**In vitro and in vivo evaluation of ranitidine hydrochloride ethyl cellulose floating microparticles**

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**Abstracts**

The real issue in the development of oral controlled release dosage forms is not just to prolong the delivery of drugs but also to prolong the presence of dosage forms in the stomach in order to improve the bioavailability of drugs with a ‘narrow absorption window’. In the present study, an anti-ulcer drug, ranitidine hydrochloride, is delivered through a gastroretentive ethyl cellulose-based microparticulate system capable of floating on simulated gastric fluid for \(4\)–\(12\) h. Preparation of microparticles is done by solvent evaporation technique with modification by using an ethanol co-solvent system. The formulated microspheres were free flowing with good packability and encapsulation efficiencies were up to 96%. Scanning electron microscopy confirmed porous, spherical particles in the size range 300–750 \(\mu\)m. Microspheres showed excellent buoyancy and a biphasic controlled release pattern with 12 h. In vivo bioavailability studies performed on rabbits and \(T_{\text{max}}\), \(C_{\text{max}}\), AUC were calculated and confirmed significant improvement in bioavailability. The data obtained thus suggests that a microparticulate floating delivery system can be successfully designed to give controlled drug delivery, improved oral bioavailability and many other desirable characteristics.

**Keywords:** Ranitidine, bioavailability, ethyl cellulose, gastroretentive system, solvent-evaporation technique

**Introduction**

Although the drug delivery system concept is not new, great progress has recently been made in this field of research and development (Majeti and Kumar 2000). The word ‘new’ or ‘novel’ in the relation to drug delivery systems is a search for something out of necessity. An appropriately designed sustained or controlled release drug delivery system can be a major advance toward solving the problems associated with the existing drug delivery systems (Brahmankar et al. 1995; Baumgastner et al. 2000; Khan 2001). Thus, a number of approaches are being developed. The common thread running through the approaches is the concept of self-administered, targeted, controlled release systems with increased bioavailability (Aulton 2002).

The most convenient and commonly employed route of drug delivery has historically been by oral ingestion (Thanoo et al. 1993). Drugs that are easily absorbed from the gastrointestinal tract and having a short half-life are eliminated quickly from the blood circulation. To avoid these problems, oral controlled release formulations have been developed, as these release the drug slowly into the gastrointestinal tract and maintain a constant drug concentration in the serum for longer periods of time (Soppimath et al. 2001; Aulton 2002). Such systems use macromolecules as carriers for drugs. This field of pharmaceutical technology has grown and diversified in recent years (Lee and Robinson 2000; Klausner et al. 2003).

However, incomplete release of drug and a shorter residence time of dosage forms in the upper gastrointestinal tract, a prominent site for absorption of many drugs, will lead to lower bioavailability. Efforts to improve oral drug bioavailability have grown in parallel with the pharmaceutical industry. As the number and chemical diversity of drugs has increased, new strategies are required to develop orally active therapeutics. The past two decades have been characterized by an
increased understanding of the causes of low bioavailability and a great deal of innovation in oral delivery technologies, marked by an unprecedented growth of the drug delivery industry (Orellana 2005). Thus, gastroretentive dosage forms which prolong the residence time of the drugs in the stomach and improve their bioavailability have been developed (Chien 1992; Chiao et al. 1995).

Targeting delivery of drugs to diseased lesions is one of the most important aspects of drug delivery systems. To convey a sufficient dose of drug to the lesion, suitable carriers of drugs are needed. Nano and microparticulate carriers thus have important applications for administration of therapeutic molecules. The present study adopted a new technique for encapsulation of an anti-ulcer drug, ranitidine hydrochloride, as gastroretentive ethyl cellulose microspheres. Microparticles were designed to float in simulated gastric fluid for >12 h and deliver the drug in a sustained manner (Wab 1987). Ethanol-dichloromethane solvent system was used to disperse the drug and encapsulation was done using solvent evaporation method.

