

**IN VITRO DISSOLUTION AND IN VIVO GAMMA SCINTIGRAPHIC EVALUATION OF GASTRO RETENTIVE METFORMIN TABLETS**

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**ABSTRACT**

The aim of present study was to study the in vitro and in vivo performance of gastro retentive metformin tablet (GR-MET) formulation for its delayed release profile. The in vitro release of GR-MET tablet prepared with polymeric mixture of HPMCK4M and HPMC K100M was observed. In vitro dissolution study of the GR-MET was carried out using USP apparatus II and distilled water as dissolution medium and compared with in vivo gamma scintigraphy studies carried out in New Zealand rabbits. In vitro studies showed that

the GR-MET released 74 % in 5 h, which matched the in vivo gastric retention half-life of 3.5 h, indicating that the designed system was retained in stomach as expected. The objective of in vivo gamma scintigraphic study was to provide a proof of concept that the floating capability of the gastro-retentive tablet was useful for increasing the gastric residence time of the dosage form. The GR-MET tablets showed significant gastro-retention at the end of 6 h.

**KEYWORDS:** Gamma scintigraphy, Metformin, gastroretentive, sustained release.

**INTRODUCTION**

Metformin hydrochloride (MET) is an oral anti-diabetic drug in the biguanide class of compounds and first line drug of choice for the treatment of type 2 diabetes. The absorption of anti-diabetic agent, MET, in humans is incomplete and the drug is excreted mainly in urine with a half-life of 4 to 6 h. MET is protonated under physiological pH condition. Ionized MET is absorbed into the negatively charged intestinal epithelium. The absorption window is predominantly in the small intestine and colonic absorption in healthy subject is poor, thus

justifying its formulation into a gastro-retentive dosages form. It has a short half-life, there by justifying its development into a sustained release formulation.

Gastro-retentive systems have some advantages over other methods of drug administration, including a longer residence time in the stomach and local action to the narrow absorption site in the upper small intestine.<sup>[1]</sup> Various methodological approaches for gastric retention have been reported in the literature, such as muco-adhesive systems, floating systems, sedimentation systems, biodegradable super porous hydrogel systems and expendable systems.<sup>[2]</sup> The floating systems are floatable dosage forms that have along-lasting intra-gastric buoyancy. This system offers a sustained action to the therapeutic window and better patient compliance.<sup>[3]</sup> Several technical methods have been used to prepare gastro-retentive floating dosage forms. Hwang et al.<sup>[2]</sup> prepared the hydrodynamically balanced system (HBS) based on hydrophilic polymers. Several researchers have investigated gas-generating systems<sup>[4-6]</sup>. These systems were formulated with carbonate or bicarbonate, citric acid and some polymers. In the single unit dosage forms, carbonates or bicarbonates react with acid such as citric acid or gastric fluid, generating CO<sub>2</sub> gas bubbles. The dosage forms are floated when the generated gas bubbles are trapped in the swollen polymer matrix of the dosage forms. However, this system has some problems. For example, the pH of the gastric fluid differs in each subject and is affected by food. Furthermore, gas-generating dosage forms have a lag time until floating occurs.

Compared to gas-generating systems, low-density systems were immediately buoyant and not affected by pH differences in the gastric fluid. Kawashima et al.<sup>[7]</sup> prepared low-density hollow microspheres using solvent evaporation methods. Streubel et al.<sup>[8]</sup> prepared low density floating matrix tablets using low-density materials such as polypropylene form powder.

