

Clarithromycin Based Oral Sustained Release Nanoparticulate Drug Delivery System

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The aim of the present work is to prepare and evaluate the oral mucoadhesive sustained release nanoparticles of clarithromycin in order to improve patient compliance by simplifying its administration, improving its therapeutic effect and reducing its dose related side effect. Clarithromycin containing gliadin nanoparticles were prepared by desolvation method using pluronic F-68® as a stabilizing agent. The results showed that this method is reproducible, very easy and led to the efficient entrapment of drug as well as formation of spherical particles ranging from 250-500 nm. Some process variables like effect of gliadin concentration and effect of surfactant were also evaluated with respect to their % drug entrapment and % yields. The maximum % drug entrapment and % yield were about 73 and 88%, respectively. The sustained release behavior of gliadin nanoparticles were evaluated both in phosphate buffer saline (pH 7.4) and simulated gastric fluid (pH 1.2), respectively, at 37±1°. Their mucoadhesive properties were determined by *in vitro* and *in vivo* methods. The shelf life of prepared nanoparticles was determined by storage at various temperatures while assessed in simulated gastric fluid (pH 1.2) with and without enzyme.

Clarithromycin is a broad spectrum macrolide antibacterial agent that is effective both *in vitro* and *in vivo* against major pathogens responsible for peptic ulcer by *Helicobacter pylori*¹ and other respiratory tract infections of *Clamidia pneumoniae*, *Mycoplasma pneumoniae*,²⁻⁵ *Streptococcus pneumoniae* and *Haemophilus influenzae*. Clarithromycin is well absorbed from the GI tract, but its systemic bioavailability (55%) is relatively low due to first-pass metabolism. It undergoes rapid biodegradation to produce the microbiologically active 14-hydroxy metabolite.

Oral drug administration represents the most convenient and common route of drug delivery. However, the bioavailability of a large number of drugs after oral administration is very low due to various reasons; too short gastric residence times, drug instability in the GI tract or lack of intestinal permeation of the drug. One possibility to improve the gastrointestinal uptake of perorally poorly absorbed drugs by their binding to colloidal particles, is nanoparticles. Nanoparticles can protect labile molecules from degradation in the GI tract and might be able to transport non-absorbable molecules into the systemic circulation.

These nanoparticulate carriers have the ability for both controlling the release and protecting the loaded drug against its degradations. Moreover, the small particle size allows them to penetrate in mucus layer and thus bind to the underlying epithelium and /or adhere directly to mucus network⁶. These adhesive-interactions of the particles with the boundary layer may improve the drug bioavailability by a number of different mechanisms. Nanoparticles may enhance the drug absorption rate by reducing the diffusion barriers between the pharmaceutical dosage form and site of action or absorption⁷. Similarly, they may prolong the residence time. Gliadin nanoparticles have a strong adhesive capacity with GI mucosa, may be due to gliadin composition. Neutral amino acids of gliadin can promote hydrogen-bonding interactions with the mucosa whereas its lipophilic components can interact with the biologic tissue by hydrophobic interactions⁸. The bioadhesive capacity of gliadin nanoparticles also has been evaluated when administered by the oral route to animals⁹. Gliadin nanoparticles show a great tropism for the upper gastrointestinal regions and their presence in other intestinal regions is very low¹⁰.

MATERIALS AND METHODS

Clarithromycin was procured as gift from Sun Pharma

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Advanced Research Centre (Vadodara) India. Gliadin was purchased from Sigma (USA). Pluronic F 68[®] was obtained from Himedia, India. All other reagents were of analytical grade.

Preparation of gliadin nanoparticles:

Gliadin nanoparticles were prepared by a desolvation procedure described previously⁸. Briefly, gliadin and Clarithromycin were dissolved in 20 ml of an ethanol: water phase (7:3 v/v) and this solution was poured into a stirred physiological saline phase (NaCl 0.9% w/v in water), containing 0.5% Pluronic F-68[®] as a stabilizer. Then ethanol was eliminated by evaporation under reduced pressure (Buchi R-140, Switzerland) and the resulting nanoparticles were purified by centrifugation at 15,000 rpm for 1 h (Eltech centrifuge, India). The supernatant was removed and the pellets were resuspended in water. The suspension was passed through a 0.45 µm pore size membrane filter and the filtrate was centrifuged again and finally, the nanoparticles were freeze-dried using 5% glucose solution as a cryoprotector. Nanoparticles were hardened by the addition of 2 mg glutaraldehyde per mg nanoparticles and stirred for 2 h at room temperature before purification and freeze-drying (Table 1).

