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# Formulation & In-Vitro Evaluation of Wax Incorporated Floating Beads of Silymarin

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**Abstract:** Among modified-release oral dosage form increasing interest has currently turned to systems designed to achieve prolonged residence at the site of drug delivery. Among different controlled release systems microbeads offer prolonged residence time and controlled release in stomach. Silymarin (SLM) is an effective hepatoprotective drug used in therapeutic practice today. However, its absorption is in stomach and needs to be retained in the same for prolonged period. So Silymarin is suitable candidate to develop into wax incorporated alginate beads. The objective of this study was to formulate and evaluate wax incorporated alginate microbeads loaded with Silymarin. The alginate microbeads of Silymarin with variable concentrations of waxes (White bees wax & Carnauba wax) were prepared by Melt Extrusion-Ionotropic gellation method. The prepared microbeads were characterized for their pre-formulation and post formulation parameters. Compatibility studies proved that there was no interaction between Silymarin and waxes used. Silymarin beads were roughly spherical in nature, which was confirmed by Scanning electron microscopy. Silymarin beads with normal frequency distribution were obtained. A maximum of 94.75% drug entrapment efficiency was obtained. The *in-vitro* performance of Silymarin beads showed controlled release up to 12 hrs depending on the wax concentration. Finally it can be concluded that the formulated sodium alginate gel beads with white bees wax were more feasible and effective than carnauba wax beads in encapsulating SLM and thereby increasing the effectiveness of the drug.
**Keywords:** Silymarin (SLM), Controlled release, Alginate gel beads, Waxes

## Introduction:

The design of oral controlled drug delivery systems should be primarily aimed to achieve more predictable and increased bioavailability. Now a day's most of the pharmaceutical scientists are involved in developing the ideal drug delivery systems. This ideal system should have an advantage of single dose for the whole duration of treatment and it should deliver the active drug directly at the specific site. Controlled release implies the predictability and reproducibility to control the drug release, drug concentration in target tissue and optimization of the therapeutic effect of a drug by controlling its release in the body with lower and less frequent dose.[1]

However, this approach is being stilled with several physiological difficulties such as inability to restrain and locate the controlled drug delivery system within the desired region of the GIT due to variable gastric emptying and motility. Furthermore, the relatively brief GET in humans which normally average 2-3 hrs through the major absorption zone, i.e., stomach and upper part of the intestine can result in incomplete drug release from the drug delivery system leading to reduced efficacy of the administered dose. Therefore, control of placement of a DDS in a specific region of the GI tract offers advantages for a variety of important drugs characterized by a narrow absorption window in the GIT or drugs with a stability problem.[2]

Microbeads are small, solid and free flowing particulate carriers containing dispersed drug particles either in solution or crystalline form that allow a sustained release or multiple release profiles of treatment with various active agents without major side effects. Additionally, the beads maintain functionality under physiological conditions, can incorporate drug to deliver locally at high concentration ensuring that therapeutic

levels are reached at the target site while reducing the side effects by keeping systemic concentration low. In the pharmaceutical field, the use of a biocompatible polymer, preferably naturally occurring shows remarkable advantages compared with the normal synthetic polymers.[3-7]

Silymarin (SLM) is one of the most powerful drugs for the hepatic diseases. It is known for its hepatoprotective action against hepatic glutathione depletion induced by ethyl alcohol and paracetamol in animal studies. Silymarin degrades as the pH increases. Hence it is necessary to dissolve it in less pH for the protection of the drug and to reduce the gastric disturbance and more over, the site of absorption of SLM is in the stomach pH. Hence it is suitable to formulate SLM as floating microbeads to reduce frequency of dosing, prevent the drug from degradation in the intestinal pH and increases its shorter biological half life.[8-10]

## Materials and Methods

### Preparation of Floating Alginate Microbeads:

For the present study, biodegradable polymer sodium alginate combined with different waxes is used with the active ingredient for preparation of floating microbeads (Table 1).