Scintigraphy is a form of imaging used in nuclear medicine, wherein radioisotopes are administered by intravenous or oral means and the emitted radiation is detected by external detectors (gamma cameras) to form two – dimensional images. Gamma scintigraphy is a non-invasive imaging technique with applications in the development of drug products and the assessment of tracer kinetics. It enables the assessment of critical performance parameters that in vitro techniques attempt. For delayed-release systems, gamma scintigraphy is a well-established and useful tool to monitor the dosage within the GI tract and assess its in vivo performance.<sup>[9]</sup>

Often, *in vitro* testing methods are not predictive of *in vivo* results. For oral dosage forms, altered gastrointestinal transit due to individual variations, physiologic or pharmacologic factors or the presence of food may influence bio-availability. Disintegration, erosion, or drug release may be premature or delayed *in vivo*. Similarly, altered deposition or clearance from other routes of administration such as nasal, ocular, or inhalation may explain drug absorption anomalies. Gamma scintigraphy combined with knowledge of physiology and dosage form design can help define these variables. The location of a radiolabeled drug in the GI tract is determined from the images obtained at various time intervals using gamma camera. External radioactive marker may be positioned at a well defined anatomical position as a reference point in the determination of the radio labeled formulation.<sup>[10]</sup>

Gamma scintigraphy is applied extensively in the development and evaluation of pharmaceutical delivery systems, particularly for monitoring formulations in the gastrointestinal track. The radiolabeling is generally achieved by the incorporation of an appropriate radionuclide such as technetium-99m or indium-111 into the formulation or by addition of a non-radioactive isotope such as Samarium-152 followed by neutron activation of the final product.<sup>[11]</sup>

Gamma-scintigraphy is simple in terms of both data acquisition and data analysis. L.Whitehead et al prepared freeze-dried calcium alginate multiple-unit FDF which demonstrated favorable *in vitro* floating characteristics.<sup>[12,13]</sup>

Ninan Ma et al prepared type of multi-unit floating alginate (Alg) microspheres by the ionotropic gelation method with calcium carbonate (CaCO<sub>3</sub>) being used as gas-forming agent. The gastrointestinal transit of optimized floating sustained-release microspheres was compared with that of the non-floating system manufactured from identical material using the technique of gamma-scintigraphy in healthy human volunteers.<sup>[14]</sup>

Sheetal Malake et al developed the method of incorporation of radioactive material during the process of coating. The technique of radio-labeling was physical entrapment of 99m pertechnetate in the barrier layer of ethyl cellulose and gelucire. Gamma Scintigraphy studies revealed maximum activity in the gastric area<sup>[15]</sup>.

## MATERIALS AND ANIMALS

MET, HPMCK4M, HPMCK100M were gift samples obtained from Flamingo Pharma. (Mumbai, India). Four healthy male New Zealand white rabbits obtained from Reliance life sciences (Mumbai, India) weighing 3.0 – 3.5 kg were used for the study. The animals used for the experiments were treated according to the protocol evaluated and approved by the Institutional Animal Ethics Committee.

### PREPARATION OF METFORMIN TABLET

A wet granulation method was used to prepare MET granules. Floating layer was prepared by mixing MET, HPMC K 4M, HPMC K100 M, Carbopol 934 P, Sodium bicarbonate and microcrystalline cellulose using a 10 % (w/v) polyvinyl pyrrolidone in isopropyl alcohol solution by mixing all ingredients mentioned in table except lubricants. After lubrication, weighed quantities of floating layer were subjected to compression. Granules were compressed with 5 mm punches on single punch machine. Hardness was kept between 4-5 kg/cm<sup>2</sup>.

MET tablet was weighed and drilled. After drilling, tablet was weighed again. The drilling was done such that tablet could retain microliter quantities of radioactive liquid solution of 99 mTc-DTPA. Quantity of 99 mTc-DTPA solution used was 5µl - 10 µl. The tablet was sealed with molten gelucire and was allowed to dry. The entire tablet was then again coated with molten gelucire.

### EVALUATION

#### Stability assessment of radio-labeled product

Stability tests were carried out to confirm that the radiolabel remained for the duration of study. Since the gamma scintigraphy procedure is intended to be carried out for a period of six h, the radio-labeled product was subjected to stability studies for a period of six h. The radio-labeled tablet was allowed to stand in 0.1 N HCl solutions. At various time intervals, the tablet was removed and activity retained was counted using CAPINTEC C II dose calibrator. The activity counted was corrected for the decay and the data was expressed as percentage of original activity.