**Materials and methods**

**Materials**

Ranitidine hydrochloride, obtained as a gift sample from Cipla Research and Development division (Mumbai), was employed as a model drug. The polymer ethylcellulose (Colorcon Asia, Goa), organic solvents dichloromethane and ethanol (S.D.Fine Chemicals, Mumbai) were used. Light and heavy liquid paraffin, individually, were used as dispersion medium along with Span 60 (Sorbitan monostearate) as an emulsifying agent.

**Rationale for selection of ingredients and processes in formulation**

Ranitidine hydrochloride (Honjec 1986), an H2 receptor antagonist, with short half-life, low single administration dose and a low oral bioavailability of 50%, was selected as a model drug to formulate a controlled release formulation with improved oral bioavailability, by prolonging the gastric residence time. Ethylcellulose (a cellulose-based derivative) is a biocompatible, hydrophobic polymer which prolongs the release of water-soluble and water insoluble drugs from its matrices.

The organic solvents chosen, dichloromethane and ethanol (95%), have lower toxicity potential compared to many other solvents and do not have any hazardous effect on the body because they evaporate during the process. Dichloromethane is partly extracted by the liquid paraffin, while the remaining evaporates. Ethanol is a good solvent for the drug. The polymer precipitates as the solvents evaporate during the formulation process to form porous microspheres.

The method used is more correctly referred to as ‘oil-in-oil’ dispersion method or ‘dry-in-oil’ method (Akbuga and Bergisadi 1996) instead of w/o (water-in-oil) emulsification method, since a polymeric solution in organic solution is considered oil-in-micro encapsulation technology (Vijaya et al. 2002). The major problem of the o/w emulsification technique is the low encapsulation efficiency of moderately water soluble drugs (ranitidine hydrochloride is water soluble) due to diffusion of drug from the organic dispersed phase into the aqueous continuous phase, which results in poor entrapment. Therefore, liquid paraffin was used as the dispersion media or external phase along with Span 60 (Sorbitan monostearate), which is a non-ionic surfactant. Span 60 has an HLB value of 4.3 and acts as a droplet stabilizer and prevents coalescence of the droplets by localizing at the interface between the dispersed phase and dispersion medium.

**Formulation of the floating microparticles**

The drug and polymers in different proportions (1 : 1, 1 : 2, 1 : 4) were weighed and co-dissolved at room temperature into a mixture of ethanol-dichloromethane (1 : 1% v/v) with vigorous agitation to form a uniform drug–polymer dispersion. This was slowly poured into the dispersion medium consisting of light/heavy liquid paraffin (200 ml) containing 0.1% Span 60 (previously melted). The system was stirred using an overhead propeller agitator at 500 rpm and room temperature over a period of 2–3 h, to ensure complete evaporation of the solvent.

The liquid paraffin was decanted and the microparticles were separated by filtration through a Whatmann filter paper, washed thrice with 180 ml of n-hexane and air dried for 24 h. The batches of microparticles prepared were labelled as RM-1, RM-2 and RM-3, respectively, for the ratios 1 : 1, 1 : 2 and 1 : 4 of drug:polymer. The process parameters were varied at two different levels to find out the effect of the various independent variables like the stirring speed, type of oil (light or heavy liquid paraffin) used as the continuous phase, type of agitating device used etc. on the properties of the end-product. The process was then optimized to get a product with desirable characteristics. The entire procedure was performed in a dark room as the drug is light-sensitive.

Percentage yield of each batch was calculated using the expression

\[
\text{Yield} = \frac{\text{Weight of microspheres}}{\text{Weight of solid starting material}} \times 100
\]

The prepared microparticles were then characterized for their various properties.
Particle size distribution

Particle size distribution was analysed by placing 5 g of the formulated microparticles in a set of standard test sieves and shaking it for a particular time interval. The particles collected in each sieve were weighed and the percentage particles retained on each sieve was calculated. The average diameter of the microparticles was represented by the geometric mean diameter obtained.