**Table 1: Formulation Design of Silymarin Loaded Floating Microbeads**

| Sl.No | Ingredients | $F_1$(gm) | $F_2$(gm) | $F_3$(gm) | $F_4$(gm) | $F_5$(gm) | $F_6$(gm) |
|---|---|---|---|---|---|---|---|
| 1 | Silymarin (SLM) | 1 | 1 | 1 | 1 | 1 | 1 |
| 2 | Sodium Alginate | 2 | 2 | 2 | 2 | 2 | 2 |
| 3 | White Bees Wax | 1 | 2 | 3 | - | - | - |
| 4 | Carnauba Wax | - | - | - | 1 | 2 | 3 |
| gm = grams | | | | | | | |

Silymarin loaded floating alginate gel beads were prepared by hot melt extrusion along with Ionotropic gellation method. Accurate quantity of polymer was dissolved in 50ml of distilled water and stirred to form dispersion. Drug was added to the above dispersion and again stirred for uniform distribution. In another beaker, various amounts of waxes (viz. white bees wax, carnauba wax) were melted in water bath at 60–85°C, depending on the melting range of the waxes used. The molten wax was added to the homogenized mixture of polymer and SLM which was already heated to same temperature and stirred until a homogenous mixture was obtained. The hot melted mixture was extruded through a 23G syringe needle into calcium chloride solution (2% w/v). The beads were allowed to remain in the same solution for 30 min to improve their mechanical strength. The formed beads were separated, washed with water and allowed to dry at room temperature overnight.[11-17]

### Evaluation of Drug Loaded Floating Microbeads:

### Drug Polymer Interaction (FTIR) Study:

Drug polymer interactions were studied by FT-IR spectroscopy. One to 2 mg of SLM alone, mixture of drug and polymer, beads were weighed and mixed properly with potassium bromide uniformly. A small quantity of the powder was compressed into a thin semitransparent pellet by applying pressure. The IR-spectrum of the pellet from 500–4000 $cm^{-1}$ was recorded taking air as the reference and compared to study any interference.[18-20]

### Surface Morphology:

Scanning electron microscopy (SEM) has been used to determine particle size distribution, surface topography, texture, and to examine the morphology of fractured or sectioned surface. SEM studies were carried out by using JEOL JSM T-330A scanning microscope (Japan).[21-23]

### Frequency Distribution Analysis:

The diameter of a sample of gel beads (300 beads) of each formulation was determined using Vernier calliper. In order to define a frequency distribution or compare the characteristics of particles with many different

diameters, the frequency distribution can be broken down into different size ranges, which can be presented as histogram.[24]

**Percentage Yield:**

The measured weight was divided by total amount of all non-volatile components which were used for the preparation of microbeads. Percentage yield can be calculated by

**% yield = Total weight of excipients and drug / Actual weight of product x 100**

**Buoyancy Behaviour:**

The time between the introduction of the FDDS into the medium and its buoyancy to the upper one third of the dissolution vessel (floating lag time) and the floating ability was determined using USP dissolution tester apparatus II (Paddle method). Fifty beads were put in the vessel and the paddles were rotated at 50 rpm in 900 ml 0.1 N HCl pH 1.2, maintained at 37±0.5 °C for 12 hours. The preparation was considered to have buoyancy, only when all beads floated on the test solution immediately or within a lag time.[25-27]

**Drug Content:**

To determine the drug content and encapsulation efficiency of the beads, 200 mg beads were crushed and dispersed in suitable solvent (methanol). The dispersion was sonicated for 15 minutes, left overnight for 24 hrs and filtered. A 1 ml sample was taken and diluted with suitable solvent (methanol), and drug content assayed using a UV-visible spectrophotometer at $\lambda_{max}$ of 287 nm. The drug content of each formulation was recorded as mg / 200 mg of gel beads.[28-30]

**Drug Entrapment Efficiency:**

The drug entrapment efficiency of prepared beads was determined by using the equation.[31, 32]

**EE (%) = Actual Drug Content / Theoretical Drug Content X 100**

***In-Vitro* Dissolution Study:**

Drug loaded silymarin gel beads equivalent to 100 mg of Silymarin was loaded into the basket of the dissolution apparatus. Dissolution study carried out for 12 hrs in 0.1N Hcl. 1 ml of the sample was withdrawn from the dissolution media at suitable time intervals and diluted to 10 ml using 0.1N Hcl and the same amount was replaced with fresh dissolution medium. The absorbance was measured at 287 nm by using UV spectrophotometer, against a blank solution. Dissolution profiles of the formulations were analyzed by plotting cumulative percentage drug release versus time. The data obtained were also subjected to kinetic treatment to understand release mechanism. [33-39]

## Results and Discussion

In the current research, floating gel beads loaded with silymarin were formulated by hot melt extrusion along with Ionotropic gellation method using sodium alginate as biodegradable polymer and bees wax and carnauba wax as rate controlling polymers. The prepared gel beads are characterized for their post formulation parameters.