Particle size and morphology:

The particle size, size distribution and zeta potential of gliadin nanoparticles were measured in a Zetasizer 3000 HS (Malvern Instrument Ltd., UK). Surface morphology and internal structure of gliadin nanoparticles were determined by scanning electron microscopy (SEM). A thin film of aqueous dispersion of nanoparticles was applied on double stick tape over an aluminum stub and air dried to get uniform layer of particles. These particles were coated with gold to a thickness of about 450 Å using Sputter gold coater. (fig.1)

Percentage drug entrapment and percentage recovery:

% Drug entrapment = Mass of drug in nanoparticles /

TABLE 1: FORMULAE FOR PREPARATION OF GLIADIN NANOPARTICLES

Drug:gliadin (ratio)	Formulation code(s)	Volume (ml) of inner ethanol:water phase (7:3 ratio)	Volume of outer phase (ml, saline)
1:0.5	GNP ₁	20	100
1:1	GNP ₂	20	100
1:2	GNP ₃	20	100
1:3	GNP ₄	20	100

GNP₁ is gliadin nanoparticles (drug:gliadin ratio,1:0.5), GNP₂ is gliadin nanoparticles (drug:gliadin ratio, 1:1), GNP₃ is gliadin nanoparticles (drug:gliadin ratio, 1:2) and GNP₄ is gliadin nanoparticles (drug:gliadin ratio, 1:3).

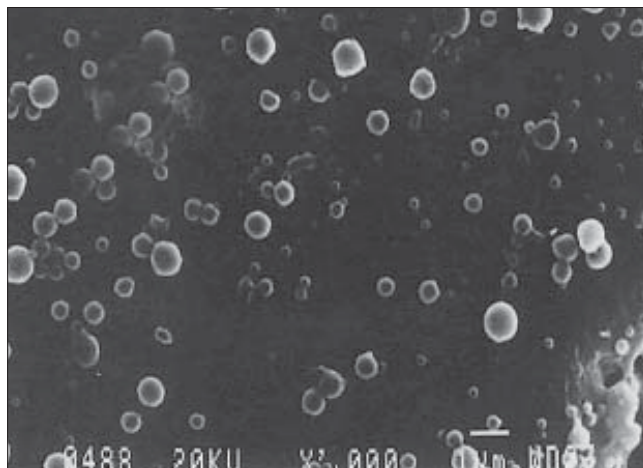


Fig. 1: SEM photomicrograph of gliadin nanoparticles

Mass of drug used in the formulation x 100
 % Nanoparticles recovery (% yield) = Concentration of drug in nanoparticles / Concentration of nanoparticles recovered x 100

The percentage drug entrapment and percentage recovery were determined using following equations¹¹. Appropriate amount of freeze-dried gliadin nanoparticles were digested with minimum amount of ethanolic solution (water:ethanol in 7:3 ratios). The digested homogenates were centrifuged at 15,000 rpm for 30 min and supernatant was analyzed for drug entrapment. The clarithromycin entrapment was measured at 760 nm using Shimadzu 1601 UV/Vis. using spectrophotometer (Table 2).

In vitro drug release studies:

Various formulations, respectively, CGNP₁, CGNP₄ were selected for the *in vitro* drug release studies¹². *In vitro* drug release of clarithromycin from gliadin nanoparticles were estimated by the dialysis cell membrane method in two different media, respectively phosphate buffer saline (PBS) (pH 7.4) (Table 3), simulated gastric fluid (SGF) (pH 1.2) (Table 4) at 37±1° for 24 h under sink condition.

