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PRODUCTION OF CLARITHROMYCIN LOADED CHITOSAN MICROSPHERES BY SPRAY DRYING: AN OPTIMIZATION STUDY

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Abstract

Helicobacter pylori (H. pylori) is one of the most common patogens in the world. Although the microorganism is susceptible to many antimicrobial agents, the eradication rate is very low due to poor permeability of the antibiotics across the mucus layer, poor stability of the drug in the acidic pH of the gastric fluid, short residence time of antibiotic in the stomach and development of resistance to antimicrobial agents. The main purpose of this study was to optimize the spray drying process for the production of chitosan based microspheres loaded with clarithromycin resulting the stomach-specific delivery system to increase the gastric residence time, to allow better antibiotic penetration through the gastric mucus layer and to act locally at the infectious site. The optimum conditions and the significance of some parameters (inlet air temperature, drug concentration and feed flow rate) on spray drying efficiency and properties of clarithromycin loaded microspheres in terms of the water activity and morphology were determined by Central Composite Design (CCD) and Response Surface Methodology (RSM).

1. INTRODUCTION

H. pylori is a spiral-shaped, microaerophilic, gram-negative bacteria which colonize gastric mucosa. *H. pylori* is related to a number of upper gastrointestinal diseases, such as chronic gastritis, duodenal and gastric ulcer, gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma and has been classified as a class 1 carcinogen by "International Agency for Cancer Research (IACR)'' in 1994 (Graham and Graham, 2002). Considering gastroduodenal diseases related to *H. pylori* infection, this bacterium has the important role in terms of public health. Although various treatment durations, doses, and drug combinations have been studied none of them have consistently reached eradication levels in excess of 90 to 95%. The reported failures of antibiotic therapy could be due to poor permeability of the antibiotics across the mucus layer or due to poor stability of the drug in the acidic pH of the stomach. Moreover, conventional tablets or capsules do not remain in the stomach long. Therefore, antibiotics might not have enough time to diffuse into the mucosa layer, and the antibiotic concentrations in the gastric mucus can not reach minimum inhibitory concentrations (Majithiya and Murthy, 2005; Zheng et al., 2006). One way to improve the efficacy in eradicating the infection is to develop mucoadhesive drug carriers which may prolong the residence time in the gastrointestinal tract (GI) because they can adhere to the mucus surface, resulting in an effective localized drug concentration

(Rajinikanth et al., 2008). Among several mucoadhesive polymers, chitosan is gaining attention in the pharmaceutical field for a wide range of drug delivery since it is known to be a natural, biocompatible, biodegradable and nontoxic. The chemical structure of chitosan is given in Figure 1. Clarithromycin is a macrolide and widely used in a standard eradication treatment of *H. Pylori* infection combined with a second antibiotic and an acid-suppressing agent (Rajinikanth et al., 2008). It has the highest eradication rate of *H. pylori* in monotherapy *in vivo* and hence was selected as a model drug in this study.

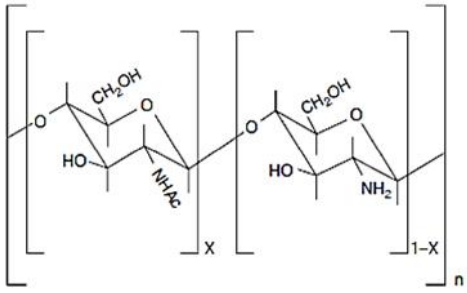


Figure 1. Chemical structure of chitosan with X=degree of deacetylation and n=number of sugar units per polymer (Source: Dadone and Vilivalam, 1998).

Spray drying is one of the microencapsulation technique in which a drug is dissolved or suspended in a melt or polymer solution and becomes trapped in the dried particle. Although several methods are available for the preparation of microparticles, spray drying is widely used in the pharmaceutical industry. The main advantage of spray drying is the ability to handle labile materials because of the short contact time in the dryer, in addition, it is a one-stage continuous process, easy to scale-up, and only slightly dependent upon solubility of drug and polymer. The particle size of the microspheres prepared by the spray drying method ranges from a micron to several tens of microns and has a relatively narrow distribution (Desai et al., 2005).

