentage mucoadhesion=Qa-Ql × 100/Qa equation (4)

where Qa=weight of microspheres applied initially and Ql=weight of microspheres leached out.

In vitro drug release study

A type I USP dissolution apparatus (Electrolab, Mehsana, Gujrat, India) was used to measure the drug release in SGF (pH 1.2, 900 mL) at 37°C±0.5°C with a rotational speed of 100 rpm. Microspheres containing 500 mg of drug were placed in SGF. Every hour, 1 mL of the sample was suitably diluted and analyzed at 229 nm (Shimadzu/UV-1700, Japan). The aliquots withdrawn were replaced with drug-free buffer. Dissolution testing was performed for 12 h (Figure 6). Kinetic models such as the zero-order,[24] first-order,[25] Higuchi,[26] and Peppas[27] were used to explore the kinetics and mechanics of drug release.

In vivo radiographic study

To determine the location and extent of gastrointestinal transit, ASP-MPs were administered orally to rats and monitored using a radiological method. The Institutional Animal Ethics Committee approved the study (protocol no: 535/02/a/CPCSEA/S/Jan.2002). Six healthy albino Wistar rats (either sex, 400-500 g) were kept in individual cages in a sanitized room maintained at 27°C±0.5°C

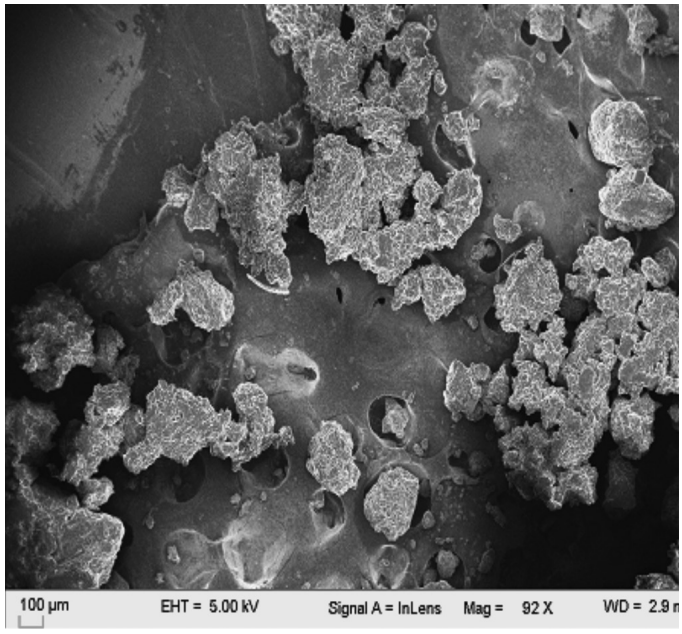
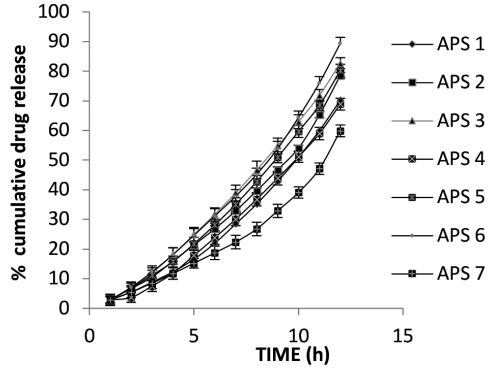
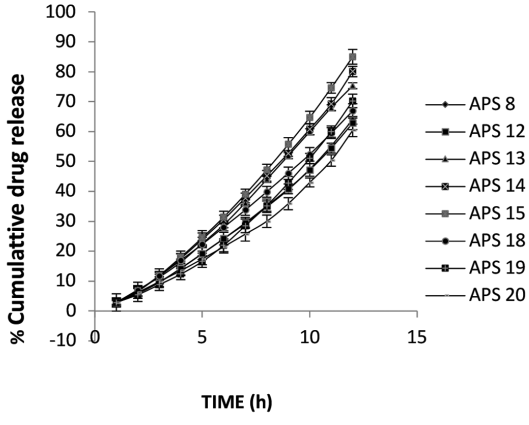


Figure 5. Scanning electron micrograph of optimized *Sterculia foetida*-pullulan microspheres loaded with amoxicillin trihydrate showing a rough surface



In vitro release profile of batches APS1-APS7



In vitro release profile of batches APS8-APS20

Figure 6. *In vitro* drug release profile of *Sterculia foetida*-pullulan microspheres loaded with amoxicillin trihydrate in simulated gastric fluid (pH 1.2) (mean ± SD; n=3)

