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Short communication

## Characterization of furazolidone-chitosan based spray dried microparticles regarding their drug release and mucin adsorptive properties



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## ABSTRACT

The present study aimed to prepare mucoadhesive microparticles of furazolidone (antimicrobial agent) using chitosan as a mucin adsorptive polymer and spray drying technology. This study recognized the potential use of furazolidone to directly target stomach (for example against *Helicobacter pylori* in order to afford low resistant anti helicobacter agent as compared to commonly used antibiotics). Therefore, local mucoadhesive drug delivery systems in the stomach by using easily tailored approach of spray drying was used in this study. In order to get sufficient drug release for effective period of time at the pH conditions of stomach under the influence of *H. pylori*, the study was conducted at two different pH levels (1.3 and 4.5). The amount of glutaraldehyde (GTA) as a crosslinking agent was also studied to get appropriate particles size, and drug release. By increasing the amount of GTA, particles size and the release were decreased at pH 1.3 as well as at pH 4.5. The pH of the media also showed significant effect on the drug release, i.e. by decreasing the pH the release was increased. Similarly, crosslinking agent showed negative effect on mucin adsorption. However, by reducing the pH from 4.5 to 1.3 the mucin adsorption was also reduced; therefore mucoadhesion increases at pH 4.5 which is desirable for targeting *H. pylori* as normal stomach pH is expected to increase by the effect of *H. pylori*. Hence, the data of this research can be considered for future in vivo study.

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## 1. Introduction

Resistance of *Helicobacter pylori* against most commonly practiced antibiotics clarithromycin and metronidazole posing serious therapeutic problems and resistance may be changed with time because of secondary resistance [1]. According to Health Protection Agency 20–63% resistance to metronidazole and 4.4–11% to clarithromycin is reported in the UK and in some urban areas it can reach up to 65%. There is no other slandered therapy recommended in case of failure [2]. Therefore, there is intense need to develop new antibiotic for *H. Pylori* with minimum resistance. A number of anti-*H. pylori* agents were being investigated and new strategies are currently under testing to minimize resistance and to make the drug available. Furazolidone emerges as potential candidate for therapeutic consideration with low documented resistance. In addition to use of low resistance antimicrobial agent, the availability of drug on site may also be considered as a promising approach to encounter *H. pylori*. There are a number of ways that can provide high concentration in gastric mucosa to enhance microorganism

eradication [3]. The diffusion of drug from gastric lumen after its adhesion to mucus layer to encounter *H. pylori* which resides deep in the stomach could be considered as oral site-specific delivery system [4].

There are a number of approaches for development of mucoadhesive drug delivery system. A large literature shows the preparation of sustained release microparticles by using spray drying approach [5,6]. A number of studies revealed that initial parameter of spray drying could influence the characteristics of finished product for example particles size increases by increasing the feed rate [7]. However, increase in inlet temperature has adverse an effect on particle size [8]. Spray drying technique for developing mucoadhesive microsphere is simple, quick and straight forward approach which can be used to optimize the number of parameters that could affect the characteristics of final formulations.

Chitosans are biodegradable, linear amino polysaccharides with a random distribution of b-1,4-N-acetyl-D-glucosamine and D-glucosamine. They are derived from the biopolymer chitin and are used in food preparations. Hence, they present safety, biocompatibility and in some studies anti-ulcer activity [9] and therefore chitosan could be considered as most appropriate polymer for development of mucoadhesive microparticles by using spray drying. Chitosan contain OH and NH<sub>2</sub> groups which are responsible for hydrogen bonding and furthermore

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being cationic polymer provide strong electrostatic interaction with negatively charged mucus surface [10].

The purpose of the current study was development of spray dried mucoadhesive microparticles of furazolidone with the ultimate aim to modulate mucoadhesion and local drug release on gastric mucosal surface. To the best of our knowledge, there are no publications available for formulation of furazolidone in mucoadhesive systems using spray drying technology.