Scanning electron microscopy

The surface topography, morphology, cross-section, particle size, etc., were determined by Scanning Electron Microscopy using a JEOL JSM-T330A scanning microscope (Japan). Dry microparticles were placed on an electron microscope brass stub and coated with gold in an ion sputter. Picture of microparticles were taken by random scanning of the stub.

Flow properties

This was assessed by measuring the angle of repose and compressibility index.

The bulk density and tapped density were measured and the compressibility index was calculated using the formula,

*Compressibility index* = \( \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100 \)

Estimation of drug incorporation efficiency

To determine the incorporation efficiency, 25 mg of drug-loaded microparticles were washed with 10 ml of SGF containing 0.1% v/v Tween so as to remove the surface-associated drug. The absorbance of the filtrate taken at 225 nm gave the surface drug content.

The filtered microparticles were then digested in a small amount of dichloromethane, to release the entrapped drug from the microspheres. The drug was then extracted into the buffer by making up the volume to 100 ml with the SGF and keeping it overnight in a metabolic shaker with slight shaking. The solution was then filtered and the drug content analysed spectrophotometrically at 225 nm.

Total incorporation efficiency

= surface associated drug + entrapped drug

In vitro buoyancy studies

The floating microparticles (300 mg) were spread over the surface of the dissolution medium (simulated gastric fluid, SGF, pH (1.2) containing 0.02% w/v of Tween 20) that was agitated by a paddle rotated at 100 rpm. After agitation for a predetermined time interval, the microspheres that floated over the surface of the medium and those settled at the bottom of the flask were recovered separately.

After drying, each fraction of the microparticles was weighed and their buoyancy was calculated by the following equation:

\[ \text{Buoyancy} \% = \frac{Q_f}{Q_f + Q_s} \]

where *Qf* and *Qs* are the weight of the floating and the settled microparticles, respectively.

In vitro drug release studies. The drug dissolution tests of micro-balloons were carried out by the paddle method specified in the US Pharmacopoeia XXI. Microparticles were weighed (weight equivalent to 50 mg of drug), gently spread over the surface of 900 ml of dissolution medium (SGF without enzymes), rotated at 100 rpm and thermostatically controlled at 37°C. Perfect sink conditions prevailed during the dissolution tests. The sample was withdrawn at a suitable interval from the dissolution vessel and assayed spectrophotometrically at 225 nm.

In vivo bioavailability studies

In vivo bioavailability studies were conducted in healthy male New Zealand rabbits weighing 2–2.5 kg. Six rabbits were divided into two groups and fasted for 24 h. One batch was fed with 35 mg Ranitidine Hydrochloride (pure drug), the second batch was given the formulation RM-3 (with drug equivalent to 35 mg). Water was given *ad libitum* during fasting and throughout the experiment.

Blood samples, 2 ml each, were collected from the marginal ear vein of the rabbits, into heparinized centrifuge tubes just before dosing and at 1, 2, 4, 6, 8, 10, 12 and 24 h during the study. Blood samples were centrifuged at 1500 rpm and the plasma was separated. One undosed plasma sample was kept as blank. To 1 ml each of the other plasma samples, 5 ml of acetonitrile was added. The tubes were then centrifuged at 2500 rpm for 15 min, 4 ml of the supernatant was pipetted out to which 0.2 ml of 1.47 M perchloric acid was added and the drug concentration was determined by UV spectroscopy at 225 nm, as validated by earlier studies. The blank consisted of 1 ml undosed plasma, 4 ml acetonitrile and 0.2 ml of 1.47 M perchloric acid (Joseph et al. 2002).