SD: Standard deviation

at a 12 h light/dark cycle. Food was abstained for 12 h prior to the study, whereas water was allowed *ad libitum*. Radiopaque ASP-MPs were prepared by adding 50 mg of barium sulfate to the polymeric solution (the composition was the same as that of the optimized formulation) following the procedure used in the formulation of ASP-MPs. ASP-MPs were administered orally through the gastric tube along with 2 mL of water. The animals were subjected to fasting throughout the study (up to 12 h) with 1 mL of water allowed every hour. ASP-MPs were monitored in the gastric region of the rats at a predetermined time interval of 10 h using X-ray imaging (Siregraph-B, Siemens, Germany) (Figure 7).[28]

Statistical analysis

Statistical optimization of the formulations was conducted using Design-Expert® version 12.0.3.0 (Stat-Ease Inc.). Each measurement was performed in triplicate (n=3) and represented as mean ± standard deviation. Student's t-test was performed to compare the two groups.[29,30]

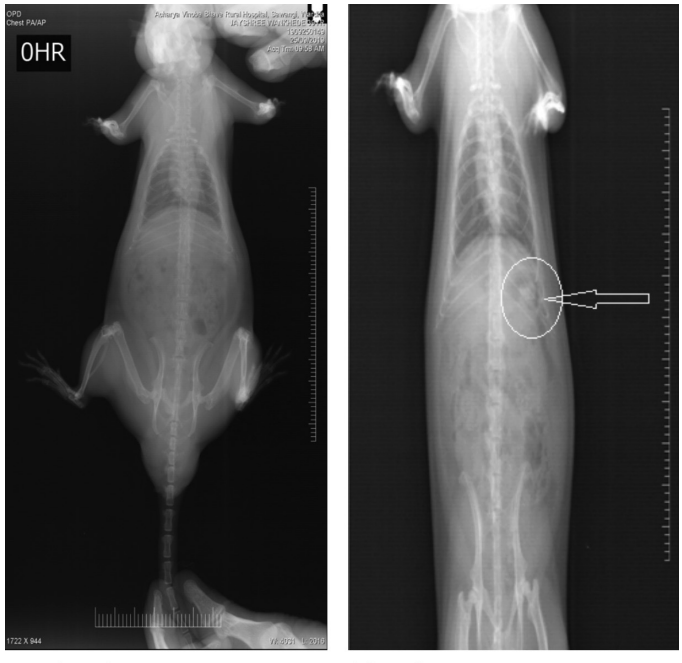
Stability testing

Stability testing of the optimized formulation was performed according to the ICH guidelines for 6 months.[31] The formulation was packed in laminated aluminum foil and placed in a stability chamber at 40°C±2°C and 75±5% relative humidity. The samples were examined at the end of 1, 3, and 6 months to check for significant differences in their mucoadhesion property, EE, and drug content. Differences were regarded as significant at $p<0.05$.

RESULTS AND DISCUSSION

Formulation of microspheres

In this study, ASP-MPs containing amoxicillin trihydrate were prepared using glutaraldehyde as a crosslinking agent.



a) 0 h **b) 10 h**

Figure 7. X-ray image of microspheres in the gastric region of rats

Pullulan is a highly water-soluble polymer; therefore, when it is used alone, it exhibits uncontrolled hydration, leading to instant release of high amounts of the drug. Therefore, to control hydration, *S. foetida* was coupled on a pullulan backbone with the help of glutaraldehyde. Water-soluble glutaraldehyde was selected as the crosslinking agent because of its ability to facilitate the formation of linkages from hydroxyl groups. The -CHO groups of glutaraldehyde (covalent crosslinker) react with the -OH groups of *S. foetida*, forming an acetal linkage that creates a three-dimensional IPN structure (Figure 2). The obtained microspheres were water-insoluble and had a three-dimensional network with amoxicillin entrapped in them. In an investigation conducted due to toxicity concerns of glutaraldehyde as a crosslinker, flavonoid-like rutin was used to crosslink gelatin microspheres, and a comparison was performed with glutaraldehyde. However, the crosslinking efficiency of rutin was low, and the glutaraldehyde-crosslinked microspheres were as safe as rutin-crosslinked microspheres.[32] The microspheres were washed several times with water to remove unreacted glutaraldehyde.

Influence of formulation composition on percent DEE (Y1)

The percent DEE increased from 60.37±1.85% to 88.24±2.46% as the concentration of polymers increased. From the response surface curve, it was determined that the encapsulation efficiency of the drug increased with an increase in the polymer concentration. The polynomial equation correlating percent DEE with independent variables is as follows.

$$EF(Y_1)= +82.11-6.30*A+5.88*B+3.81*C-0.3525*AB+0.7350*AC-1.08*BC-2.36*A^2-2.46*B^2-1.48*C^2 \quad \text{equation (5)}$$

where A is the volume of glutaraldehyde and B and C are the amounts of pullulan and *S. foetida*, respectively. The randomized central composite design showed a good fit. A high f value of 28.94 and a p value of <0.0500 indicated that the model was significant.

