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Short Communication

# STABILITY OF FLOATING MICROSPHERES AT NORMAL AND ACCELERATED CONDITIONS

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**ABSTRACT**

**Objective:** To study the stability profile of floating microspheres of repaglinide as per ICH guidelines under normal (25±2°C/60±5% RH) and accelerated condition (40±2°C/75±5% RH and 5-8°C/65±5% RH) for a period of six mo.

**Methods:** Floating microspheres were prepared using ethylcellulose (EC) and hydroxypropyl methylcellulose (HPMC) and subjected to stability studies. Physical appearance, scanning electron microscopy (SEM), % buoyancy, % residual drug content and drug release of stored formulation were evaluated after every two mo.

**Results:** Change in color, size, and residual drug content showed no significant variations in formulations stored at a different set of conditions. SEM images showed no morphological transformation during the study. Less than 5% change was observed in buoyancy and drug release.

**Conclusion:** The data depict that the formulation is sufficiently rugged for marketing worldwide under various climatic conditions including normal, oven and freezing temperature.

**Keywords:** Stability, Microspheres, Buoyancy, Physical, Freezing



Drug development process plays a key role in achieving scientific success and commercial launching of the drug product. During the developmental stage, pharmaceutical analysis and stability studies are the most important steps required to determine and assure the identity, potency and purity in terms of ingredients, as well as formulated products [1].

Definition of stability for a pharmaceutical product is the capability of specific formulation in a prescribed container/closure system to maintain its physicochemical, microbiological, toxicological, protective and informational integrity [2]. Thus, the effect of environmental factors on the quality of the drug substance or a formulation which is utilized for prediction of its shelf life is studied thoroughly. For regulatory approval of formulation predetermined storage conditions and proper instructions regarding labeling of a product is prime important during stability studies [3].

Drug formulation's stability testing is a complex process involving a variety of factors during which it may undergo a change in appearance, consistency, content uniformity, particle size and shape, moisture contents, pH, package integrity thereby affecting its stability. Various chemical reactions may occur in the pharmaceutical product such as reduction, oxidation, racemization and may leads to the formation of the degradation product, loss of excipient and active pharmaceutical ingredient (API) potency, loss of activity etc [4].

Various regulatory authorities in several countries have made provisions for submission of data generated during stability studies by the manufacturers to assure the formulation of stable product from stable molecule to be available for patients use. ICH and WHO issue guidelines for smooth and planned conduction of stability studies and must be followed strictly for quality results [5, 6].

Repaglinide is an oral hypoglycemic agent and the first member of meglitinide class, used to treat type-2 diabetes mellitus. Floating microspheres have been known for increasing therapeutic efficacy and enhance physical as well as the chemical stability of many drugs [7]. *In vitro* and *in vivo* evaluation of the formulation have been extensively studied and prolific results thus obtained have been reported previously [8].

The objective of the present investigation is to assess the stability profile of optimized formulation of EC and HPMC at the normal and accelerated condition. As reported earlier *in vitro* characterization results shows prolonged drug release from spherical, highly entrapped and good buoyant floating microspheres. Excellent *in vivo* floating behavior in rat stomach for appreciable time with good results during histopathological studies have been reported [8]. Now, during stability studies, extensive work on physical appearance, SEM, % buoyancy, % residual drug content and drug release studies of the formulation at normal and accelerated condition for a period of six mo have been conducted.

Repaglinide was received as gift sample from Torrent Pharmaceuticals, Ahmadabad, India. Ethylcellulose, Hydroxypropyl methylcellulose were purchased from Himedia Chemicals, India. Analytical grade ethanol, dichloromethane, polivinyl alcohol (PVA) was procured from SD fine chemicals Mumbai, India.

Microspheres were prepared by solvent diffusion-evaporation technique [9]. Repaglinide (10 mg), EC: HPMC (5cps) 1:2 ratio and 0.1% of polyethylene glycol (as surfactant) were mixed in 1:1 ratio of ethanol and dichloromethane. The slurry was slowly introduced into 80 ml of 0.46% w/v of PVA as an emulsifier and stirred at 900 rpm for about 1 h. The floating microspheres were thoroughly washed 3-4 times with distilled water, dried for 1 h at room temperature and stored in desiccators over fused calcium chloride [8].

The prepared formulation was studied for stability profile at normal and accelerated conditions as per ICH guidelines. The formulation was placed separately in amber colored borosilicate screw capped glass container and stored at normal room temperature (25±2 °C/60±5% RH), freezing temperature (5-8 °C/65±5% RH) and for accelerated testing at oven temperature (40±2 °C/75±5% RH) respectively for a period of 6 mo. After every two mo the stored formulations were evaluated for various parameters.

Change in color was visualized and size of the formulation was determined by optical microscopy (Dolphin ASI-22 5354) using an ocular micrometer. Morphological transformation, if any during the study was checked using scanning electron microscope (SEM) (Jeol JSM-1600, Tokyo, Japan). Floating ability was studied by placing the

microspheres in SGF (pH 1.2) and stirred at 100 rpm for 12 h. % buoyancy was calculated using formula as reported [8]. % residual drug content was determined by crushing the microspheres and dissolved in ethanol followed by filtration and analyzed at 247 nm using UV spectrophotometer (Shimadzu 1700).