## 2. Materials

Furazolidone, glutaraldehyde (GTA) and chitosan were purchased from sigma Aldrich. Mucin Type I (from bovine submaxillary glands), Schiff reagent periodic acid and pepsin (partially purified from porcine stomach) were purchased from sigma Aldrich. Trisma buffer and calibration particles CPC (300, 400, 1000 and 2000 nm) were purchased from Izon Science, Oxford UK. All other chemicals, reagents and solvents used were of either analytical or pharmaceutical reagent grade.

## 3. Preparation of microparticles

Chitosan microparticles were prepared by using spray dryer (Bucci B-290 Switzerland) technique. 0.1% of chitosan (1 mg per ml) in 15 ml of 1% acetic acid solution was prepared by continuous stirring for 6 h. Different volumes of 1% glutaraldehyde as crosslinking agent were added to make different formulations listed in Table 1. For drug loading 10 mg or 15 mg of furazolidone was suspended in distilled water (~85 ml) or dissolved in acetonitrile/distilled water mixture (3:82) in different formulations as shown in Table 1. The volume to be spray dried was 100 ml per batch.

The applied spray drying conditions were inlet temperature of 160 °C, pressure of 4 bars, flow rate of 2 ml/min, aspirator 100% and pump at 65%. The resultant dry powder was collected via cyclone separator in a dry collection bottle.

## 4. Characterization of chitosan microsphere

Microspheres were characterized by particle size analysis, mucoadhesion, in vitro drug release, process yield and drug content.

### 4.1. Drug content

The powder collected contains drug and polymer was dissolved in specified amount of distilled water to make suspension. 1 ml of suspension was centrifuged. Acetonitrile and acetic acid was added to pellets to dissolve the drug and chitosan and then 0.5 ml of the solution was subjected to HPLC (as discussed below). The concentration was calculated from AUC by using HPLC standard calibration curve of furazolidone.

### 4.2. HPLC method

The HPLC analysis was carried out using Agilent Chem Station LC-DAD, with UV detector (Agilent technologies, (76,337), Germany). The column (4.60 mm × 150 cm) was used for analysis. Gradient system of mobile phases consisted of 0.5% phosphoric acid water with pH 7.4 and acetonitrile, flow rate of 1 ml/min was applied. Temperature of the column was maintained at 30 °C and wavelength used was 320 nm. Retention time was determined at 4.388 min.

### 4.3. Process yield

The yield recovered from spray drying was calculated by the formula

$$= \frac{\text{powder recovered (mg)}}{\text{Total amounts of drug, glutaraldehyde and chitosan (mg)}} \times 100.$$

### 4.4. Particle size analysis

Particle size was determined by using Izon qNano particle sizer (Izon Science Ltd. NewZealand) in which particles size was measured in terms of current block that is proportional to the diameter of particle size. Two different nanopores (1000 and 2000) were selected for each formulation to get more reliable data. Selection of nanopores for each formula was based on the expected size and particle size distribution.

### 4.5. Muco-adsorption

For mucin adsorption, calorimetric method was used in which periodic acid and Schiff reagent were used for the determination of remaining free mucin after its adsorption on the chitosan microparticles. 0.1 g of sodium metabisulfate was added to every 6 ml of Schiff reagent and incubated at 37 °C till it turned into pale yellow color. 10 µl of 50% periodic acid was added to 7 ml of 7% acetic acid to make periodic acid reagent. Periodic acid reagent (0.2 ml) was added to mucin samples and incubated at 37 °C for 2 h followed by addition of 0.2 ml of Schiff reagent at room temperature (~22 °C) and kept for 30 min. Absorbance was measured at 555 nm by UV/visible spectrophotometer (Model M501, Camspec, Biochrom, UK). The concentration of free mucin was determined by using standard calibration curve of mucin (Type-I from sigma Aldrich). Standard solutions for calibration curve (0.1, 0.25, 0.5, 0.75 and 1 mg/2 ml) were made and absorbance was measured by UV/visible spectrophotometer at 555 nm.

Mucin concentration of 1 mg/2 ml was prepared and 2 ml of micro-particle (F1 to F3) suspensions (in pH 1.3 or pH 4.5) containing different amount of chitosan and crosslinking agent were centrifuged. Supernatant was discarded and pellets of F1–F3 were dispersed in to standard mucin solution separately and vortexed for 5 min and later analyzed for free mucin concentration at predetermined time intervals (1.5, 3, 4.5 and 6 h). Separate Eppendorf tubes with mucin and microparticles formulations were used for each time interval. The dispersion was centrifuged at 4000 rpm for 5 min, supernatant was used for the measurement of free mucin by using the method stated above.

