

Pharmaceutical suspension containing both immediate/sustained-release amoxicillin-loaded gelatin nanoparticles: preparation and in vitro characterization

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Abstract: Pharmaceutical suspension containing oral dosage forms delivering both immediate-release and sustained-release amoxicillin was developed as a new dosage form to eradicate *Helicobacter pylori*. Amoxicillin-loaded gelatin nanoparticles are able to bind with the mucosal membrane after delivery to the stomach and could escalate the effectiveness of a drug, providing dual release. The objective of this study was to develop amoxicillin nanoparticles using innovative new technology – the Büchi Nano Spray Dryer B-90 – and investigate such features as drug content, particle morphology, yield, in vitro release, flow properties, and stability. The nanoparticles had an average particle size of 571 nm. The drug content and percentage yield was $89.2\% \pm 0.5\%$ and $93.3\% \pm 0.6\%$, respectively. Angle of repose of nanoparticle suspension was 26.3° and bulk density was 0.59 g/cm^3 . In vitro drug release of formulations was best fitted by first-order and Peppas models with R^2 of 0.9841 and 0.9837 respectively; release profile was 15.9%, while; for the original drug, amoxicillin, under the same conditions, 90% was released in the first 30 minutes. The nanoparticles used in this study enabled sustained release of amoxicillin over an extended period of time, up to 12 hours, and were stable for 12 months under accelerated storage conditions of $25^\circ\text{C} \pm 2^\circ\text{C}$ and $60\% \pm 5\%$ relative humidity.

Keywords: nanoparticles, drug delivery, nano spray dryer, amoxicillin, *H. pylori*

Introduction

β -Lactams are one of the best weapons clinical physicians have against *Helicobacter pylori*. Amoxicillin is a semisynthetic penicillin that is presently the only β -lactam used to treat *H. pylori* infection. It is unchanged in gastric acid with a short drug residence time in the stomach. It prevents synthesis of the bacterial cell wall and has good sensitivity to *H. pylori* in vitro.¹ These triple therapies contribute to the usefulness of amoxicillin in therapeutic applications. An effective drug concentration cannot be achieved in the gastric mucosal layer or epithelial cell surface where *H. pylori* reside.²⁻⁴ However, there is some information highlighting that management with amoxicillin cannot completely eliminate *H. pylori*, signifying that further research is necessary to expand its therapeutic effect.^{5,6} Therefore, management of large doses of amoxicillin on an everyday basis is essential for eradication of *H. pylori*. Poor patient compliance due to adverse effects such as nausea, vomiting, and diarrhea are not unusual. Subsequently, it is expected that if local delivery of antimicrobial agents from the gastric lumen into the mucus layer can be achieved, then the *H. pylori* eradication rate would be increased.⁷

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Several dosage forms have been recommended to help retain amoxicillin in the stomach, including nanocapsules,⁸ gellan beads,⁹ floating microballoons,¹⁰ hydrogel nanoparticles,¹¹ and mucoadhesive microparticles,¹² which are able to stay in the gastrointestinal tract for an extended time, and can therefore prove more effective for *H. pylori* eradication. Various methods can be used to prepare polymer nanospheres, such as microemulsion, interfacial polymerization, miniemulsion, salting out, dialysis, supercritical fluid technology surfactant-free emulsion, and solvent evaporation.¹³ Spray drying is a well-known, quick, one-step process frequently used in the pharmaceutical industry to produce a dry powder from a liquid form.¹⁴

In light of the above, there is a pressing need to develop a novel formulation that carries amoxicillin into the stomach to bind with the gastric mucosa, to intensify the efficiency of the drug, and to offer a sustained action. To date, to the author's knowledge, no study has been carried out on the formulation of a suspension containing a dual delivery formulation of amoxicillin. The purpose of this study was to design amoxicillin-loaded gelatin nanoparticles as a new dual delivery suspension containing pure amoxicillin as well as a granulated nanoparticle form of amoxicillin using a Büchi Nano Spray Dryer B-90 (BUCHI Labortechnik AG, Flawil, Switzerland) for *H. pylori* eradication management.

Materials and methods

Amoxicillin trihydrate was kindly gifted for this research by Karnataka Antibiotics and Pharmaceuticals Ltd (Bangalore, India). Gelatin bloom strength 175, xanthan gum, D-sorbitol powder, citric acid, sodium citrate, sodium lauryl sulfate, and sodium benzoate were obtained from Sigma-Aldrich (St Louis, MO, USA). All other reagents and chemicals used were of analytical grade.

