CHAPTER 7

IN VIVO GASTRORETENTIVITY STUDIES

7.1. Introduction

In vitro performance parameters obtained from conventional and modified dissolution methods need to be further substantiated using in vivo evaluation in any product development programme. Such studies provide an insight into the in vivo fate of the delivery system and its efficacy and enable the relationship to be established between in vitro and in vivo performance of a dosage form. The in vitro floating and/ or mucoadhesive performance of gastroretentive/ floating dosage forms is difficult to be reproduced in vivo due to non-reproducible/ unpredictable GI physiology. Animal models that have been used for gastroretentivity studies include rats, dogs and pigs. While animal models have obvious advantages in assessing gastroretentivity of delivery systems, human subjects are increasingly utilized for in vivo evaluation with visualization techniques such as γ-scintigraphy, endoscopy, radiology (X-ray imaging), magnetic resonance imaging (MRI) and ultrasonography [Timmermans and Moes, 1994; Whitehead et al., 1998; Kedzierewicz et al., 1999; Wilding et al., 2001; Klausner et al., 2003; Sato and Kawashima, 2003; Säkkinen et al., 2004; Sato et al., 2004a; Sato et al., 2004b; Patel et al., 2005; Frances et al., 2006; Rahman et al., 2006; Xu et al., 2006].

Amongst these methods, the technique of γ-scintigraphy is most commonly employed to investigate the GI performance of pharmaceutical dosage forms, but incorporation of radioactive substance in the dosage form may lead to alteration in dosage form behaviour and also absorption of radioactivity to bones and tissues of the subjects. Endoscopic analysis appears to be another viable method, but the invasive nature of the endoscopy procedure can cause significant discomfort to the subject. Use of radiological procedures, which involves inclusion of a radioopaque material into an oral drug delivery system was first reported in 1933 and is still being used [Wilding at al., 2001]. However, X-ray studies suffer from some major disadvantages. To assess accurately the gastric retention, the subjects must be exposed to the repeated risk of serial X-rays and it becomes necessary to modify the physical state of the dosage form in order to make it radioopaque, which is

particularly more critical in case of floating devices. MRI, despite of its accuracy, also requires incorporation of dense markers and thus, has tendency to alter nature of the dosage form. Ultrasonic imaging is another non-invasive and safe method, which may be employed for in vivo evaluation [Kimmey et al., 1987; Wilding et al., 2001].

Ultrasonic imaging was utilised in a pilot study to assess the shape and size of a tablet in the stomach with a view to use this approach for future assessment of bioadhesive dosage forms [Aiache et al., 1988; Maublant et al., 1988]. A swelling hydrogel type GRDF was imaged using ultrasound in the stomach of a dog, by repetitive administration of a large volume of water via a gastric tube. When water was present in the stomach, ultrasound imaging was very effective in monitoring the position of the hydrogel in the stomach, solvent penetration into the gel and interactions between gastric wall and the GRDF during peristalsis [Shalaby et al., 1992; Klausner et al., 2003].

Conventional sonographic assessment of location and retention of dosage forms in the GIT is limited by the gas-filled bowel, which produces shadowing artifacts and thus, interferes with ultrasound imaging. However, with technological advancements in ultrasonography and the growing expertise of ultrasonographers, ultrasound is now being recognized as a valuable imaging technique. Ingestion of degassed water has been used to improve ultrasound imaging of the GI tract, but with inconsistent results. Alternatively, investigators have studied oral ultrasound contrast agents designed to adsorb and displace stomach and bowel gas. One such agent is SonoRx from Bracco, consisting of simethicone-coated cellulose, which has been recently approved for clinical use by the FDA. Another approach to improve the image quality of sonography is to deploy contrast enhanced ultrasound (CEU) [Fleischer et al., 1981; Miller and Kemberling, 1984; Taylor, 1985; Stringer et al., 1986; Kimmey et al., 1987].