**FTIR Studies:**

From the FTIR studies given in Fig 1-3, showed no chemical interaction between the drug molecule and polymers used.

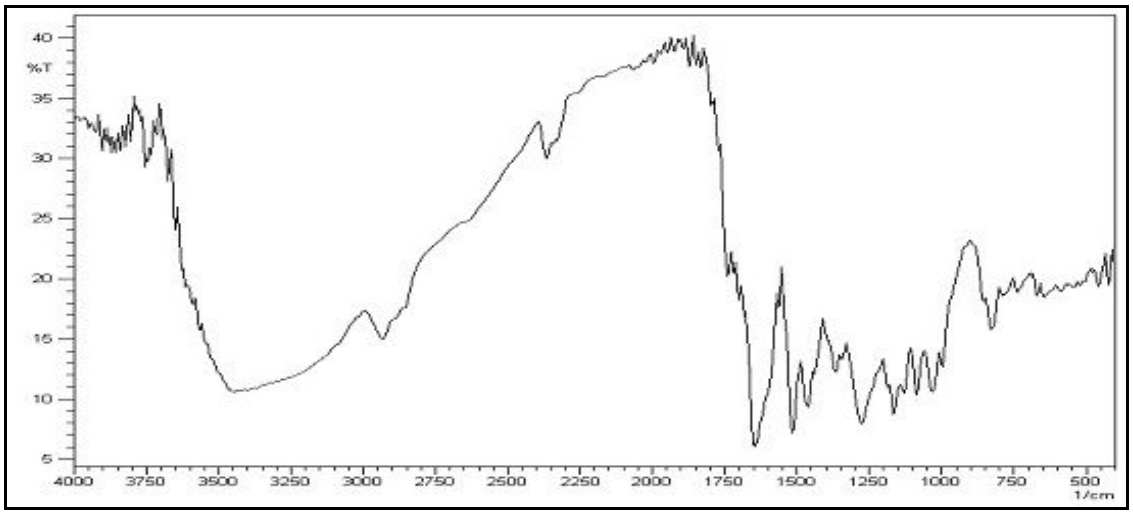


**Fig 1: FTIR of pure Silymarin**

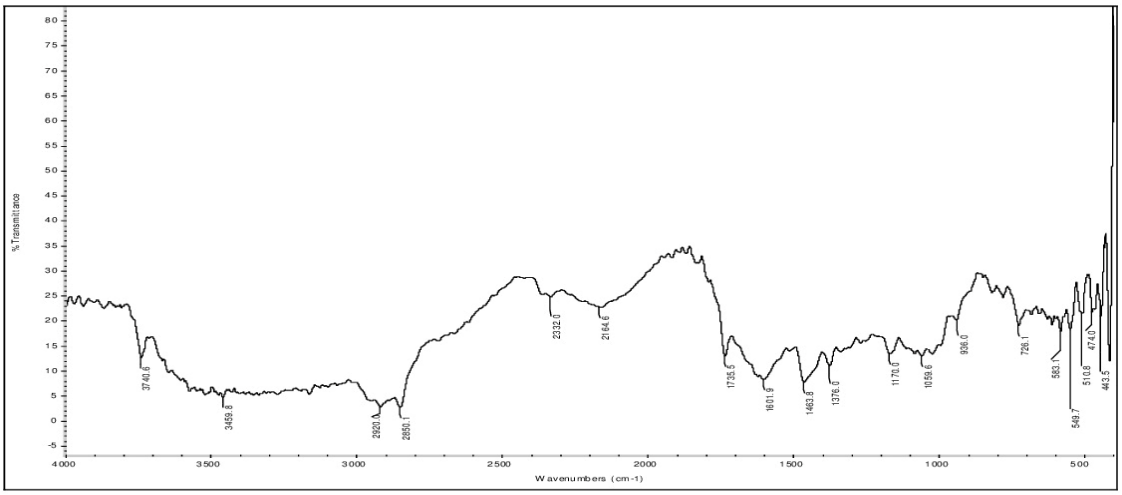


**Fig 2: FTIR spectra of blank Sodium alginate gel beads**

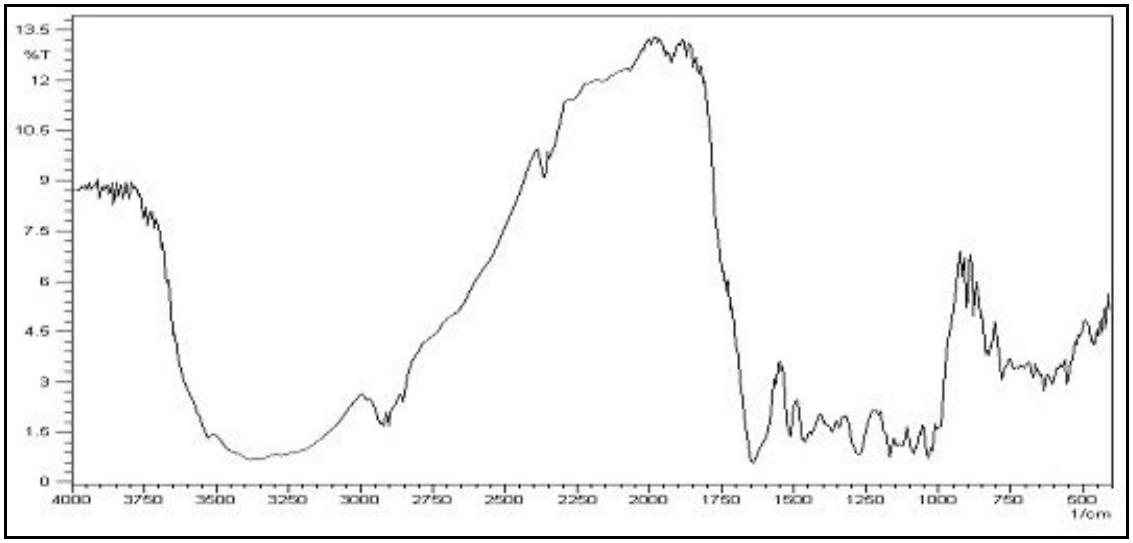


**Fig 3: FTIR spectra of sodium alginate beads of Silymarin**

**Particle Size:**

As the ratio of wax was increased, the mean particle size of SLM beads had also increased (Fig 4). The significant increase may be due to the increase in the viscosity of the droplets. SLM beads having a size range