2. MATERIALS AND METHODS

2.1. Materials

High molecular weight chitosan (Sigma-Aldrich Chemie, Germany, cat. # 419419, deacetylation degree % 86) was used in the microsphere manufacturing. Clarithromycin was supplied by Ranbaxy Pharmaceuticals Inc. Acetic acid was purchased from Merck, Darmstadt

2.2. Production of Clarithromycin Loaded Chitosan Microspheres

Chitosan microspheres were produced by spray dryer using Buchi® Mini Spray Dryer B-290 (Switzerland) with a 0.5 mm standard nozzle and a parallel flow. 1 % (w/v) chitosan solution was prepared by dissolving chitosan in 2 % acetic acid and control microspheres were obtained by drying this solution at 140 °C in a spray dryer. Clarithromycin and chitosan solutions prepared by dissolving them in separate beakers were mixed and stirred for 1 h. The final concentrations of clarithromycin and chitosan in this solution were 0.1% and 1% (w/v), respectively. Clarithromycin loaded chitosan microspheres were obtained by drying this solution in a spray dryer at an inlet air temperature of 180°C and a feed rate of 4 ml/min.

2.3. Optimization of the Microsphere Production

Optimization of the spray drying process could be very important phenomenon in

terms of product quality and process efficiency in the development of clarithromycin and cinnamon bark oil loaded chitosan microspheres. Since the particle size distribution, shape and surface properties, water activity and the spray drying efficiency are governed by the inlet air temperature, feed rate and clarithromycin or cinnamon bark oil concentration of feed solution, the significance of these variables, as well as interactions between them, were examined using a central composite design and response surface methodology (RSM).

Water activity of the spray dried microspheres was measured by HygroLab 3 (Rotronic). The particle size distribution, shape and surface properties were examined by scanning electron microscope (Philips XL 30S FEG, FEI Company, Eindhoven, Netherlands). Spray drying efficiency (SE) was calculated to measure how much of microspheres were obtained after the manufacturing process as in Eqn (1);

$$SE(\%) = \frac{amount.of\ spray.dried\ microspheres}{amount.of\ initial\ .polymer} \times 100 \quad (1)$$

Three factors as process inputs were the drying air temperature, solution feed rate and clarithromycin or cinnamon bark oil concentration of solution. The response variables were the moisture content, particle size distribution and surface morphology and spray drying efficiency. The levels of the factors were coded as -1 and +1, corresponding to the low and high levels respectively. Alpha was taken as 1.68. Actual values are 170°C-190°C and 185°C-195°C for drying air temperature; 8-10 ml/min and 6-10 ml/min for solution feed rate and 0.2%-0.6% (w/v) and 0.7%-1.3% (w/v) for clarithromycin or cinnamon bark oil concentration of the solutions, respectively. All statistical analyses were performed by MINITAB Statistical Software, Release 15.

3. RESULTS AND DISCUSSIONS

3.1. Characterization of Spray Dried Microspheres

Spray drying of chitosan solution having 0.2-0.6% (w/v) clarithromycin concentration was carried out in the specified drying air temperatures and feeding rates. The synthesized particles

were graded in four categories (Table 1) according to their shapes (wrinkled or smooth) and particle size distributions (monodisperse or polydisperse) which indicate the adequacy grade of the particles for the specific use. The surface properties, particle size and shape are very important for the practicability of the particles. The particle size in the range of 1-5 μm isexpected to have good aerodynamic properties that bring also the tendency of small particles to agglomerate due to the Van der Waals forces. The stickness of a particle is related to the moisture content. Moisture content represents a measure of the quantity of water in a product. It provides information about yield, quantity and texture of product. A portion of the total water content present in a product is strongly bound to specific sites on the chemicals that comprise the product. These sites may include the hydroxyl groups of polysaccharides, the carbonyl and amino groups of proteins, and other polar sites. Hydrogen bonds, ion-dipole bonds, other

strong chemical bonds tightly bound water. Some water is bound less tightly, but is still not available. Water activity is a measure of how efficiently the water present can take part in a chemical reaction. It is defined as "free" or "unbound" water in a system and determined as the vapor pressure of water in a sample divided by the vapor pressure of pure water at the sample temperature (Beristain et al., 2002). Water activity affects the surface charge and stickiness of the particles. It provides valuable information about microbial spoilage, chemical stability and physical stability. Besides particle size, shape and moisture content, spray drying efficiency is also a important parameter in a spray drying process. Spray drying is a solvent evaporation process. The solvent in the droplets is removed very quickly due to heat energy provided in the spray dryer. The maximum spray drying efficiency can be achieved from a balance of the amount of heat energy input and the amount of heat energy used in the evaporation process which is related to the amount of the sample input.