### 4.6. In-vitro drug release

Modified dispersion method stated by [11] was used to perform in-vitro dissolution. Release was determined, in triplicate, in water bath at 37 °C for F1–F3 formulations at (0, 0.5, 1, 1.5, 2, 3, 4 and 5 h) at pH 1.3 and pH 4.5; hence the study was conducted on two different pH levels, one of them was pH condition that mimics post proton pump inhibitors microenvironment in stomach in the presence of *H. pylori* and the other pH was using natural stomach pH. In this release study method, microspheres equal to 2 mg of the drug was centrifuged at 4000 rpm for 5 min. Supernatant was discarded and pellets were transferred to new vials without disturbing the yellow colored precipitated drug at the bottom. Pellets were washed with water three times and suspended

**Table 1**  
Composition of furazolidone-chitosan formulations prepared by spray drying.

Formulation code	Chitosan (0.1%)	Drug (mg)	Glutaraldehyde (1%)	Solvent used	Total volume (ml)/batch
F1	15 mg	10	2 mg	Acetonitrile/distilled water (3:82)	100
F2	15 mg	15	4 mg	Acetonitrile/distilled water (3:82)	100
F3	15 mg	15	6 mg	Distilled water	100

**Table 2**

Particle size, percent drug content and process yield of spray dried furazolidone-chitosan formulations. For formulation composition refer to Table 1.

Formula	Nanopore (µm)	Particle size (µm)				Drug content (%)	Process yield (%)
		Maximum	Minimum	mode	mean		
F1	1.000	3.640	0.987	1.137	1.616	60.0	49.2
	2.000	4.248	1.866	2.116	2.377		
F2	1.000	2.195	0.723	0.939	1.163	66.6	57.3
	2.000	4.240	1.688	1.818	1.860		
F3	1.000	2.407	0.670	0.805	0.990	51.5	44.3
	2.000	3.386	1.274	1.424	1.729		

**Table 3**

Correlation coefficient squared of release kinetic models for furazolidone-chitosan formulations at different pH levels. For formulation composition refer to Table 1.

Formulations	pH 1.3		pH 4.5	
	Zero order r <sup>2</sup>	First order r <sup>2</sup>	Zero order r <sup>2</sup>	First order r <sup>2</sup>
F1	0.980	0.971	0.991	0.988
F2	0.992	0.989	0.997	0.977
F3	0.996	0.980	0.997	0.990

in 10 ml of SGF at the desired pH. After specified time 0.5 ml of sample was withdrawn from and replaced by equal volume of fresh SGF which was filtered by 0.2 µm filter and then analyzed by HPLC analysis.

4.7. Particle morphology

Scanning electron photomicrographs of all microspheres were taken by microscope (Hitachi S3000N, Hitachi High-Technologies UK-Electron Microscopes, Wokingham Berkshire, UK). Small amount of each sample was attached to a 15 mm diameter aluminum specimen stub using double sided carbon adhesive tabs (Mikrostik adhesive, Agar Scientific), and the powder samples were sputter-coated with a thin layer of gold/palladium mixture to allow them to be electrically conductive. This was carried out using a Quorum Technology (Polaron range) SC760, whereby the samples are exposed to argon atmosphere at 10 Pa. The samples are coated at a process current of 18–20 mA for 2 × 105 s, with a turning through 180° in between.