Contrast enhanced ultrasound is the application of ultrasound contrast agents like, gas-filled microbubbles to traditional sonography. Ultrasonic imaging using microbubble contrast agents enhances the ultrasound backscatter, or reflection of the ultrasound waves, to produce a unique sonogram with increased contrast due to the high echogenicity difference between the gas in the microbubbles and the soft tissue surroundings of the body. When gas bubbles entrapped in polymeric sheath/ matrix are caught in an ultrasonic frequency field, they compress, oscillate, and reflect a characteristic echo, which generates the strong and unique sonogram in contrast enhanced ultrasound. Gas cores can be composed of air, carbon dioxide, nitrogen or perfluorocarbon. Contrast enhanced ultrasound can be used to image

blood perfusion in organs, measure blood flow rate in the heart and other organs, and has other applications as well [Goldberg et al., 2001; McCulloch et al., 2000; Lindner, 2004].

To study the expected performance of the designed formulations, in vivo, preliminary studies were carried out for proof-of-concept. Contrast enhanced ultrasonography was applied to visualize one of the best floating matrix based placebo tablets, based on gas generation, in the stomach of a healthy human volunteer after oral ingestion. In addition, floating microencapsulated product showing best in vitro release profile was evaluated for its in vivo gastroretentivity in Wistar rats. These two studies and their findings have been presented in this chapter.

7.2. Experimental section

7.2.1. Materials

Drugs, polymers, excipients and chemicals/ reagents used in the studies were obtained from the same sources as mentioned in the Chapters 3 and 4. Sudan black B, employed for colouring microencapsulated delivery, was purchased from HiMedia Laboratoties Ltd., Mumbai.

7.2.2. Equipments

Ultrasonographic imaging was carried out in Anita Sonography Clinic, Pilani, Rajasthan using an Ansaldo-Hitachi Ultra sonograph (Model- AU-450; 2-15 mHz). UV-visible-NIR spectrophotometer (*Jasco*, Tokyo, Japan, model V-570) was used for analysis required for drug content estimation as mentioned in chapter 3.

7.2.3. Methodology

The gastroretentivity study of SU-GR-CRDF on human subject was approved by Institutional Human Ethics Committee, B.I.T.S., Pilani *(Approval no.: IHEC-11/ 06-07)* and the in vivo gastroretentivity study in Wistar rat model of MU-GR-CRDF was approved by Institutional Animal Ethics Committee, B.I.T.S., Pilani *(Approval no.: IAEC/RES/8/2)*. The techniques employed for the studies have been well established, but the exact protocol for each study was customized as per the requirement(s) for evaluating the dosage forms.

Gastroretentivity study of floating tablet in human subject

To study the gastroretentivity of floating tablet formulation, contrast enhanced ultrasonography (CEU) was employed. Healthy male human subject aged 26 years with normal body mass index (BMI of 22) participated in the study. Formulation was perorally administered with sufficient quantity of water to the subject who was fasted overnight. The study was carried out for 6 hours post administration under fasting condition with regular

intake of water. After administration of the formulation, ultrasonic images of the stomach were taken at 10, 30, 90, 180 and 320 min post administration in supine position.

Gastroretentivity study of floating microcapsules in Wistar rats

In vivo floating/ gastroretentivity study was carried out for one of the best formulations of celecoxib [Cele(31)-EC:PVP(1:3)21] which was marked with Sudan black B for easier identification. Male Wistar rats, weighing 280-320 g, fasted overnight with access to only water *ad libitum* were used for the study. The animals were divided into two groups (control & formulation), each group having five rats.

In the control group, each rat was administered 20 mg of celecoxib, while in the formulation group, each group was administered 100 mg (equivalent to 20 mg of celecoxib) of the microencapsulated product suspended in 0.125 w/v % of sodium carboxy methyl cellulose perorally using a modified intrathecal syringe fixed with 18 mm gauge needle. Rats were allowed access to only water *ad libitum* throughout the study. At 0.5, 1.0, 2.0, 4.0 and 6.0 h respectively, rats were sacrificed by cervical dislocation, decapitated and dissected. Stomach was removed, cut open and imaged. The drug/ microencapsulated granules were scrapped out and stomach walls were washed (to remove any adhering formulation) to quantify the total drug/ formulation present at each time point.