The calibration curve for Ranitidine Hydrochloride was calculated as follows. Ranitidine solutions in acetonitrile were prepared at concentrations of 1, 2, 4, 6, 8 up to 20 ml. Each ml of this solution was made up to 5 ml using acetonitrile. To each of these solutions, 1 ml of undosed rabbit blood was added and the contents centrifuged at 2500 rpm for 15 min. Supernatant 4 ml was then pipetted out to which 0.2 ml of 1.47 M Perchloric acid was added and the
The absorbance was measured at 225 nm. The blank was prepared using plasma from the undosed animal, acetonitrile, perchloric acid in exactly the same way. The calibration curve for Ranitidine hydrochloride plotted as absorbance at 225 nm was linear over the range of 1–16 μg ml⁻¹, with a correlation coefficient of 0.9996.

The dose for rabbit was calculated as follows:

\[
\text{Total dose (in humans)} = 0.07 \times 300 \times \left( \frac{0.07 \times 2.5}{1.5} \right) = 35 \text{ mg of 2.5 kg rabbit}
\]

Pharmacokinetic parameters were derived from the plasma concentration vs time plot. The area under the curve (AUC), the peak plasma concentration (\(C_{\text{max}}\)) and the time to attain the peak concentration (\(T_{\text{max}}\)) were obtained. The elimination rate constants \(k_{\text{el}}\) for the different dosage forms were determined from the semi-logarithmic plot of plasma concentration vs time. \(k_{\text{el}}\) was calculated from the terminal linear portion of the curve.

Results and discussion

Percentage recovery yield

The percentage practical yields slightly decreased as the polymer ratio increased. The maximum yield was found to be 89.31% in RM-1 with light liquid paraffin.

A better yield of microparticles was obtained when light liquid paraffin was used. The porosities were also higher with light liquid paraffin when compared to that with heavy liquid paraffin. Discrete free-flowing microparticles were produced at 500 rpm. The type of mechanical device used for stirring also influenced the size of particles formed. The magnetic stirrer often gave larger particles and at times sticky masses, due to its comparatively lower stirring speed when compared to the propeller agitator, which has a higher stirring speed. Thus, discrete, free-flowing particles were formed with the propeller agitator.

Particle size distribution

The particle size distribution was found by passing 5 g of the microparticles through a set of standard sieves. The values of the percentage of particles retained on each sieve and average particle size was calculated.

The mean particle diameter of the microspheres was between 360–620 μm. As the polymer concentration increases, the particle size also increases. This is because the viscosity of the polymer solution increases with increasing polymer concentration, which in turn decreases the stirring efficiency. The polymer rapidly precipitates, leading to hardening and

avoiding further particle size reduction during solvent evaporation.

Scanning electron microscopy

Surface topography, particle size, morphology and internal cross-sectional structure of the microspheres were investigated with a scanning electron microscope. SEM is one of the common methods used, owing to the simplicity of sample preparation and ease of operation. Often 3-dimensional information about macro (0.1–10 mm), meso (1–100 μm) and microstructure (10–1000 nm) is often found in the same micrograph.

Scanning electron photomicrographs of the formulation RM-2 are shown in Figure 1. The microspheres were porous, rough, grossly spherical and slightly aggregated. The surface topography reveals that the microspheres were highly porous due to the rapid escape of the volatile solvents during formulation. Inward dents were seen on the surface, probably due to collapse of the walls of the microspheres during the in situ drying process. Thus, the rate of removal of the solvent from the embryonic microspheres influences the morphology of the product.

Very less particulate matter of the drug were seen on the surface of the microspheres, indicating uniform distribution of the drug in the polymeric network. Usually the drug is present in the amorphous form in microspheres (as suggested by X-ray studies) and the drug present on the surface at lower polymer concentrations was responsible for the ‘burst effect’ or ‘burst release’.

Both large (>100 μm) and small (<10 μm) microparticles are formed by solvent evaporation process. Reports suggest that orally administered microspheres of less than 10 μm are taken up by the Peyer’s patches and thus may increase their retention time in the stomach while the larger particles are retained in the stomach by virtue of their buoyancy due to the pores and cavity in them. Thus, the particle size plays an important role in targeting the drug to the stomach and upper part of small intestine.