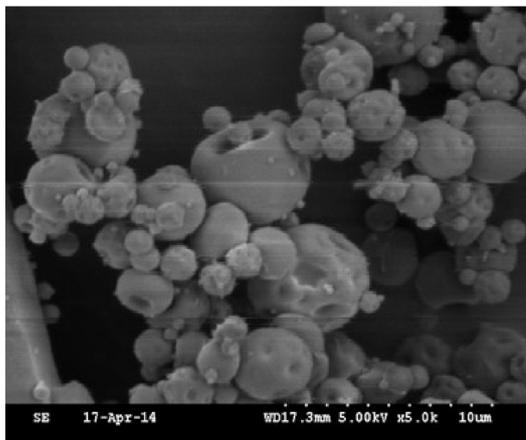
4.8. Statistical analysis

The statistical software SPSS 21 (SPSS Inc., Chicago, USA) was applied to carry out the statistical analysis by using ANOVA test and Post hoc multiple comparisons were applied when necessary. A p value of <0.05 was considered significant.

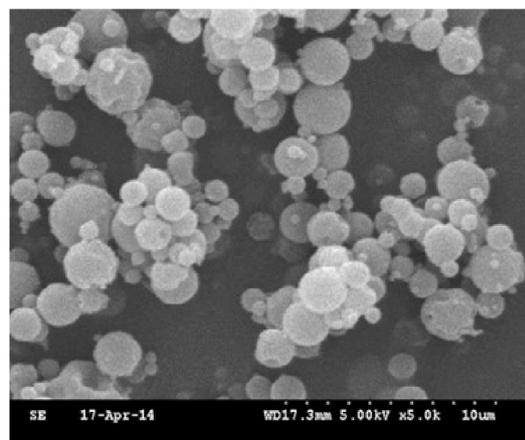
5. Results and discussion

5.1. Characteristics of microparticles

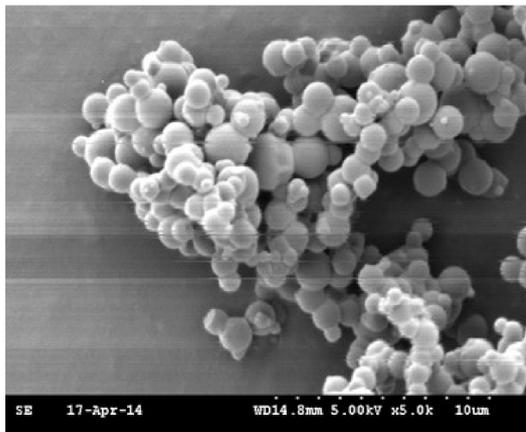
Characteristics of microparticles prepared by spray drying method are listed in Table 2. Particle size ranges from 1.0 µm to 2.4 µm. By adding



F1



F2



F3

Fig. 1. SEM photomicrographs of mucoadhesive spray dried furazolidone microparticles. For formulation composition refer to Table 1.

the cross linking agent particle size was decreased which is in agreement with study conducted by [10,12]. Formulation F3 with highest amount of glutaraldehyde having particle size of 0.805/1.424  $\mu\text{m}$  (mode value, Table 2) however it increased by decreasing the amount of glutaraldehyde. (See Table 3.)

According to study conducted [13] increase in viscosity of the solution, resulting in low recovery percentages. Similarly, in the current study increasing the amount of chitosan in formulation decreases the process yield. F1 having chitosan to drug ratio 1.5: 1 showed process yield of 49% in contrast to F2 having 1:1 ratio with process yield 57.3%, Table 2.

The selection of solvent also affects the process yield. Furazolidone being poorly water soluble drug was not completely dissolved when only distilled water was used as solvent before spray drying in case of formulation F3 that gave 44.3% process yield. However the yield was increased in F1 and F2 shown in Table 2, when drug was first dissolved in acetonitrile to make it completely soluble. The use of co-solvent described by other studies bring two possibilities; solvent facilitates evaporation process that in turn decreases the time and energy required and shows positive effect on the percentage recovery [13]. It was reported that selection of solvent type influences the structure of resultant microparticles which influences different characteristics [14].

Similarly, drug content of microparticles was most probably affected by the selection of solvent i.e. in F1 and F2 when acetonitrile was used as co-solvent with distilled water the drug content was 60 and 66.6% respectively. But in case of F3 when distilled water was only used as solvent drug content was only 51% as shown in Table 2. Regarding particle morphology, the microparticles were sphere and smooth as shown in Fig. 1, but the morphology of microspheres with low concentration of glutaraldehyde showed slightly wrinkled and somewhat distorted surface.