Preparation of nanoparticles by spray drying

Amoxicillin-loaded gelatin nanoparticles (GLAN) were formulated using a spray drying technique.^{15,16} The experiments were designed according to our previous published work.¹⁶ The optimized parameters included the concentration of gelatin (500 mg), inlet temperature (80°C), and flow rate (21 mL/hour). A solution of gelatin (gelatin soaked for 1 hour in 50 mL of water and warmed before spraying) containing a predetermined quantity of amoxicillin was sprayed using spray caps incorporated with mesh of 4 µm hole size. All samples were filtered prior to the spray drying process to

avoid blockage of the mesh hole. The piezo-driven spray head generates ultrafine droplets, which are gently dried into solid particles. The dried powder is electrostatically charged and collected at the collecting electrode, and the particles were scraped and stored in a desiccator at 25°C for further investigation.

Morphology of spray-dried particles

The surface morphology of the nanoparticles was observed using scanning electron microscopy (FEI Quanta 200; Quanta Technology, LLC, Raleigh, NC, USA). The nanoparticles were placed in metal stubs and coated with gold using an ion sputter machine (JEOL, Tokyo, Japan) and visualized at 12 kV.^{17,18}

Particle size analysis

A study of amoxicillin nanoparticle size was done using laser light diffraction and an ultrasonic method. The nanoparticles were diluted 5-fold with deionized water using a Malvern Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK).^{17,19}

Determination of amoxicillin drug content in nanoparticles

A known amount of amoxicillin nanoparticles was dissolved in 0.1 M hydrochloric acid. The amoxicillin content was assayed using a spectrophotometer (UV-1601 spectrophotometer; Shimadzu, Tokyo, Japan) at 272 nm by constructing a calibration curve. The best-fitting straight line equation of the calibration curve was found to be $A = 0.294x + 0.0071$.¹⁶ The investigations were performed in triplicate. The drug content was considered as the ratio between the actual and theoretical quantity of amoxicillin loaded for an optimized preparation.

$$\begin{aligned} \text{Amoxicillin content} \\ = \frac{\text{Weight of amoxicillin in nanoparticles}}{\text{Total weight of the nanoparticles}} \times 100 \quad (1) \end{aligned}$$

Product yield/recovery

The efficiency of the nanoparticle yield was determined as the percentage weight of the nanoparticle achieved compared with the total amount of drug and polymer initially used in the formulation procedure.¹⁷

$$\begin{aligned} \text{Nanoparticle yield/recovery} \\ = \frac{\text{Total weight of nanoparticles recovered}}{\text{Entire weight of the polymer + drug}} \times 100 \quad (2) \end{aligned}$$

Angle of repose

The angle of repose was assessed to understand the flow ability of the nanoparticle suspension powder by passing the powder suspension through a funnel on a horizontal surface. The height (h) of the heap formed was measured and the radius (r) of the cone base was also determined. The angle of repose (ϕ) was calculated as:²⁰

$$\phi = \tan^{-1} \left(\frac{h}{r} \right) \quad (3)$$

Bulk density

The spray-dried powder was introduced into a 100 mL graduated cylinder. The volume of the material was noted on the graduated cylinder. The bulk density was determined by following the formula:

$$\text{Bulk density g/cm}^3 = \frac{W}{V} \quad (4)$$

where “w” is the weight of the nanoparticle powder and “v” is the volume of the nanoparticle powder.

In vitro drug release and kinetics

In vitro drug release studies were carried out according to a previously published article.¹⁶ Briefly, both the nanoparticles and the dry powder suspensions were carried out using the paddle method specified in United States Pharmacopeia (USP) XXVII.¹⁶ The in vitro release studies were carried out using the USP I basket method (50 rpm, 500 mL, and 37°C ± 1°C). Amoxicillin-loaded nanoparticle suspensions equivalent to 250 mg of drug were positioned in a dialysis cellulose membrane bag (D9402-100FT; Sigma-Aldrich) with a molecular weight cutoff of 12,400 Da, retained and placed into the baskets, and transferred to 500 mL of 0.05 M phosphate buffer. Aliquots were withdrawn at preset time intervals and an equal volume of dissolution medium was substituted into the flask to maintain a constant volume. The drawn samples were examined at 272 nm, and the results were used to determine a collective drug release profile for the nanoparticles. Sink conditions were maintained throughout. Examination of the suspension comprising of only amoxicillin was carried out in a similar manner.^{21,22}