Appropriate volume of gliadin nanoparticles dialysed was taken in dialysis tube against 100 ml of media, continuously

TABLE 2: PERCENTAGE DRUG ENTRAPMENT AND% RECOVERY

Formulation code(s)	% drug entrapment	% yield
GNP ₁	43.7±2.3	63.6±1.7
GNP ₂	55.6±3.4	65.2±2.3
GNP ₃	73.1±1.3	88.7±2.8
GNP ₄	68.6±2.1	76.1±1.9

GNP₁ is gliadin nanoparticles (drug:gliadin ratio,1:0.5), GNP₂ is gliadin nanoparticles (drug:gliadin ratio, 1:1), GNP₃ is gliadin nanoparticles (drug:gliadin ratio, 1:2) and GNP₄ is gliadin nanoparticles (drug:gliadin ratio, 1:3).

TABLE 3: IN VITRO DRUG RELEASE PROFILE IN PBS (PH 7.4) AT 37±1°

Time (h)	% cumulative drug released			
	CGNP ₁	CGNP ₂	CGNP ₃	CGNP ₄
1	8.3±1.2	9.1±2.3	5.2±2.1	5.0±2.2
2	12.9±3.2	9.9±2.4	5.7±2.3	6.8±2.3
3	13.4±2.2	11.6±3.0	6.1±1.8	8.1±2.6
4	15.2±1.3	13.9±1.6	6.9±2.3	11.7±1.3
5	18.7±3.0	16.7±1.9	8.3±2.6	14.2±2.9
6	21.2±2.9	17.2±2.4	10.5±1.6	17.6±1.5
7	25.8±2.3	19.1±3.3	19.7±2.4	18.5±2.4
8	26.2±1.8	19.9±2.1	21.8±3.2	20.7±3.6
24	65.7±2.3	58.2±2.6	63.4±1.6	49.7±2.3

GNP₁ is gliadin nanoparticles (drug:gliadin ratio,1:0.5), GNP₂ is gliadin nanoparticles (drug:gliadin ratio, 1:1), GNP₃ is gliadin nanoparticles (drug:gliadin ratio, 1:2) and GNP₄ is gliadin nanoparticles (drug:gliadin ratio, 1:3).

TABLE 4: IN VITRO DRUG RELEASE PROFILE IN SGF (PH 1.2) AT 37±1°

Time(h)	% cumulative drug released			
	CGNP ₁	CGNP ₂	CGNP ₃	CGNP ₄
1	6.0±2.3	6.3±3.1	3.2±1.4	6.4±0.9
2	9.3±2.6	7.8±2.2	10.5±2.1	8.2±1.2
3	10.9±1.9	11.6±1.3	20.2±1.9	12.9±3.1
4	17.2±1.6	24.9±2.0	29.8±2.3	16.2±2.1
5	24.2±3.2	27.8±3.2	35.1±2.6	19.3±1.6
6	36.4±2.7	38.4±1.2	40.1±1.6	23.1±2.1
7	41.2±3.4	43.2±1.7	42.2±2.3	25.7±3.1
8	54.3±1.8	49.8±3.1	53.9±1.9	38.9±2.1
24	73.2±2.7	68.2±1.6	62.3±2.5	52.6±1.9

GNP₁ is gliadin nanoparticles (drug:gliadin ratio,1:0.5), GNP₂ is gliadin nanoparticles (drug:gliadin ratio, 1:1), GNP₃ is gliadin nanoparticles (drug:gliadin ratio, 1:2) and GNP₄ is gliadin nanoparticles (drug:gliadin ratio, 1:3).

stirred with magnetic stirrer at 37±1°. After appropriate time intervals (one h) one ml sample was withdrawn and analyzed for clarithromycin content at 760 nm, in a Shimadzu 1601 UV/Vis. spectrophotometer. Equal volume of fresh media preheated to 37° was added to replace the withdrawn sample.