## 5.2. Mucin adsorption/mucoadhesion

*H. pylori* inhabitates in the mucosal layer of the stomach and not in the deeper muscularis and serosal layer, and because the major component of stomach mucosa is mucin therefore this study was conducted on mucin to investigate mucin adsorption characteristics of spray dried furazolidone-chitosan microparticles at two different pHs. The amount of chitosan microparticles adsorbed on mucin was determined indirectly by measuring the concentration of free mucin after reaction between chitosan and mucin at different time intervals. The crosslinking agent shown the negative effect on mucin adsorption as depicted by the Fig. 2. The possible explanation could be that, increasing glutaraldehyde concentration make microparticles harder which consequently decrease the percentage of mucin adhesion ascribed in another study [15]. In the current study, at pH 4.5 the formulation F1 having lowest amount of glutaraldehyde attain the maximum adsorption after 4 h, and then became steady. However 50% was achieved in first 2 h. In contrast, the formulation F2 achieves 50% after 3 h and could maximally reach to 87% in 6 h. Formulation F3 with highest amount of glutaraldehyde attained 50% at fourth hour and shown maximum adsorption of 75% after 6 h as shown in Fig. 2a. However, at pH 1.3 similar formulations demonstrated different adhesion behavior. In current study the decrease in the pH value, decrease the amount of the mucin adsorbed, results shown in Fig. 2b; this fact ascribed in other studies [16,17], that acidic environment had negative influence on the adsorption of mucin. At pH 1.3 F1 gave only 7% adhesion in 2 h and maximally reached to 60%. However, F2 and F3 the adhesion was very low after 2 h and hardly achieved 50% after 6 h as shown in Fig. 2b. The results at two different pH levels were significant ( $p < 0.05$ ). Similarly, variable amount of crosslinking agent also has significant impact on mucin adsorption.

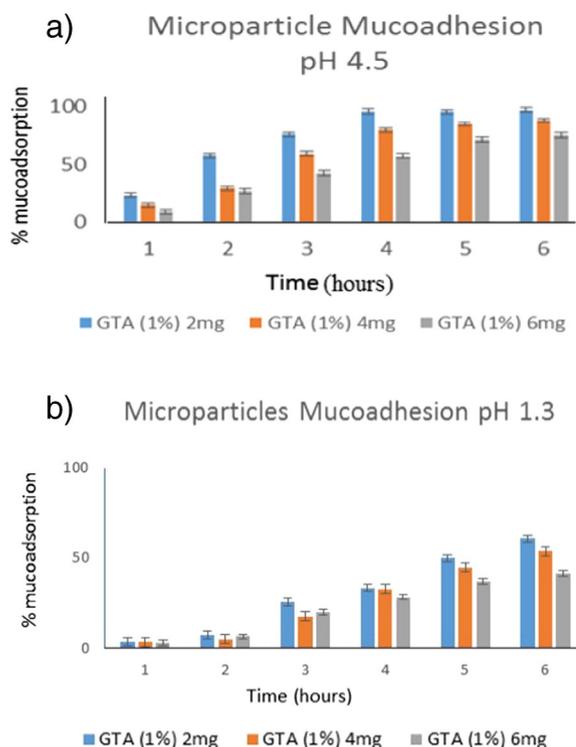


Fig. 2. Mucin adsorption of furazolidone-chitosan microparticle formulations at a) pH 4.5 and b) pH 1.3. Glutaraldehyde is abbreviated by GTA. For formulation composition refer to Table 1.

## 5.3. In-vitro drug release

In vitro drug release of microparticles are shown in Fig. 3. Results showed that release was increase by decreasing pH F1 released 91% of drug after 5 h at pH 1.3, however the same formulation released only 73% at pH 4.5. Similarly, F2 and F3 released 76% and 79% of drug respectively at pH 1.3 while the release was 62% and 51% at pH 4.5 as shown in Fig. 3. Chitosan used as a polymer for microparticles becomes soluble under low pH condition due to protonation of amino group that leads to the release of drug at lower pH [18]; it was reported that microspheres almost released all entrapped drug at pH 1.2, however, nearly 70% of the drug was released over 3 h at pH 3.0 [18]. These results are in agreement with the current study showing that increase in pH leads to slower release of the drug.