Formulation of dry syrup for reconstitution

Oral suspensions for reconstitution were prepared using the formulae shown in Table 1. To formulate a dry blend for

Table 1 Formulation of dry suspension of amoxicillin-loaded nanoparticles for reconstitution

Solution number	Components	Concentration
1	Amoxicillin-loaded nanoparticles	Equivalent to 500 mg amoxicillin
2	Xanthan gum	0.6%
3	D-sorbitol powder	20%
4	Citric acid	1.5%
5	Sodium citrate	0.75%
6	Sodium lauryl sulfate	0.05%
7	Sodium benzoate	0.2%

reconstitution, the powder constituents were decreased to nearly the same particle size. Xanthan gum was incorporated as the efficient suspending agent in the suspensions. D-Sorbitol powder was added to impart a slightly sweet taste. Citric acid and sodium citrate were used to vary the pH of the study preparations. Components present in small quantities (sodium lauryl sulfate [0.05%], sodium benzoate [0.2%], and buffer components) were mixed equally. The same procedure was followed for xanthan gum (0.6%) and amoxicillin-loaded nanoparticles (comprising the equivalent of 500 mg amoxicillin). The ingredients were blended with a portion of D-Sorbitol (20%) according to the geometric dilution procedure. To prepare the reconstituted suspension, approximately 10 mL of water was added to the bottle containing dry suspension powder and stirred with a spoon until a homogeneous product was obtained.²³

Stability of dry suspension

The stability model was planned based on the International Conference On Harmonization (ICH) Q2B guidelines.^{17,24,25} The nanoparticles were stored at 25°C ± 2°C and 60% ± 5% relative humidity for a period of 12 months. This is the long-term storage temperature for products that are intended to be refrigerated when considering the thermosensitivity and thermolability of the polymer.^{17,24,25} Gelatin is a biopolymer produced by denaturing collagen, which is the major protein present in animal connective tissue; it is sensitive to degradation as long as no gel has formed. The stored samples were confirmed for their physical change, particle size distribution, and drug content. The drug content was analyzed by a UV spectrophotometric method. The testing was carried out at 0, 2, 4, 6, and 12 months (as per the FDA’s guideline for submitting papers on stability of human drugs and biologics, DHHA, February 1987) for long-term storage conditions and at 3-month intervals for a period of 12 months for long-term storage conditions as per ICH guidelines (ICH Q6A) for stability studies.¹⁶

Results and discussion

Spray-dried particle morphology

The amoxicillin nanoparticles designed under optimized experimental conditions were shriveled sphere structures and were a free-flowing powder (Figure 1). Other pharmaceutical scientists have also stated that spray-dried particles developed a shriveled shape and led to high surface area, which is advantageous for dissolution enhancement, and that shrinking may also be due to quick drying in the spraying compartment.^{26,27} A shriveled shape might also be due to the lack of a plasticizer. We did not use a plasticizer in our experiments due to possible irritation in the gastrointestinal tract as the dosage form was intended to stay in the body for a longer time.²⁸

Drug content, yield, angle of repose, and bulk density

The drug content and percentage yield of the formulation were found to be $89.2\% \pm 0.5\%$ and $93.3\% \pm 0.6\%$, respectively. This is possible in practice only with the use of the Büchi Nano Spray Dryer B-90, which can create much higher yields compared to some traditional preparations.^{29,30} The minimal losses may be due to particle collection with the manual particle scraper from the collecting electrode. The angle of repose of the nanoparticle suspension powder was 26.3°C , indicating that the formulation was free-flowing in nature and that the bulk density was freely settled having a value of 0.59 g/cm^3 .

Analysis of nanoparticle size

Spray drying has recently been gaining greater consideration as it is a gentle, continuous, and scalable drying method by

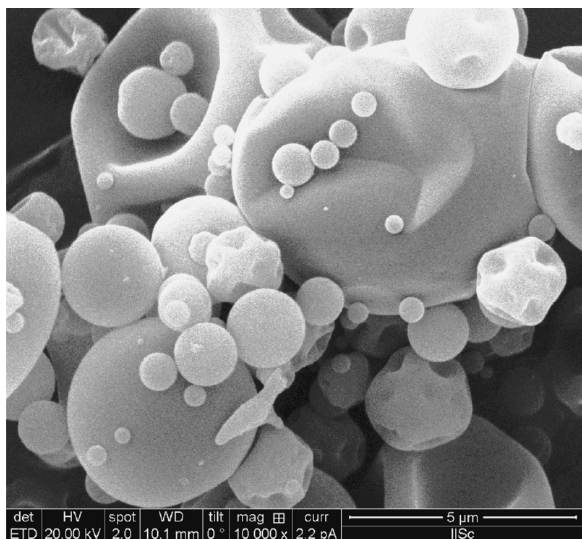


Figure 1 Scanning electron microscopy image of amoxicillin-loaded gelatin nanoparticles prepared by a spray dryer.