Several factors were hypothesized to see the effects on the properties of drug loaded chitosan microspheres. The properties of the synthesized microspheres that were monitored were "water activity", "particle type" and "spray drying efficiency". Statistical analysis based on a central composite design was performed to examine the influence of the three variables (inlet air temperature, drug concentration and feed flow rate) on the final properties of clarithromycin and cinnamon bark oil loaded chitosan microspheres. These variables were examined at three levels: upper, medium and lower limits.

Table 1. Categorization of spray dried particles

Type 1	smooth, 1-5 micron, monodisperse
Type 2	smooth, 1-5 micron, polydisperse
Type 3	Wrinkled, 1-5 micron, polydisperse
Type 4	sticky, 1-5 micron, polydisperse

Table 2 lists the results of the experiments performed according to the experimental design for the production of clarithromycin loaded microspheres. The analysis was done using coded units.

3.2. Water Activity

The estimated regression coefficients for water activity of clarithromycin loaded microspheres were given in Table 3.

The regression equation (Equation 2) for water activity was as follows, with a regression coefficient R2 of 83.37 %:

$$\hat{y}_1 = 25.088 + 4.876x_1 + 4.5x_1x_2 - 5.25x_2x_3 \quad (2)$$

Coefficient estimates and the p-values verified the significance of the main factor of inlet air temperature, second order interactions of inlet air temperature and feed rate and clarithromycin concentration and feed rate. The inlet air temperature was found to be the most effective parameter on water activity. Feed rate alone was not an effective parameter, but the interactions with inlet air temperature and concentration indicated significance.

Table 2. Definition and results of randomized experiments in coded factors

Run Number	Inlet Air Temperature (A)	Feed Rate (B)	Clarithromycin Concenration (C)	Water Activity (a_w)	Particle Type	Efficiency (%)
1	-1	-1	-1	0.262	2	58.87
2	1	-1	-1	0.260	3	47.18
3	-1	1	-1	0.476	2	46.49
4	1	1	-1	0.922	4	34.59
5	-1	-1	1	0.483	2	65.40
6	1	-1	1	0.414	2	62.29
7	-1	1	1	0.524	4	47.84
8	1	1	1	0.675	4	61.92
9	-1.68	0	0	0.652	1	58.76
10	1.68	0	0	0.659	2	42.71
11	0	-1.68	0	0.320	1	52.54
12	0	1.68	0	0.281	4	14.19
13	0	0	-1.68	0.429	2	53.48
14	0	0	1.68	0.652	4	58.95
15	0	0	0	0.410	2	51.57
16	0	0	0	0.412	2	51.41
17	0	0	0	0.408	2	52.58

Table 3. Estimated regression coefficients for water activity of clarithromycin loaded microspheres

Term	Coef	SE Coef	T	P
Constant	25.0882	2.957	8.484	0.000
A	1.4029	1.389	1.010	0.346
B	4.8760	1.389	3.511	0.010 *
C	-1.5344	1.389	-1.105	0.306
A*A	0.6684	1.529	0.437	0.675
B*B	0.2265	1.529	0.148	0.886
C*C	-1.1877	1.529	-0.777	0.463
A*B	4.5000	1.814	2.480	0.042 *
A*C	-4.0000	1.814	-2.204	0.063
B*C	-5.2500	1.814	-2.893	0.023 *

* The terms with P value less than or equal to 0.05 have been considered as significant.

3.3. Particle Morphology

Morphology of clarithromycin loaded microspheres was investigated by scanning electron microscopy to decide particle classifications according to Table 7.3. Mostly, all particles were smooth and uniformly distributed in the size range of 1-5 μm. (Figure 2). However, more sticky particles with low sphericity were also observed due to the insufficient drying. The micrographs depicted different particle shaped and sized microspheres, obtained at each run are given in Figure 2.