In vitro evaluation of gastric mucoadhesion of nanoparticles:

Male Sprague Dawley rats weighing 200-250 g were fasted overnight before the experiments, but allowed free access to water *ad libitum*. The stomach was excised under anesthesia and perfused with physiological saline to remove the contents of stomach. The cleaned stomach was used immediately after preparation. A 100 mg of nanoparticles sample that were suspended in SGF (pH 1.2) was filled into the cleaned stomach, ligated and then incubated in physiological saline at 37° for 30 min. The liquid content of stomach was then removed by injecting the air and the stomach was perfused with SGF (pH 1.2) for 30 min, at a flow rate of 1 ml per min. The stomach was cut to open and nanoparticles that remained in

stomach were recovered with SGF (pH 1.2). The final volume of washing solution was mixed with 10 ml of ethanolic solution and kept for 2 h for complete digestion of gliadin nanoparticles. After filtration through 0.45 µm filter paper, absorbance was determined spectrophotometrically at 230.6 nm (gliadin) and gastric mucoadhesion was determined as the % of nanoparticles remaining in stomach after perfusion.

In vivo evaluation of gastric mucoadhesion of nanoparticles:

Sprague Dawley rats (200-250 g) were fasted for 24 h before experiment but were allowed free access to water *ad libitum*. Nanoparticles (100 mg) that were filled in capsule were administrated to rats using a gastric sonde. Four hr post administration, the rats were sacrificed and stomach was removed and washed with SGF (pH 1.2) to recover the remaining nanoparticles. The amount of nanoparticles that remained in the stomach was determined using the same methods as described above under *in vitro* methods.

For X-ray studies, gliadin nanoparticles were prepared containing barium sulfate as contrast agent for *in vivo* studies. The study was carried out with albino rats free of detectable gastrointestinal diseases or disorders, each animal, having fasted overnight. After 30 min, each subject was ingested with nanoparticles formulation together with 2 ml of water. The intragastric behavior of formulations after dosing was observed by taking a series of X-ray photographs at suitable time intervals (figs. 2-7).

Stability studies:

The stability of gliadin nanoparticles was evaluated in PBS (pH 7.4) and in SGF (pH 1.2) with and without pepsin. Ten milligrams of formulations were incubated at 37±1° with 20 ml of PBS (pH 7.4) and SGF (pH 1.2) with and without pepsin, for a period of 2, 4, 8 and 24 h. After specified time intervals, the suspension was centrifuged at 15,000 rpm for 1 h, supernatant was removed and nanoparticles were dissolved in ethanolic solution (7:3 ratio). The gliadin content was determined by taking absorbance at 230.6 nm against blank (Table 6). The shelf life of all the formulations was determined by storage in amber colored vials, closed with rubber closers at room temperature, 0° and at 8° and % residual drug contents were determined after 10, 20 and 30 d (Table 7).