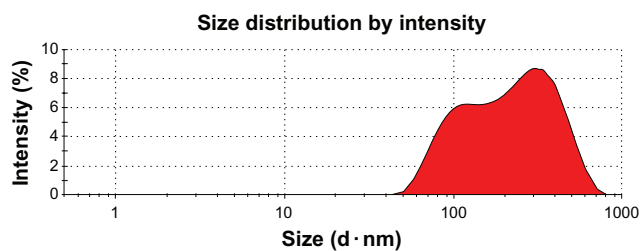


Figure 2 Particle size distributions for the amoxicillin nanoparticle formulation.

which to transform liquids to dry powders. It also benefits from marginal loss of high-value powder due to exceptionally high yields, and is an efficient and fast process.¹⁶ Figure 2 shows the formation of amoxicillin nanoparticles with heterogeneous particle size distribution. This is important because, when the particles are of uniform size, the spacing is the greatest, but when a range of sizes is used, the void spaces are filled in the target region (stomach). Similar results have been reported elsewhere of nanoparticles formulated by a spray-drying process.³¹ The average diameter of a nanoparticle was 571 nm.

Release behavior of amoxicillin

In the drug-manufacturing industry, drug-dissolution testing is routinely done and it is extremely important to know the release pattern and release kinetics of the formulation. Figure 3 illustrates the in vitro drug release from the amoxicillin suspension and the gelatin nanoparticles containing amoxicillin. The formulation shows a close to biphasic release profile. Around 15.9% of the overall amount of amoxicillin in the nanoparticles was released in the first hour. This initial burst of release may be attributed to the drug molecules positioned within the shell or at the interface between the core and the shell. This system can produce a rapid rise in the plasma concentration

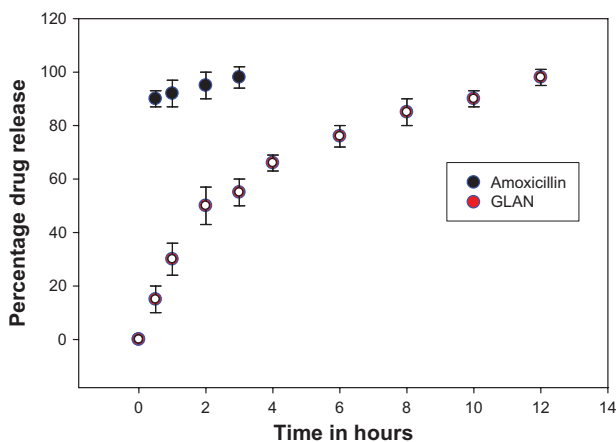


Figure 3 Drug-release profile of amoxicillin alone and of amoxicillin-loaded gelatin nanoparticles (GLAN).

Table 2 In vitro release models with correlation coefficient value (R^2)

Release model	Equation	R^2 values
Baker and Lonsdale	$= \frac{2}{3} \left[1 - \left(1 - \frac{F}{100} \right)^{\frac{2}{3}} \right] - \frac{F}{100} = Kt$	0.9789
Peppas	$= F = Kt^n$	0.9837
Hixson and Crowell	$= F = 100[1 - (1 - Kt)^3]$	0.9554
Higuchi, square root of time	$= F = K\sqrt{t}$	0.9790
First-order	$= 100[1 - e^{-Kt}]$	0.9841

Abbreviations: F, amount of drug released in time (t); K, release rate constant; n, release exponent.

for some drugs that are required to have a timely therapeutic effect. However, complete drug release from the amoxicillin nanoparticles was prolonged up to 12 hours. During this period, the amount released was 98.0%. In comparison with the amoxicillin nanoparticles, the amoxicillin suspension showed faster amoxicillin release, with 90% of the amoxicillin released in 30 minutes.¹⁶ This result specifies that nanoparticles have potential in a controlled-release formulation of amoxicillin. Further studies in animal models are necessary to confirm the in vivo activity of amoxicillin nanoparticles with respect to the eradication of *H. pylori* infection.