The influence of independent formulation variables on EE is depicted using response surface plots is shown in Figure 1a. The EE increased, possibly due to the high viscosity of the polymer blend, consequently reducing drug seepage during microsphere preparation. Further, the high polymer concentration may have caused increased entrapment of the drug inside the *S. foetida*-pullulan network. However, the encapsulation efficiency decreased with an increase in the glutaraldehyde concentration; this decrease might be due to an increase in the crosslinking density of the polymer matrix. This caused the formation of a rigid structure with a consequent reduction in free volume within the polymer matrix for drug entrapment.[33,34]

Influence of formulation composition on in vitro mucoadhesion

The percent mucoadhesion in SGF of all batches was between 60.78±1.62% and 87.35±1.83%. As the polymer concentration increased, mucoadhesion increased. The polynomial equation

correlating percent mucoadhesion with the independent variables is shown below:

Percent mucoadhesion= +76.072.81*A+6.59*B+3.86*C

equation (6)

The model f value of 35.43 and p value of <0.05 indicated that the model was significant. The predicted R^2 of 0.7856 was in agreement with the adjusted R^2 of 0.8446 as the difference between them was <0.2. The influence of independent formulation variables on mucoadhesion (%) is depicted using response surface plots in Figure 1b. An increase in mucoadhesion with polymer concentration may have occurred due to the availability of more OH groups for hydrogen bond formation with the mucus membrane.[29] Mucoadhesion decreases with an increase in glutaraldehyde levels. The decrease in mucoadhesion may be because of the formation of a strongly crosslinked polymeric matrix, causing decreased swelling and weaker interactions with the mucosal surface. The mucoadhesive properties of *S. foetida* are attributable to its capacity to form a mucoadhesive bond with mucin and interpenetration of the polymer chain in the interfacial area.[2,33,34]

Influence of formulation composition on percent drug release

All the formulations showed sustained release of amoxicillin over 12 h in SGF (pH 1.2). The cumulative drug release from semi-IPN microspheres after 12 h was between 60.32±2.09% and 89.41±1.99% (Table 2, Figure 6). The drug release rate decreased with an increase in polymer concentration. The correlation between drug release at 12 h and different independent variables is explained by the following polynomial equation:

Drug release= +82.15-6.18*A-4.17*B-4.53*C+1.30*AB+0.1912*AC-0.2513*BC-2.148A^2-4.54*B^2-3.23*C^2

equation (7)

The impact of formulation variables on percent drug release is depicted using surface response plots in Figure 1c. Increased *S. foetida* content in the blend membrane reduced the rate of drug release. The hydrophobicity of the microsphere matrix increased with *S. foetida* content due to an increase in ester linkages and methyl groups. Additionally, the reduction in the rate of drug release could be caused by a decrease in the molecular volume of *S. foetida* in the polymer matrix due to its decreased swelling and hydration capacity. Similarly, the drug release pattern was highly dependent on glutaraldehyde levels.[33] When the glutaraldehyde volume was increased from 2 mL to 4 mL, percent drug release was decreased from 89.41±1.99% to 60.32±2.09% in 12 h. This behavior may be a result of fewer free spaces in the glutaraldehyde-treated IPN matrices for unhindered drug diffusion through the polymer network.[34] The drug release followed Hixson-Crowell kinetics as it showed maximum R^2 (0.9964). The drug release mechanism followed super case-II (n=1.33 of Korsmeyer-Peppas), i.e., diffusion and chain relaxation. There could be a dominant role of chain relaxation at later time points due to waning of crosslinks over time.

Optimization by desirability function

Optimization was perfomed based on the desirability function. Microspheres were prepared under the model-predicted optimal condition with glutaraldehyde 4% v/v, pullulan 8.28% w/v, and *S. foetida* 2.14% w/v. Optimization was performed by placing constraints on the dependent variables. The response variables of the formulated microspheres under optimized conditions were nearly equal (low percentage bias) to the predicted values, ensuring reliability of the model (Table 3).

Influence of formulation composition on particle size

The microspheres had sizes of 57.99±1.53-121.90±1.38 µm. The variation in particle size due to formulation variables indicates that microsphere size was dependent on the glutaraldehyde amount and polymer concentration. When the amount of glutaraldehyde increased, the particle size decreased. This may be the result of the increased crosslink density, which caused shrinking of the internal phase to a greater extent, creating a low void space. Alternatively, when the polymer ratio was increased at a given quantity of glutaraldehyde, the particle size also increased. This could be because of increased drug entrapment in the voids present in the polymeric network. Additionally, when the drug concentration was increased, it needed more space in the polymeric networks and voids, consequently increasing the size.