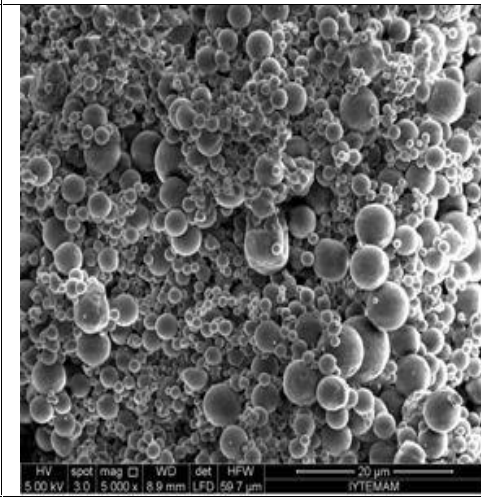
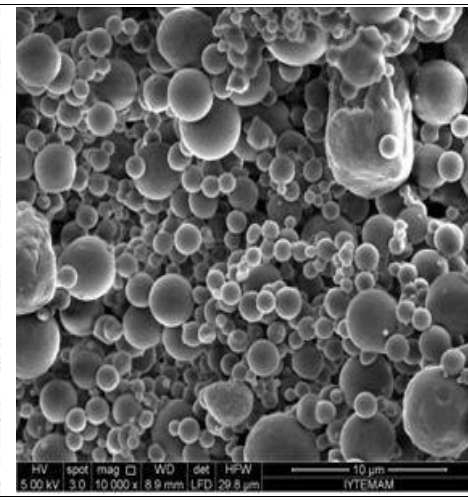
Run No	5000x	10000x
10		

Figure 2. SEM micrographs of clarithromycin loaded microspheres manufactured by spray drying according to central composite design; run number: 10.

The estimated regression coefficients for particle type of clarithromycin loaded microspheres were given in Table 4.

Table 4. Estimated regression coefficients for particle type of clarithromycin loaded microspheres

Term	Coef	SE Coef	T	P
Constant	1.96639	0.3063	6.419	0.000
A	0.34282	0.1439	2.383	0.049 *
B	0.73556	0.1439	5.113	0.001 *
C	0.31952	0.1439	2.221	0.062
A*A	-0.06101	0.1583	-0.385	0.711
B*B	0.29254	0.1583	1.848	0.107
C*C	0.46932	0.1583	2.964	0.021 *
A*B	0.12500	0.1880	0.665	0.527
A*C	-0.37500	0.1880	-1.995	0.086
B*C	0.37500	0.1880	1.995	0.086

* The terms with P value less than or equal to 0.05 have been considered as significant.

The regression equation (Equation 3) for particle type was as follows, with a regression coefficient R2 of 89.15 %:

$$\hat{y}_2 = 1.966 + 0.343x_1 + 0.736x_2 + 0.469x_3^2 \quad (3)$$

This equation revealed that the key parameters that had dominant effect on particle type of microspheres as inlet air temperature, feed rate, and pure quadratic term (Table 4).

In spray drying, it was observed that some of the liquid droplets were attached inside the wall of the main chamber. Once the inlet air temperature was set below 170°C, or the pump rate was chosen to be faster than 8 ml/min, the solvent in the droplets could not be fully evaporated and the spray drying efficiency was calculated below 50%.

The estimated regression coefficients for drying efficiency of clarithromycin loaded microspheres were given in Table 5.

The regression equation (Equation 4) for efficiency was as follows, with a regression coefficient R^2 of 83.04 %:

$$\hat{y}_3 = 51.428 - 7.865x_2 \quad (4)$$

The analysis designated that the only significant factor was feed rate for efficiency.

The optimum conditions for the manufacturing of clarithromycin loaded microspheres by spray drying were determined as 168°C for inlet air temperature, 4.64 ml/min for feed rate and 0.064 % (w/v) for clarithromycin concentration with desirability of 0.76.

Table 5. Estimated regression coefficients for the efficiency of clarithromycin loaded microspheres

Term	Coef	SE Coef	T	P
Constant	51.4277	4.382	11.737	0.000
A	-2.8988	2.058	-1.409	0.202
B	-7.8651	2.058	-3.822	0.007 *
C	4.3578	2.058	2.118	0.072
A*A	1.0723	2.265	0.473	0.650
B*B	-5.0694	2.265	-2.238	0.060
C*C	3.0083	2.265	1.328	0.226
A*B	2.1214	2.688	0.789	0.456
A*C	4.3198	2.688	1.607	0.152
B*C	0.8786	2.688	0.327	0.753

* The terms with P value less than or equal to 0.05 have been considered as significant.

CONCLUSION

Clarithromycin loaded chitosan microspheres can be used in stomach-specific delivery systems to increase the gastric residence time, to allow better antibiotic penetration through the gastric mucus layer and to act locally at the infectious site. In further studies, crosslinked microspheres should be produced for prolonged release of clarithromycin from the microspheres. Then the drug release profiles and mechanisms should be investigated to reveal the potential of controlled release of clarithromycin from chitosan microspheres.

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