Analysis of release kinetics

In order to understand the release kinetics of amoxicillin from GLAN nanoparticles, the in vitro release data were fitted to all well-known kinetic equations (Table 2).^{16,32,33} The release mechanism was calculated from the slope of the appropriate plots and the regression coefficient (R^2) was determined by Sigma Plot software (v 9.01; Systat Software, Inc, San Jose, CA, USA). It was found that the in vitro drug release of

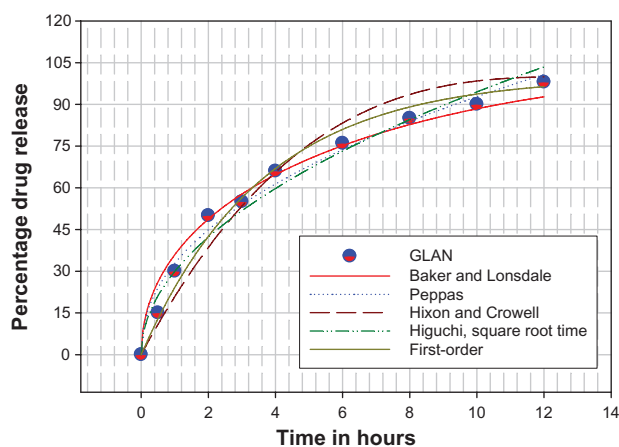


Figure 4 In vitro release profile for amoxicillin nanoparticles (different model fitting). **Abbreviation:** GLAN, amoxicillin-loaded gelatin nanoparticles.

Table 3 Interpretations of stability test readings under long-term storage conditions ($25^\circ\text{C} \pm 2^\circ\text{C}$ and $60\% \pm 5\%$ relative humidity)

	Months				
	0	3	6	9	12
Physical change	–	–	–	–	–
Particle size (nm)	571	576	578	580	582
Drug content (%)	100.5	99.8	99.3	98.9	96.9

Note: “–” indicates no physical change.

GLAN formulations was best explained by two models (Figure 4). A novel observation has been made in this study that the drug release agreed well with the first-order and Peppas models. The values of the correlation coefficient (R^2) of the first-order and Peppas models for the obtained release data were approximately 0.9841 and 0.9837, respectively. The first-order kinetics equation describes the dissolution of amoxicillin that is not successfully bound in the gelatin matrix and which is ready to dissolve from the nanoparticle surface. This rapid progression occurs immediately after dipping a sample into release medium and is called “the burst effect.” The Peppas kinetic model defines the drug-release mechanism depending on its data; the release process can be governed by Fickian diffusion ($n = 0.5$), by gelatin polymeric matrix erosion, or by the combination of both mechanisms. The release pattern was more controlled and was sustained for a longer period of time, up to 12 hours.

Stability studies of GLAN

The amoxicillin content under long-term storage conditions did not vary to a large extent in the nanoparticle formulations; the maximum variation of 1.7% from the initial concentration was seen in GLAN 24 months from the date of production. The preparation was stable for 24 months under the long-term

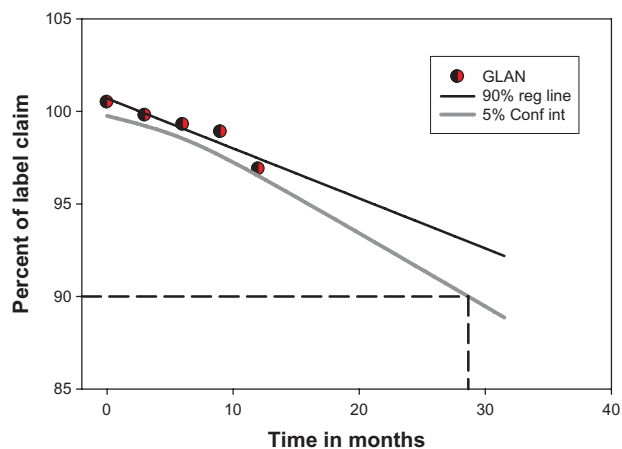


Figure 5 Drug-content stability plot for amoxicillin nanoparticles under long-term storage conditions.