RESULTS AND DISCUSSION

The protein and polysaccharide from aqueous phase can

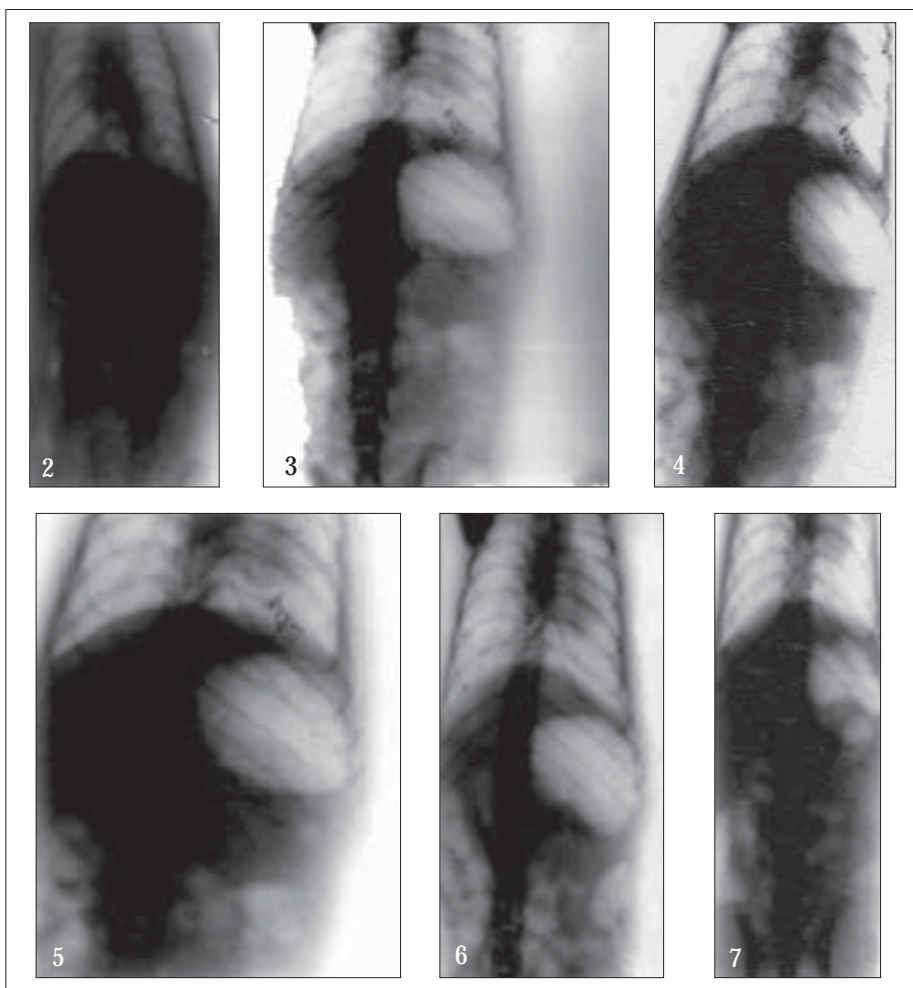


Fig. 2: X-ray photographs of gliadin nanoparticles formulation (At 0.0 time)
Black portion of stomach shows the absence of gliadin nanoparticles at 0.0 time.

Fig. 3: X-ray photographs of gliadin nanoparticles formulation (After 0.5 h)
White patches at stomach site show the presence of gliadin nanoparticles which indicates its mucoadhesive properties.

Fig. 4: X-ray photographs of gliadin nanoparticles formulation (After 1.5 h)
White patches at stomach site show the presence of gliadin nanoparticles which indicates its mucoadhesive properties.

Fig. 5: X-ray photographs of gliadin nanoparticles formulation (After 2 h)
White patches at stomach site show the presence of gliadin nanoparticles which indicates its mucoadhesive properties.

Fig. 6: X-ray photographs of gliadin nanoparticles formulation (After 4.5 h)
White patches at stomach site show the presence of gliadin nanoparticles which indicates its mucoadhesive properties.

Fig. 7: X-ray photographs of gliadin nanoparticles formulation (After 6 h)
White patches at stomach site shows the presence of gliadin nanoparticles which indicates its mucoadhesive properties.

be desolvated by pH change or change in temperature or by adding some appropriate counter ions or by any other desolvating agents like ethanol, isopropanol. Desolvation deaggregates the protein and turns the suspension colloidal and hence milky in appearance.

Gliadin nanoparticles were prepared by desolvation method for macromolecules, by adding a solvent phase of gliadin to a non-solvent phase, which has low viscosity

and high mixing capacity in all proportions. Addition of a desolvating agent resulted in the desolvation of macromolecules. The process is commonly known as coaservation, a new phase, the coaservate phase when treated with a cross linking agent (glutraldehyde), produced nanoparticles of macromolecules.

The desolvation process can be regarded as a gradual removal of the solvent molecules from around the

macromolecules and the molecules “rolling up” in the less friendly environment. The protein macromolecules would be a discrete, poorly solvated, rolled up molecules, thus able to produce colloidal particles.

The gliadin defines a group of polymorphic proteins extracted from gluten, which are soluble in ethanolic solution and show a remarkably low solubility in water except at extreme pH. Due to these physicochemical properties, gliadin nanoparticles can be prepared by desolvation method for macromolecules, using environmentally acceptable solvent such as ethanol and water. These macromolecules showed a high capacity for loading drug and were soluble without further chemical or physical cross linking treatment. In the present system, the diffusion of ethanol (good solvent for gliadin), from the gliadin solution in to the aqueous medium, drastically reduced the solubility of gliadin solution, forming nanoparticles in the solution. Gliadin nanoparticles have shown the average particles size range of 250-500 nm with positive zeta potential of 22.8 mV. The gliadin nanoparticles were spherical with smooth surface. (fig. 1.) The % drug entrapment of nanoparticles indicated that at different gliadin concentration, the % drug entrapment were 23.7±2.3, 41.6±3.4, 67.1±1.3 and 63.6±2.1, respectively.