The release rate of the drug was examined in 0.1 N HCl using dissolution test apparatus (Veego, VDA-6DR, USP Std) of paddle type. 1 ml sample was withdrawn after 12 h filtered and analyzed spectrophotometrically at 247 nm.

The stability of the pharmaceutical product is essential in a number of ways such as: safety of the patient, legal requirement concerned with the quality and purity of drug and to prevent the economic repercussion of marketing of the undesirable product. Several researchers have studied the stability profile of floating microspheres as per ICH guidelines [10, 11, 12]. In the present study stability of optimized formulation was carried out at three different temperatures (5-8 °C, 25±2 °C and 40±2 °C) and evaluated continuously after every two mo.

Physical appearance showed no significant variation and change in color (table 1). Similarly, no significant change in the size of microspheres from initial day was reported up to 3 mo while smaller non-significant change was observed at end of 6 mo for the samples stored at refrigeration and oven temperature respectively (data not shown). The Little increment in particles size might be due to aggregation of microspheres stored in refrigerated conditions was observed. Formulation stored at high temperature shows no aggregation of microspheres as the probability of melting of the polymer is negligible owing to their high melting point. The particle size of the microspheres was found to decrease slightly at 40±2 °C may be due to the evaporation of residual amount of organic solvent at a higher temperature from the microspheres [11]. During surface morphology, SEM images indicate the retention of the spherical shape of microspheres, there was no sign of morphological transformation with the development of cracks or rupturing of the surface of stored formulations (fig. 1).

The floating capacity was assured as the % buoyancy was not changed much (<5%) for the stored formulation. The % buoyancy of initial sample at the start of the study was 84.36±0.9 % which changes to 82.85±0.3, 84.02±0.4 and 82.91±0.5 % for increasing order of temperature selected during the study (n=3). Thus, sample stored at room temperature shows maximum buoyancy. The histogram was plotted between % buoyancy and time for formulations stored at different temperatures [12] and the results were almost similar to initial day of storage (fig. 2). To determine % residual drug content [13], the initial drug content of all the formulations was taken to be 100%. Graph between % drug content and time was plotted which shows no significant effect from the initial day (fig. 3). All datas are represented as mean±SD (n=3). A non-significant loss of drug was found in the sample stored at 40±2 °C as compared to 5-8 °C and 25±2 °C which may be due to slight loss of integrity of the system [14].

Drug release was studied, and the histogram was plotted between % drug release and time for different formulations, which showed no significant change. At the end of study after 12 h release of stored formulation was taken and compared by plotting a graph between cumulative % drug release and time (fig. 4). There was no change in release rate at freezing and room temperature. The release is slightly increased (84.5±2.1 to 86.10±2.1) for formulation kept at oven temperature as compared to initial day release which might be due to the formation of more pores in the microspheres due to evaporation of residual amount of solvent. The results of the stability studies indicated that the repaglinide loaded floating microspheres was stable at all conditions but most stable at room temperature.

The optimized formulation of repaglinide using EC and HPMC (5 cps) having good *in vitro* and *in vivo* characteristics was formulated and evaluated for stability profile at three different temperature conditions (freezing, room and oven). The storage conditions employed do not have a drastic effect on microspheres integrity. Stability of product is thus, justified by observing the results of physical and chemical stableness of dosage form. Thus, it may be concluded that EC and HPMC microspheres of repaglinide are a suitable delivery system for prolonged activity having significant stability without losing its therapeutic activity when stored at different temperatures.

**Table 1: Physical appearance of formulation during stability study after six mo**

| | Condition | Color change | Size | Inference |
|---|---|---|---|---|
| 1. | Normal | No change | No change | Complies with stability condition |
| 2. | Accelerated | No change | No change | Complies with stability condition |