Abbreviations: GLAN, amoxicillin-loaded gelatin nanoparticles; reg, regression; Conf int, confidence interval.

storage conditions of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $60\% \pm 5\%$ relative humidity. A maximum decrease of 2.6% from the original concentration was observed (Table 3). The average particle size was 571 nm and did not vary appreciably. It was more or less shriveled in nature and no physical changes, such as liquefaction, formation of bulges, or bruising, were observed in the nanoparticle preparations throughout the 24-month study period. Nevertheless, the observed changes were found to be insignificant and had no influence on the quality of the preparation.²⁵ Figure 5 shows the fall of drug content of GLAN in long-term storage environments.

Conclusion

In the present study, a pharmaceutical suspension containing oral dosage forms for delivering both immediate-release and sustained-release amoxicillin was developed as a new dosage form with which to eradicate *H. pylori*. Our results can also be used to develop an effective outline for targeted stomach-specific drug delivery, and can be explored further in vivo with other drugs, using a slight alteration in the methods used here for formulation of the nanoparticles.

The amoxicillin nanoparticle suspension established for this investigation was found to have suitable physical properties or chemical properties and to have a satisfactory particle size. The nanoparticles were found to release the amoxicillin to a maximum extent in vitro. This unusual dual-release formulation supplements the already existing domain of site-specific drug delivery systems and holds promise as a substitute to conventional drug delivery, allowing that bacteria, such as *H. pylori*, are extremely challenging to treat and to manage efficiently.

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Disclosure

The author reports no conflicts of interest in this work.

References

- Romano A, Cuomo A. Eradication of *Helicobacter pylori*: a clinical update. *Med Gen Med*. 2004;6:19.
- Axon AT. The role of acid inhibition in the treatment of *Helicobacter pylori* infection. *Scand J Gastroenterol Suppl*. 1994;201:16–23.
- Fontana G, Licciardi M, Mansueto S, Schillaci D, Giammona G. Amoxicillin-loaded polyethylcyanoacrylate nanoparticles. influence of PEG coating on the particle size, drug release rate and phagocytic uptake. *Biomaterials*. 2001;22:2857–2865.
- Patel JK, Chavda JR. Formulation and evaluation of stomach-specific amoxicillin-loaded carbopol-934P mucoadhesive microspheres for anti-*Helicobacter pylori* therapy. *J Microencap*. 2009;26:365–376.
- Lin CK, Hsu PI, Lai KH, et al. One-week quadruple therapy is an effective salvage regimen for *Helicobacter pylori* infection in patients after failure of standard triple therapy. *J Clin Gastroenterol*. 2002;34:547–551.
- Liu Z, Lu W, Qian L, Zhang X, Zeng P, Pan J. In vitro and in vivo studies on mucoadhesive microspheres of amoxicillin. *J Control Release*. 2005;102:135–144.
- Nagahara N, Akiyama Y, Nakao M, Tada M, Kitano M, Ogawa Y. Mucoadhesive microspheres containing amoxicillin for clearance of *Helicobacter pylori*. *Antimicrob Agents Chemother*. 1998;42:2492–2494.
- Jain P, Jain S, Prasad KN, Jain SK, Vyas SP. Polyelectrolyte coated multilayered liposomes (nanocapsules) for the treatment of *Helicobacter pylori* infection. *Mol Pharm*. 2009;6:593–603.
- Narkar M, Sher P, Pawar A. Stomach-specific controlled release gellan beads of acid-soluble drug prepared by ionotropic gelation method. *AAPS Pharm Sci Tech*. 2010;11:267–277.
- Awasthi R, Kulkarni GT, Pawar VK, Garg G. Optimization studies on gastroretentive floating system using response surface methodology. *AAPS Pharm Sci Tech*. 2012;13:85–93.
- Moogooee M, Ramezanzadeh H, Jasoore S, Omid Y, Davaran S. Synthesis and in vitro studies of cross-linked hydrogel nanoparticles containing amoxicillin. *J Pharm Sci*. 2011;100:1057–1066.
- Sambathkumar R, Venkateswaramurthy N, Vijayabaskaran M, Perumal P. Formulation of clarithromycin loaded mucoadhesive microspheres by emulsification-internal gelation technique for anti-*Helicobacter pylori* therapy. *Int J Pharm and Pharm Sci*. 2011;3:173–177.
- Sham JO, Zhang Y, Finlay WH, Roa WH, Lööbenberg R. Formulation and characterization of spray-dried powders containing nanoparticles for aerosol delivery to the lung. *Int J Pharm*. 2004;269:457–467.
- Pilcer G, Vanderbist F, Amighi K. Preparation and characterization of spray-dried tobramycin powders containing nanoparticles for pulmonary delivery. *Int J Pharm*. 2009;365:162–169.
- Li X, Anton N, Arpagaus C, Belleteix F, Vandamme TF. Nanoparticles by spray drying using innovative new technology: the Buchi nano spray dryer B-90. *J Control Release*. 2010;147:304–310.
- Harsha S. Dual drug delivery system for targeting *H. pylori* in the stomach: preparation and in vitro characterization of amoxicillin-loaded Carbopol® nanospheres. *Int J Nanomedicine*. 2012;7:4787–4796.
- Harsha NS, Rani RH. Drug targeting to lungs by way of microspheres. *Arch Pharm Res*. 2006;29:598–604.
- Nowacek AS, Balkundi S, McMillan J, et al. Analyses of nanoformulated antiretroviral drug charge, size, shape and content for uptake, drug release and antiviral activities in human monocyte-derived macrophages. *J Control Release*. 2011;150:204–211.
- Lu B, Zhang JQ, Yang H. Lung-targeting microspheres of carboplatin. *Int J Pharm*. 2003;265:1–11.
- Gaba P, Singh S, Gaba M, Gupta GD. Galactomannan gum coated mucoadhesive microspheres of glipizide for treatment of type 2 diabetes mellitus: in vitro and in vivo evaluation. *Saudi Pharmaceutical Journal*. 2011;19:143–152.
- Singh SK, Chidrawar VR, Ushir YV, Vadalala KR, Sheth NR, Singh S. Pharmaceutical characterization of amoxicillin trihydrate as mucoadhesive microspheres in management of *H. Pylori*. *Int J Pharm Tech Res*. 2010;6:348–358.
- Nayak AK, Malakar J. Formulation and in vitro evaluation of hydrodynamically balanced system for theophylline delivery. *J Basic Clin Pharm*. 2011;2:133–137.
- Raula J, Eerikäinen H, Kauppinen EI. Influence of the solvent composition on the aerosol synthesis of pharmaceutical polymer nanoparticles. *Int J Pharm*. 2004;284:13–21.
- Lacoulonche F, Gamisans F, Chauvet A, Garcia ML, Espina M, Egea MA. Stability and in vitro drug release of flurbiprofen-loaded poly-epsilon-caprolactone nanospheres. *Drug Dev Ind Pharm*. 1999;25:983–993.
- Harsha S, R C, Rani S. Ofloxacin targeting to lungs by way of microspheres. *Int J Pharm*. 2009;380:127–132.