Figure 1(c and d) shows the SEM of the formulations before and after dissolution of RM-2. Diffusion seemed to be the predominant mechanism, as shown by the pores in the polymer.

Flow properties

The flow properties of all the formulations were found out by measuring the angle of repose and compressibility index. The results are shown in Table I. The values of angle of repose were of the range 24–29°, which are within the normal acceptable range of 20–40°. The porous microspheres thus showed reasonably good flow potential.

This is further substantiated by the values of Compressibility index (I) which was in the range 15–18, indicating good flow characteristics of the microspheres.
This also implies that the microspheres are non-aggregated. The improved micrometric properties of the prepared microspheres when compared to that of the pure drug alone suggest that they can be easily handled and filled into a capsule. Therefore, capsules loaded with microspheres can be suggested as a floating micro-particulate drug delivery system. Moreover, the soft gelatin capsules easily absorb water and disintegrate and do not hinder the floating capability of the microspheres.

**Estimation of drug incorporation efficiency**

The values of total % drug incorporation efficiency are shown in Table II. High incorporation efficiencies are seen with lower concentrations of polymer with the drug.

**In vitro buoyancy studies**

**In vitro** buoyancy studies reveal that, in spite of stirring the dissolution medium for more than 12 h, ~85–90% of RM-1 to RM-3 still continued to float without any apparent gelation, thus indicating that microspheres exhibit excellent buoyancies which can be attributed to the pores and cavities present in them. Figure 2 shows the buoyant microsphere formulations. The relative buoyancies are also shown in Table II.

The relative density of the microparticles is higher at higher polymer concentrations and the porosity is less.
So the microparticles having higher polymer concentrations were less buoyant than those with lower polymers concentrations. The formulation RM-1 showed highest buoyancy of 94.18 ± 4.4%. The porosity was found to be less at higher polymer concentrations, probably due to the slowing down of solvent evaporation due to increase in viscosity of the polymer solution.

Table II shows the porosities of the microsphere formulations. The particle size also has an influence on buoyancy when compared to smaller particles (<500 µm), the particles with size range 500–1000 µm exhibited higher buoyancies. Thus, the sizes of particles also play an important role in buoyancy.

In vitro drug release studies

Dissolution studies on all the formulations of the floating microspheres were carried out using a USP XXIII Type II, i.e. Paddle Type dissolution apparatus. As the microspheres floated in the stomach and released the drug, simulated gastric fluid (pH 1.2) was used as the dissolution medium.

The results obtained in the in vitro drug release studies were plotted in five kinetic models of data treatment, that is cumulative percentage drug release vs time (zero order kinetics), Log cumulative percentage drug retained vs time (first order kinetics), Higuchi’s classical diffusion equation (Higuchi’s matrix) in which cumulative percentage release was plotted against square root time, according to Hixson Crowell’s erosion equation cube root of cumulative percentage drug retained vs time (Mathiowitz 1999).

The cumulative percentage drug release after 12 h was found to be 95.83%, 96%, 93% for the formulations RM-1–RM-3. It was found that the drug release was prolonged up to 12 h. It was also observed that as the polymer ratio increased the drug release decreased. Ranitidine hydrochloride is water soluble and the release of the drug was retarded due to the hydrophobic and insoluble nature of the polymer used.

Also at lower polymer concentration a ‘burst release’ was seen due to surface drug present on the micro-particles. Among formulations RM-1–RM-3 it was seen that RM-1 shows the highest burst release of 36% in 1 h because of the high amount of drug present on the surface. This was substantiated by the higher values obtained in the percentage drug entrapment efficiencies. RM-2 shows ~33% burst release which is optimum and close to the theoretical kinetic profile of the drug. The burst release was least in the formulations RM-3 (28% in the first hour). After the first hour the drug release in the formulations followed a steady pattern approximating zero order release. An ideal controlled release formulation of ranitidine hydrochloride should release ~32% of the drug in the first hour, like the conventional tablets 6.16% per hour up to 12 h. These values were obtained by calculating a theoretical drug release profile for 12 h.