7.3 Results and discussion

Gastroretentivity study of floating tablet in human subject

In this study, contrast enhanced ultrasonography, in which the gas entrapped matrix based formulation itself acted as a self-marker, was employed for visualization. The microbubbles of CO_2, which were generated on interaction of the gastric fluid with gas generating agent and entrapped in the matrix of polymer-gel acted as an excellent contrast media. Without adding any external marker, which would have otherwise altered the density/ nature of the formulation, the formulation was visualized at different time points. A continuous swelling of the tablet was observed with time. Within 10 min, the diameter increased from 12 mm to about 18.6 mm. In half an hour, the diameter of the tablet was observed to be about 22 mm, which increased to about 34 mm in 3 h. Studied placebo formulation was visualized in the stomach for more than 5 h 20 min in human subject in fasted state. However, the intensity of marker decreased with time due to swelling of the formulation and loss of entrapped gas in the polymeric matrix [Figures 7.1 to 7.5].

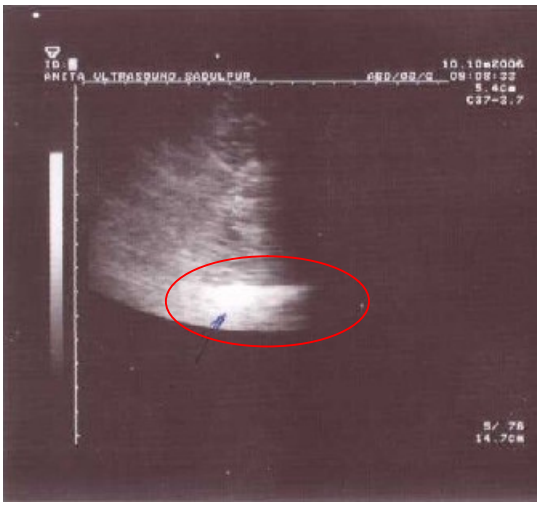

Figure 7.1: Ultrasonic image of tablet formulation retained in human stomach 10 min post peroral administration

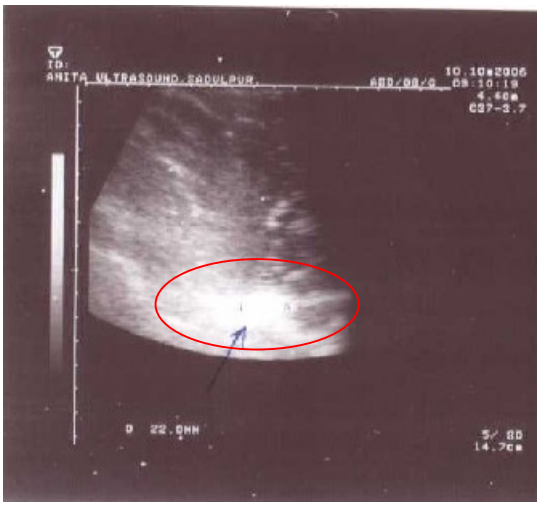

Figure 7.2: Ultrasonic image of tablet formulation retained in human stomach 30 min post peroral administration