The release of clarithromycin mainly depends upon the gliadin concentration. The burst release of clarithromycin from nanoparticles at initial stage resulted from the dissolution of drug crystals on the surface of nanoparticles; with increasing gliadin concentration, the release rate of clarithromycin from nanoparticles decreased drastically. The smaller nanoparticles released the drug more rapidly than larger ones in PBS and SGF at initial stage, as the smaller devices possess the larger surface area.

Mucoadhesion involves different kind of interaction forces between mucoadhesive materials and mucous surface, such as electrostatic attraction, hydrogen bonding, Van der Waals forces and mechanical interpenetration and entanglement.

Spectrophotometric method (λ_{\max} 230.6 nm) was used to measure *in vitro* mucoadhesive capacity of developed formulations. Table 5 shows the % gastric retention of gliadin nanoparticles in the rat gastrointestinal mucosa. The adhesion properties of nanoparticles increased with increasing concentration of gliadin among various gliadin concentrations, better mucoadhesion was observed for GNP₄ formulations (3%).

TABLE 5: % GASTRIC RETENTION OF NANOPARTICLES

Formulation code(s)	Average% gastric retention	
	<i>In vitro</i>	<i>In vivo</i>
GNP ₁	74.9±2.7	62.4±2.9
GNP ₂	76.1±3.4	66.3±1.3
GNP ₃	81.5±2.3	69.7±2.4
GNP ₄	70.4±1.6	67.1±3.1

GNP₁ is gliadin nanoparticles (drug:gliadin ratio,1:0.5), GNP₂ is gliadin nanoparticles (drug:gliadin ratio, 1:1), GNP₃ is gliadin nanoparticles (drug:gliadin ratio, 1:2) and GNP₄ is gliadin nanoparticles (drug:gliadin ratio, 1:3).

TABLE 6: REMAINING % OF GLIADIN CONTENT IN DIFFERENT MEDIA

Media	% remaining of gliadin content			
	2h	4h	8h	24h
PBS (pH 7.4)	98.3±1.4	98.1±1.1	97.8±2.6	97.5±2.7
SGF (pH 1.2)	98.4±1.8	97.6±2.3	97.4±1.5	93.2±1.3
SGF (pH 1.2)+ enzyme (pepsin)	96.3±0.9	95.8±1.2	92.9±3.1	71.4±2.2

GNP₁ is gliadin nanoparticles (drug:gliadin ratio,1:0.5), GNP₂ is gliadin nanoparticles (drug:gliadin ratio, 1:1), GNP₃ is gliadin nanoparticles (drug:gliadin ratio, 1:2) and GNP₄ is gliadin nanoparticles (drug:gliadin ratio, 1:3). PBS is phosphate buffer saline and SGF is simulated gastric fluid.

The fact that disulfide bonds of GNP₄ gliadin interact with the fucose and sialic acid groups, which are present in the gastric mucosa. The mucolytic activity of thiol, such as N-acetyl cysteine, disulfide exchange reactions take place between mucin glycoprotein in mucous and mucolytic agent. Due to exchange reaction intra as well as intermolecular disulfide bridge within the glycoprotein structure are cleaved leading to a breakdown of the mucous. Based on the observation that the mucolytic agent is thereby covalently bound to mucin glycoprotein in mucus, also other thiols bearing compounds in particular polymers with thiols group should be covalently bound to the mucus.

Further, bioadhesive behavior *in vivo* was confirmed by taking the X-ray photographs of nanoparticles containing barium sulfate in the stomach of rat (figs. 2-7). To deepen the radio-graphical contrast *in vivo*, sufficient barium sulfate should be enclosed in the nanoparticles. To meet this opposing requirement, barium sulfate nanoparticles having a particle density of 1.099/cm³ were prepared.