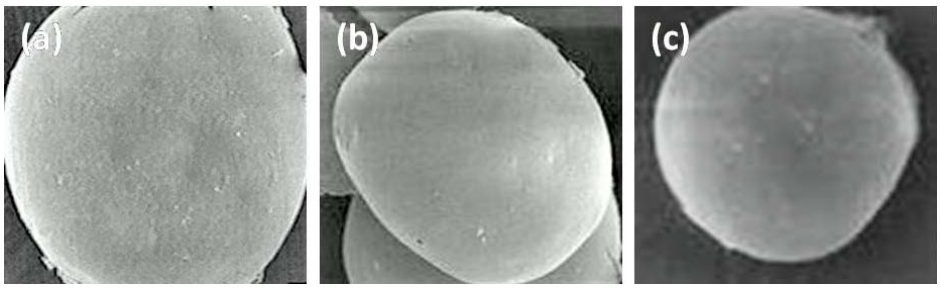


**Fig. 1: SEM images of microspheres: (a) Freezing temperature (b) Room temperature (c) Oven temperature**

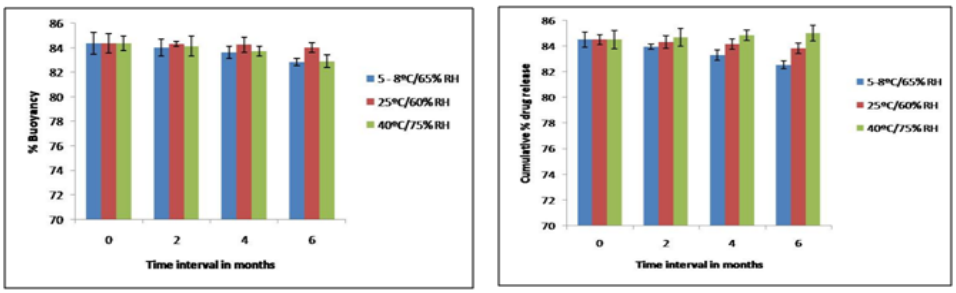


**Fig. 2: Comparison of % buoyancy and % drug release at different conditions. All datas are represented as mean±SD (n=3)**

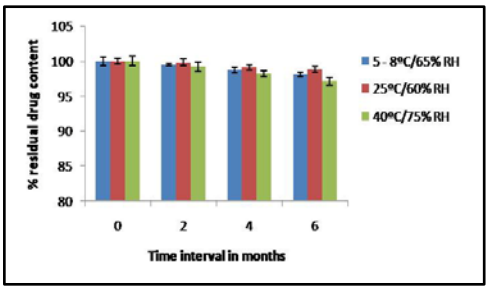


**Fig. 3: % residual drug content of formulations at different temperatures. All datas are represented as mean±SD (n=3)**

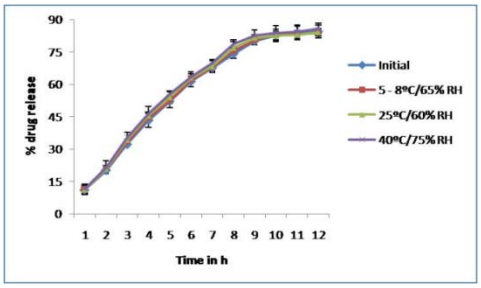


**Fig. 4: % cumulative drug release after six mo storage at different temperatures for 12 h All values are represented as mean±SD (n=3)**

## CONFLICT OF INTERESTS

All authors have none to declare

## REFERENCES

1. Singh S, Bakshi M. Guidance on the conduct of stress test to determine the inherent stability of drugs. Pharm Technol Asia 2000;1-14.
2. Kommanaboyina B, Rhodes CT. Trends instability testing, with emphasis on stability during distribution and storage. Drug Dev Ind Pharm 1999;25:857-67.
3. Singh S. Stability testing during product development, in: Jain NK, Pharmaceutical Product Development. CBS publisher and distributors India; 2000. p. 272-93.
4. Carstensen JT, Rhodes CT. Rationale policies for stability testing. Clin Res Regulatory Affairs 1993;10:177-85.
5. Guideline IH. Stability testing guidelines: stability testing of new drug substances and products. ICH Q1A (R2) (CPMP/ICH/2736/99); 1999.
6. WHO. Stability studies in a global environment. Geneva meeting working document QAS/05.146; 2004.
7. Menon A, Ritschel WA, Sarkar A. Development and evaluation of a monolithic floating dosage form for furosemide. J Pharm Sci 1994;83,239-45.
8. Sharma M, Kohli S, Dinda A. *In vitro* and *in vivo* evaluation of repaglinide loaded floating microspheres prepared from different viscosity grades of HPMC polymer. Saudi Pharm J 2015;23:675-82.
9. Kawashima Y, Niwa T, Takeuchi H, Hino T, Itoh Y. Hollow microspheres for use as a floating controlled drug delivery system in the stomach. J Pharm Sci 1992;81:135-40.
10. Tyagi LK, Kori ML. Stability study and *in vivo* evaluation of lornoxicam loaded ethyl cellulose microspheres. Int J Pharm Sci Drug Res 2014;6:26-30.
11. Tyagi LK, Kori ML. Stability study and *in vivo* evaluation of lornoxicam loaded ethyl cellulose microspheres. Int J Pharm Sci Drug Res 2014;6:26-30.
12. Panwar MS, Tanwar YS. Evaluation of stability of diltiazem hydrochloride is floating microspheres at normal and accelerated conditions. J Pharm Biol Sci 2015;5:57-60.
13. Nath B, Nath LK, Mazumdar B, Sharma N, Sarkar M. Design and development of metformin HCl floating microcapsules using two polymers of different permeability characteristics. Int J Pharm Sci Nanotechn 2009;2:627-37.
14. Gawde P, Agrawal S. Design and characterization of eudragit coated chitosan microspheres of deflazacort for colon targeting. J Pharm Res 2012;5:4867-70.