Characterization of ASP-MPs

FTIR

Figure 2 depicts the FTIR spectrum of amoxicillin trihydrate, showing peaks at 1616.24 cm^{-1} for aromatic C=C stretching and 1685.67 cm^{-1} and 1577.66 cm^{-1} for C=O stretching and N-H deformation of amide, respectively. The peak at 1774.39 cm^{-1} was due to β-lactam C=O stretching, and the peaks at 2968.24 and 3039.60 cm^{-1} corresponded to C-H stretching for CH_3 and aromatic C-H stretching, respectively. All the prominent peaks of amoxicillin trihydrate were retained in the spectrum of a physical mixture of *S. foetida* and pullulan, indicating no interaction of the drug with the polymer. The FTIR spectrum of ASP-MP formulation revealed a broad absorption band from 3527.56^{-1} to 3105 cm^{-1} due to the OH group of *S. foetida* and CHO group of glutaraldehyde, which proves the formation of glutaraldehyde-

Table 3. Optimization by desirability function

Optimized formulation	Optimized level	Responses	Predictive value	Experimental value	% bias
Glutaraldehyde	4% v/v	Entrapment efficiency (%)	88.751	86.92	-2.06
Pullulan	8.28% w/v	Drug release in 12 h (%)	81.924	80.43	-1.81
Sterculia foetida	2.14% w/v	Mucoadhsion (%)	82.498	81.73	-0.92

crosslinked semi-IPN microspheres. Furthermore, bands from 3475^{-1} to 3620 cm^{-1} due to O-H stretching in the FTIR spectrum of *S. foetida* had shifted to the lower wavenumber from 3423^{-1} to 3519 cm^{-1} in the FTIR spectrum of APS-MPs. This shift can be attributed to hydrogen bond formation between *S. foetida* and amoxicillin in the microspheres. A similar finding was reported by Üstündağ Okur et al.[35]

DSC

Thermograms of amoxicillin, pullulan, *S. foetida* gum, physical mixture of *S. foetida*-pullulan with amoxicillin trihydrate, and ASP-MPs are shown in Figure 3. The pure drug exhibited a characteristic broad peak starting from 60°C to 140°C due to trihydrate dehydration and a broad endothermic peak starting from 185°C to 210°C with a peak at 199°C due to melting. DSC of pullulan revealed an endothermic peak from 0°C to 110°C, whereas *S. foetida* showed an endothermic peak from 0°C to 110°C due to dehydration. The endothermic peak of amoxicillin trihydrate due to the loss of water molecule is revealed in the DSC of the physical mixture, showing a lack of incompatibility. In contrast, the endothermic melting peak was diminished due to the masking of the drug in the polymers. The absence of a characteristic endothermic peak of amoxicillin in the ASP-MP thermogram might have accurred because of the entrapment of the drug in the microspheres, making it undetectable.

XRD

Sharp peaks with high intensity from 2ɵ 10˚-30˚ in the amoxicillin trihydrate XRD pattern indicated crystalline properties. The characteristic peaks present in the drug from 2ɵ 15˚-30˚ had broadened in microspheres, indicating a loss of crystallinity upon entrapment in microspheres (Figure 4).

SEM

SEM was performed to visualize the morphology of the ASP-MPs. The formed microspheres were slightly irregular and aggregated, with a rough surface (Figure 5). The rough surface might be due to the shrinkage of crosslinked *Sterculia* gum during drying of the IPN microspheres.

In vivo radiographic studies

X-ray images revealed the presence of microspheres in the stomach at 6 h after administration. The ASP-MPs were retained for an extended period of time due to mucoadhesion to the stomach mucosal lining (Figure 7).

CONCLUSION

Semi-IPN microspheres loaded with amoxicillin trihydrate were successfully prepared using *S. foetida* and pullulan by the emulsion crosslinking method using glutaraldehyde as the crosslinker. Microspheres prepared under optimized conditions (2.14% w/v *S. foetida*, 8.28% w/v pullulan, and 4% v/v glutaraldehyde) showed predicted values of EE, drug release, and mucoadhesion, with low percentage bias. Additionally, X-ray images revealed the presence of microspheres for 10 h. Thus, the prolonged retention of microspheres in the stomach with sustained drug release could effectively act against the *H. pylori* reservoir in the stomach and improve the therapeutic effectiveness of amoxicillin trihydrate against *H. pylori.*

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