26. Miller DA, Gil M. Spray-drying technology. In: Williams RO III, Watts AB, Miller DA, editors. *Formulating Poorly Water Soluble Drugs*. New York: Springer; 2012:363–442.
27. De Jaeghere F, Allémann E, Cerny R, et al. pH-Dependent dissolving nano- and microparticles for improved peroral delivery of a highly lipophilic compound in dogs. *AAPS Pharm Sci*. 2001;3:E8.
28. Coowanitwong I, Arya V, Kulvanich P, Hochhaus G. Slow release formulations of inhaled rifampin. *AAPS J*. 2008;10:342–348.
29. Sansone F, Rossi A, Del Gaudio P, De Simone F, Aquino RP, Lauro MR. Hesperidin gastroresistant microparticles by spray-drying: preparation, characterization, and dissolution profiles. *AAPS Pharm Sci Tech*. 2009; 10:391–401.
30. Atuah KN, Walter E, Merkle HP, Alpar HO. Encapsulation of plasmid DNA in PLGA-stearylamine microspheres: a comparison of solvent evaporation and spray-drying methods. *J Microencapsul*. 2003;20: 387–399.
31. Maas SG, Schaldach G, Litringer EM, et al. The impact of spray drying outlet temperature on the particle morphology of mannitol. *Powder Technology*. 2011;213:27–35.
32. Rosario-Meléndez R, Ouimet MA, Uhrich KE. Formulation of salicylate-based poly(anhydride-ester) microspheres for short- and long-term salicylic acid delivery. *Polymer Bulletin*. 2013;70:343–351.
33. Nochos A, Douroumis D, Bouropoulos N. In vitro release of bovine serum albumin from alginate/HPMC hydrogel beads. *Carbohydrate Polymers*. 2008;74:451–457.

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