Kinetic models with the highest correlation coefficient were judged to be the most appropriate model for the dissolution data. For the formulations RM-2 and RM-3 the best fitting linear parameter was that of the Higuchi model and their correlation coefficients were 0.9909, 0.9982 and 0.9961, respectively. This indicates that the drug release is controlled by diffusion of the drug through the pores. On the other hand, the formulation RM-1 best fitted into the Peppas model (Singh et al. 2000; Sankar et al. 2003).

In vivo bioavailability studies

The in vivo evaluation of the floating microspheres of ranitidine hydrochloride was conducted in two groups of New Zealand rabbits. Rabbit has been chosen as the model for study because there have been many bioavailability studies done using this animal model.

Figure 3 shows the plot of plasma concentration of the drug vs time for the pure drug and formulation RM-2 of the floating microspheres. The pure drug gives a small peak at the first hour and a pronounced secondary peak at the third hour. A ‘peak and valley’ fluctuating plasma
profile is seen in the case of the pure drug. On the other hand, in the case of the formulations of floating microspheres of the pure drug, a controlled release kinetic profile is observed. The mean pharmacokinetic parameters of the pure drug and the formulation were estimated and are given in Table I.

Assessment of the AUC showed that the bioavailability was lesser for the pure drug (normal oral bioavailability being only 50%) and increased to ~2.4-times with formulation. The elimination was less rapid with the microparticulate formulation when compared to the free drug. Thus, the bioavailability of an anti-ulcer drug like ranitidine hydrochloride has been significantly increased by formulating it into a multiple unit floating dosage form.

Conclusion

The present study has been a satisfactory attempt to formulate a floating microparticulate system of an anti-ulcer drug like ranitidine hydrochloride with a view of controlled delivery of the drug and also targeting the drug to its local site of action, the stomach, thus improving its oral bioavailability.

From the experimental results it can be concluded that biocompatible and cost-effective polymers like ethyl cellulose can be used to formulate an efficient floating microparticulate system with good percentage entrapment efficiency and practical yield. The particle size analysis revealed that the particles were of the size range of 350–600 m, showed good flow properties and packability, indicating that it can be successfully handled and filled into a capsule dosage form. The microparticles were very porous, as revealed by the scanning electron microscopic studies and had low densities, thus exhibiting excellent buoyancies in simulated gastric fluid. As their surfaces were rough and porous, this microparticulate polymeric network could be referred to as ‘micro sponges’ (Figure 1).

In vitro drug release studies showed a biphasic release pattern for all formulations with an initial ‘burst effect’ which may be attributed to the drug present on the surface. Formulation RM-2 showed a better controlled release while RM-1 showed the maximum cumulative release. The formulation RM-2 showed a kinetic release profile similar to the theoretical controlled release profile of the drug and could be regarded as the optimum formulation. The overall curve fitting into various mathematical kinetic models was found to be on an average. The formulations RM-2 and RM-3 best fitted into the Higuchi model while RM-1 fitted into the Peppas model.

The in vivo studies revealed that the oral bioavailability of the drug increased by more than twice by formulating it into microspheres. The pure drug showed stability problems with light and moisture, which was also overcome by formulating it into a microparticulate drug delivery system. Further pre-clinical and clinical trials are required to warrant the use of the dosage form in human beings. Elaborate in vivo studies in human beings need to be done to correlate the in vivo data with in vitro values.

Thus, the formulated floating microspheres seem to be a potential candidate as an oral gastro retentive controlled drug delivery system in this era of patenting of novel and controlled drug delivery systems.

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