The other factor that influenced the release rate is the concentration of crosslinking agent (glutaraldehyde). Formulation that contained higher concentration of glutaraldehyde (Table 1), showed less amount of drug release as shown in Fig. 3; these results are in agreement with a study by He et al. [10] that showed by increasing glutaraldehyde the release was reduced. The effect of both pH and crosslinking agent have significant impact on release from zero time till five hours' time.

All the formulation at both pH levels demonstrated zero order kinetics as the squared correlation coefficient values of zero order kinetics are higher than those of first order kinetic (Table 3). Zero order release model describes the system where the rate of drug release is independent of its concentration and the data are designed as the amount of drug released against time [19].

## 6. Conclusion

This study demonstrated that mucoadhesive furazolidone microparticles were successfully prepared by spray drying process. Those particles showed better adherence to mucin at low acidic condition (pH 4.5), this favors the local gastric delivery of furazolidone against

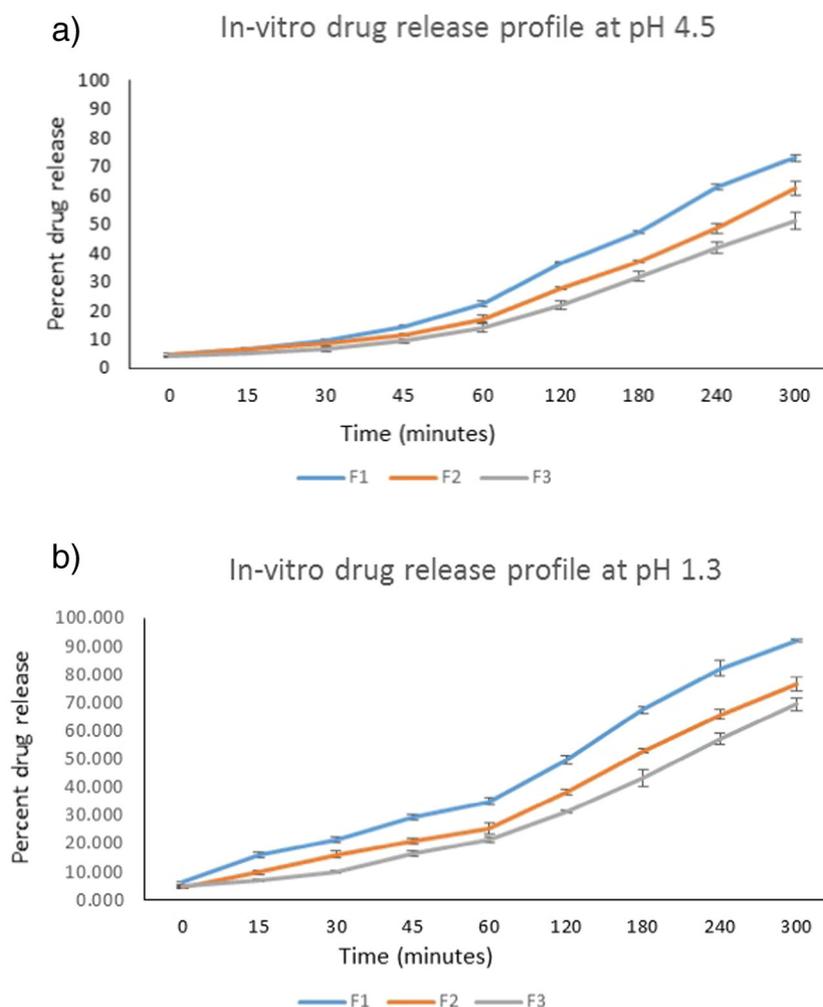


Fig. 3. In-vitro drug release of furazolidone-chitosan microparticle formulations at a) pH 4.5 and b) pH 1.3. For formulation composition refer to Table 1.

for example *H. pylori* which increases gastric pH. At pH 4.5, formulations F1, F2 and F3 accomplished 50% mucin adsorption after 2 h, 3 h and 4 h, respectively. Therefore, this research is worth considering future *in vivo* study.

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