At the early stages, within 30 min, after dosing, the nanoparticles were found to adhere on the stomach (fig. 3). After 6 h, some nanoparticles still remained, since the present bioadhesive system, could delay their arrival at the pylorus. The prolonged residence time of nanoparticles in the stomach might be explained, by their mucoadhesive properties as well as by random emptying

TABLE 7: EFFECT OF STORAGE ON RESIDUAL DRUG CONTENT

Formulation code (s)	Initial			% residual drug content								
				10 days			20 days			30 days		
	0	8°	RT	0	8°	RT	0	8°	RT	0	8°	RT
CGNP ₃	100	100	100	97.9±2.1	98.7±1.9	98.6±2.2	93.9±2.9	94.3±3.6	91.8±1.3	83.4±1.5	89.2±4.3	88.1±1.6
CGNP ₂	100	100	100	98.9±2.5	98.5±3.9	95.0±5.6	93.3±5.7	94.8±1.4	91.2±3.4	82.9±2.4	88.6±4.2	89.3±4.2
CGNP ₃	100	100	100	99.6±5.9	98.1±4.9	97.4±2.1	91.7±5.2	91.4±1.8	90.1±3.2	84.6±5.7	85.8±7.1	88.9±5.7
CGNP ₄	100	100	100	97.4±5.4	97.7±3.6	95.2±2.9	95.5±2.4	94.3±4.8	92.4±3.4	81.1±3.5	87.2±5.8	90.4±4.8

GNP₁ is gliadin nanoparticles (drug:gliadin ratio, 1:0.5), GNP₂ is gliadin nanoparticles (drug:gliadin ratio, 1:1), GNP₃ is gliadin nanoparticles (drug:gliadin ratio, 1:2) and GNP₄ is gliadin nanoparticles (drug:gliadin ratio, 1:3).

effect due to the presence of multiple unit system. Due to their wide spreading over the antrum, the subunits approached to the pylorus might pass through the stomach individually and release the drug at once in gut, leading to decreased variability of drug action among patients compared with that occurring in the case of single unit dosage form.

This study has suggested that the gliadin nanoparticles could serve as candidate novel delivery device to improve the bioavailability of clarithromycin and possibly other compounds, which are aimed to produce a local and specific effect in the stomach and are specifically observed through the upper region of the stomach.

In this study, we could prove that clarithromycin resided in the stomach for a longer period of time when it was administered in the form of the mucoadhesive nanoparticles than when administered as suspension or conventional system. The clarithromycin containing gliadin nanoparticles provided greater anti-bacterial activity than the plain drug formulations.

REFERENCES

- Williams, M.P. and Pounder, R.E., *Amer. J. Gastroenterol.*, 1991, 94, S11.
- Longtry, H.D. Brogden, R.N., *Drugs*, 1997, 53, 973.
- McCartry, J.M. *Clin. Ther.*, 2000, 22, 281.
- Alvarez Elcoro, S. and Enzler, M.J. *Mayo. Clin. Proc.*, 1999, 74, 613.
- Corbon, C. and Poole, M.D., *J. Chemother.*, 1999, 11, 107.
- Durrer, C., Irache, J.M., Puisieux, F., Duchene, D. and Ponchel, G., *Pharm. Res.*, 1994, 11, 680.
- Gupta, P.K., Leung, S.H. and Robinson, J.R., In: *Bioadhesive drug delivery Systems*. CRC Press, Boca Raton, FL, 1990, 65, 92.
- Arangoa, M.A., Ponchel, G., Orecchioni, A.M., Renedoi, M.J., Duchene, D. and Irache, J.M., *Eur. J. Pharm. Sci.*, 2000, 11, 333.
- Arangoa, M.A. Campareso, M.A., Popineau, Y., Trache, J.M., Campareso, M.A., Popineau, Y. and Irache, J.M., *Chromatographia*, 1999, 50, 43.
- Arangoa, M.A., Companero, M.A., Renedo, M.J., Ponchel, G. and Irache, J.M., *Pharm. Res.*, 2001, 11, 1521.
- Goverder, T., Stolnc, S., Garnet, M.C.L. and Davises, *Int. J. Pharm.* 2000, 199, 95.
- Kreuter, J; *Acta Pharm. Helv.* 1983, 58, 196

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