#### Dissolution Studies

The release rate of MET from floating tablets was determined using USP dissolution testing apparatus II (Paddle type). The dissolution test was performed using 900 ml distilled water, at 37 ± 0.5°C and 75 rpm. A sample (10 ml) of the solution was withdrawn from the dissolution

apparatus hourly for 12 h, and the samples were replaced with fresh dissolution medium. The samples were filtered through Whatman filter paper and the absorbance of these solutions was measured at 235 nm. The cumulative percentage drug release was calculated.

### Gamma scintigraphy studies

The objective of in vivo gamma scintigraphic study was to provide a proof of concept that the floating capability of the gastro-retentive tablet was useful for increasing the gastric residence time of the dosage form. The in vivo transit behavior was monitored in four healthy male New Zealand white rabbits weighing 3.0 – 3.5 kg. The rabbits were housed individually in stainless steel cages, fed on a carrot, sprouted pulses and lucern grass and had a free access to water. They were subjected to an acclimatization period of one month. A wash out period of one week was maintained between two studies. Radio-labeling of the MET tablet was carried out as mentioned. Radio-labeled tablet was fed to rabbits orally. The rabbits were allowed to get up and move around and repositioned under the camera for each image. No food was permitted for the duration of six hours test period.

Animals were placed in ventro dorsol position under gamma camera. Anterior and Posterior static images were acquired in 128×128 matrix at different time intervals (0 minutes, 0.5, 1, 2, 3, 5 and 6 h) post administration of labeled MET tablet using Siemens ECam Gamma Camera fitted with low energy high resolution collimator. Regions of interest were drawn on both anterior and posterior images. The percentage of retained gastric activity of the labeled drug was calculated using geometric mean counts. Decay corrected counts were used to assess gastro retentive ( $T_{1/2}$ ) behavior of labeled MET tablet.

## RESULT AND DISCUSSION

### Assessment of stability of optimized radio-labeled product

From the stability results we can say that 27 % of radioactivity was retained at the end of 5 h in 0.1 N HCl indicating good stability for period of five h.

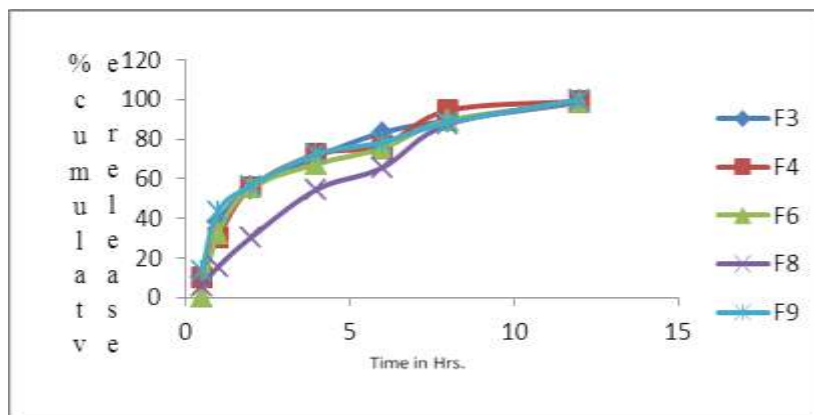
**Table 2: Results of stability study of radio-labeled formulation**

Time (h)	% of radioactivity retained	% of radioactivity released
1	92.4	7.6
2.5	63.2	36.8
4	43.6	56.4
5	27.4	72.6

As the activity with time reached to very low microcurie levels after 6 h, the amount of activity at 6 h could not be measured as it was beyond the range of the dose calibrator to measure such small amounts of activity.

### Dissolution studies

The results of dissolution studies can be seen from the graph of % Cumulative release v/s time (h), as shown in **Fig. 1**.



**Fig.1: Dissolution profile for optimized batches.**

The in vitro drug release profile was subjected to various kinetic models in order to find out the mechanism of drug release. The best fit model with the highest coefficient of regression was seen with Higuchi model. The rate constants were calculated from the slope of the respective plots. Drug release followed Higuchi kinetic model which describes the diffusion controlled drug release. Half life of MET calculated for optimized formulation was 3.54 h.