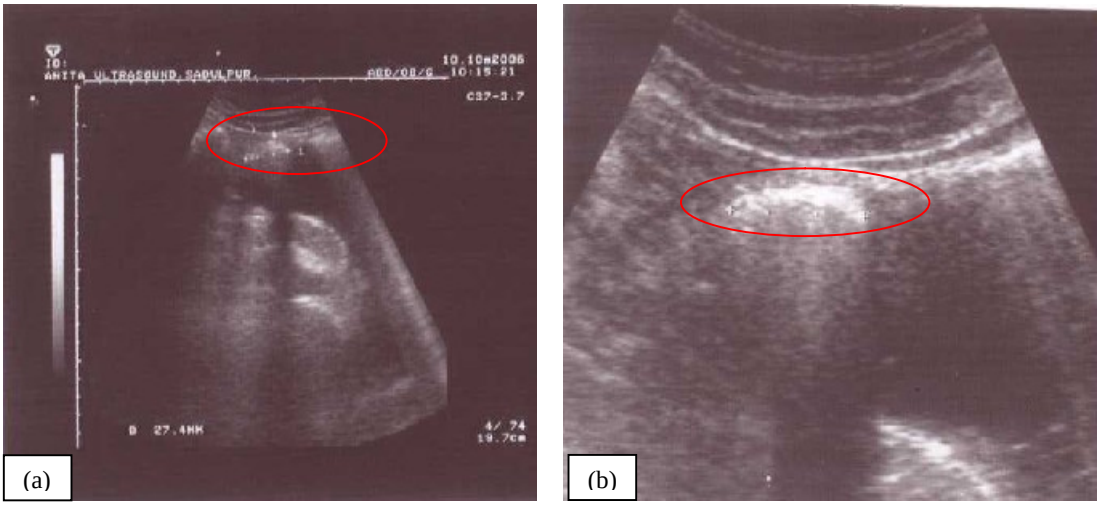

Figure 7.3: Ultrasonic image of tablet formulation retained in human stomach (a) 90 min post peroral administration, (b) 1.5 times magnification of tablet formulation retained 90 min post peroral administration

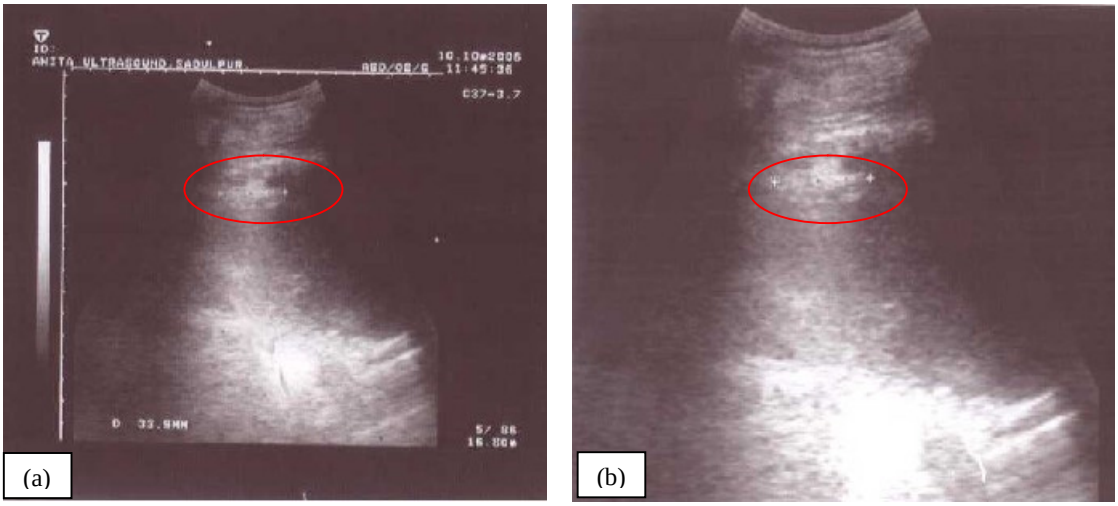

Figure 7.4: Ultrasonic image of tablet formulation retained in human stomach (a) 180 min post peroral administration, (b) 1.5 times magnification of tablet formulation retained 180 min post peroral administration