**Table 1: Diffusion kinetics model fitting data of bilayer tablet**

Model	Equation	R <sup>2</sup>
Zero order	Y=6.048 X +12.25	0.866
First order	Y=0.046+1.934	0.936
Korsmeyer - Peppas	Y=0.639+1.315	0.982
Higuchi Square Root	Y=33.38X -15.76	0.983
Hixon crowell cube root	Y=0.166X +5.050	0.973

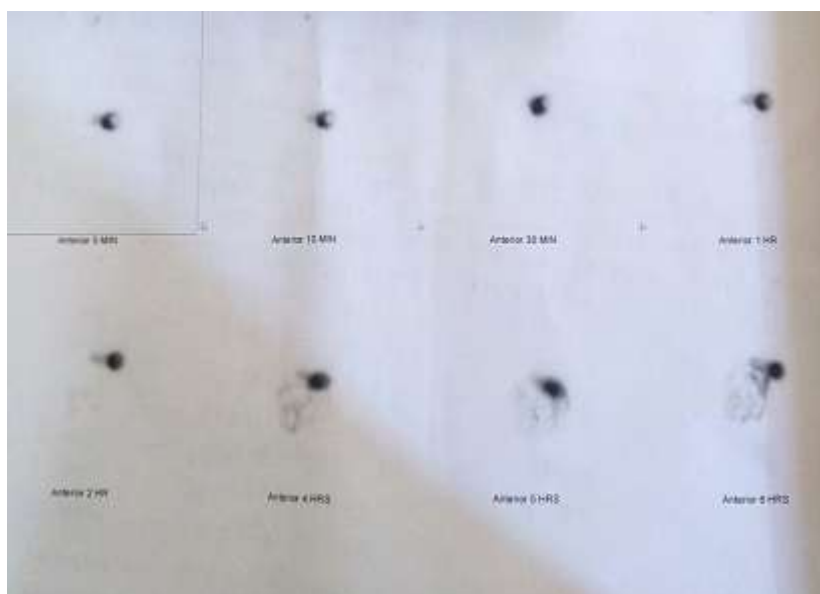
### Gamma scintigraphy studies

The percentage of region of interest i.e. gastric area was calculated and a representative result is shown in the table below. In three studies, there was significant retention of labeled MET tablet ( $T_{1/2}$  not reached at the end of study) and in remaining seven studies the gastric emptying  $T_{1/2}$  was found to be 125, 140, 215, 215, 210, 225, 105 mins.

From the gamma images (**Fig. 2**) and gastro-retention percent, we can conclude that the formulation shows gastro-retention (**Table 3**). The aim of the investigation was to establish the proof of concept of gastro-retention of the formulation designed. With this aim, the drug formulations were radio-labeled instead of the drug.

**Table 3: Percentage gastric retention at various time intervals**

Imaging points (in h)	Gastric retention percentage
0	92.2
0.5	86.7
1	79.5
2	75.6
4	71.2
5	50
6	45.6



**Fig. 2: Gamma images of GRT formulation.**

It was observed from the images that the MET formulation was retained in the stomach for 6 h and the drug was gradually released in the intestinal region over time. The results also indicated that the gastric peristalsis properties did not affect the tablet's retention in the stomach and the active drug was released gradually within 6 h. The floating lag time (FLT) of the size-reduced MET containing tablet was measured. The test was performed under the same conditions as in the other batches. The result indicated that size reduction did not affect the FLT of the tablets. The in vivo results from gamma scintigraphy correlated to the obtained results from the in vitro dissolution. The images obtained from gamma scintigraphy

study showed that gastric emptying did not affect the retention properties of the formulations in the stomach due to its floating property.<sup>[16]</sup>

## CONCLUSION

GR-MET formulation was unequivocally shown by *in vivo* scintigraphy to have good gastric retention with a mean retention  $T_{1/2}$  of 3.5 h. This matched well with the *in vitro* dissolution studies, using the USP paddle method, which showed significant retention at 5 h. GR-MET tablet formulation can be a successful drug for programmable release of MET over a period of time as shown by *in vivo* gamma scintigraphic study.

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