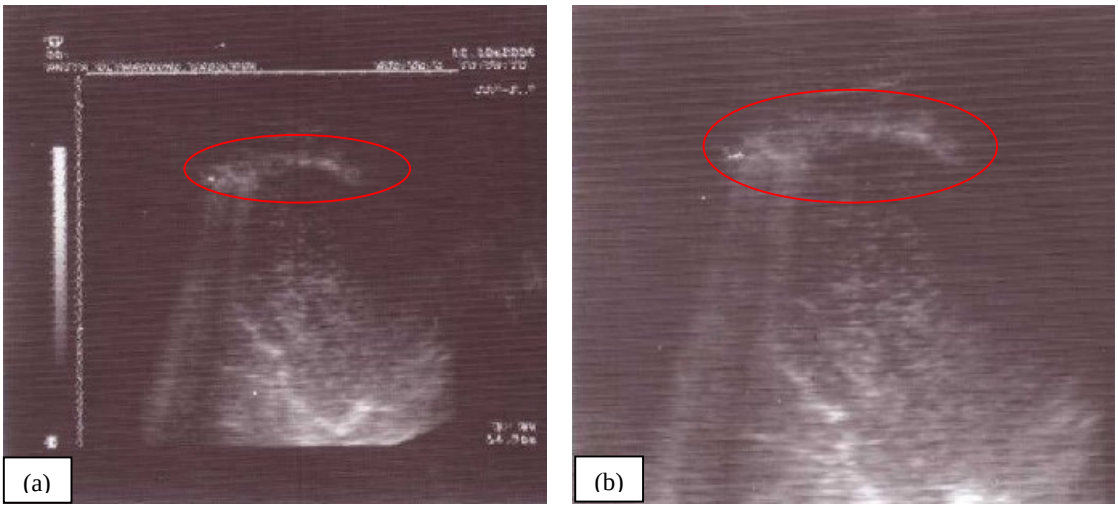

Figure 7.5: Ultrasonic image of tablet formulation retained in human stomach (a) 320 min post peroral administration, (b) 1.5 times magnification of tablet formulation retained 320 min post peroral administration

Gastroretentivity study of floating microcapsules

Microencapsulated product of celecoxib [Cele(31)-EC:PVP(1:3)21] was retained in the stomach of male Wistar rats beyond 6 h. Floating as well as mucoadhesive nature of the product aided in longer residence in the stomach. At initial time points, microcapsules were deep gray in colour, more or less intact, less swollen and easier to scrap out. However, as the time increased, their colour faded and they became highly swollen, strongly adhered to the stomach and very difficult to remove from the walls. At the 4^{th} and 6^{th} h, there was very little fluid in the stomach, but microcapsules (though buff in colour) were still found to be tenaciously adhering to the stomach wall.

In the control group, very little amount of celecoxib (0.46 mg) was left in the stomach after 30 min of oral ingestion. Total amount of celecoxib present in the stomach after 1, 2 and 4 h was 0.13, 0.05 and 0.02 mg respectively with no amount remaining at the 6^{th} h. In contrast to this result, total amount of celecoxib present in the stomach in case of formulation group were significantly higher and were 14.65, 13.49, 11.40, 6.30 and 3.51 mg respectively after 0.5, 1, 2, 4 & 6 h [Table 7.1]. Photographic image of split open stomach of Wistar rat at different time for pure drug and formulation is presented in Figure 7.6.

However, the total weight of formulation and amount of celecoxib was found to decrease with time. This was observed probably because negligible amount of fluid was present in the stomach at later time points and the drug content was also found to decrease with time. Fed state and/ or continual presence of water is likely to give better results.

Table 7.1: Results of in vivo floating/ gastroretentivity study of peroral administration of microencapsulated product in Wistar rat

Time (h)	**Control (20 mg of pure drug)**	**Cele(31)-EC:PVP(1:3)21 (100 mg of formulation)**			
	Total amount of celecoxib retained in stomach (mg)	Weight of microcapsules retained in stomach (mg)	Drug content per 10 mg of product retained (mg)	Amount of celecoxib present in washings (mg)	Total amount of celecoxib retained in stomach in microcapsules (mg)
0.5	0.46	80.58	1.81	0.09	14.65
1.0	0.13	83.57	1.61	0.07	13.49
2.0	0.05	75.48	1.50	0.06	11.40

4.0	0.02	47.60	1.31	0.06	6.30
6.0	0.00	29.46	1.17	0.05	3.51

Panel-1

Panel-2

Figure 7.6: Photographic image of split open stomach (Wistar rat) at different time for pure drug control group (Panel-1) and for formulation group (Panel-2)

7.4 Conclusions

Contrast enhanced ultrasonography (CEU), in which the gas entrapped matrix based formulation itself acted as a self-marker, was used for visualization of matrix based tablet and image of the tablet was observed for 5 h 20 min in fasted condition. However, the intensity of image decreased with time due to loss of entrapped gas from the matrix of the tablet. Designed placebo SU-GR-CRDF was retained in the stomach for more than 5 h 20 min under fasted condition. In addition, a representative batch of MU-GR-CRDF of celecoxib [Cele(31)-EC:PVP(1:3)21] was retained in the stomach of male Wistar rats beyond 6 h under fasted condition. These observations can be considered as proof-of-concept of the whole formulations design.

7.5 References

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