of 1.0 to 2.1 mm (Fig 5) with normal frequency distribution was obtained. Compared to bees wax gel beads, carnauba wax beads were larger in size.

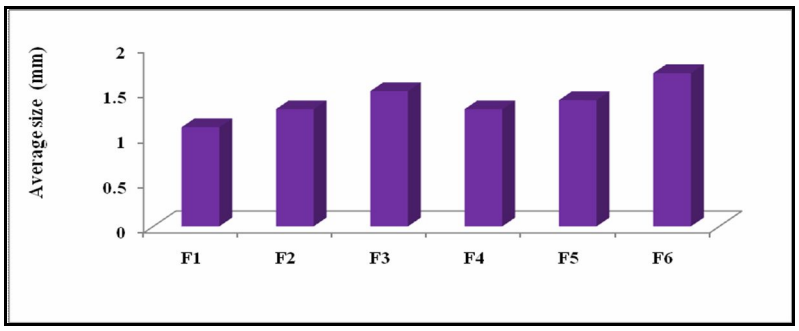


**Fig 4: Average diameter of SLM gel beads**

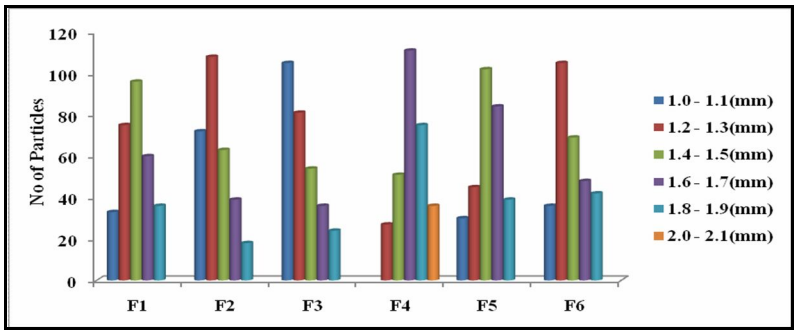


**Fig 5: Frequency distribution of SLM gel beads**

**Scanning Electron Microscopy (SEM):**

The SEM analysis was done on the prepared gel beads of bees wax and carnauba wax to access their morphological and surface characteristics. Surface smoothness was observed with beeswax incorporated SLM beads when compared to carnauba wax incorporated beads which was found to have a slightly rough surface. (Fig 6, 7)

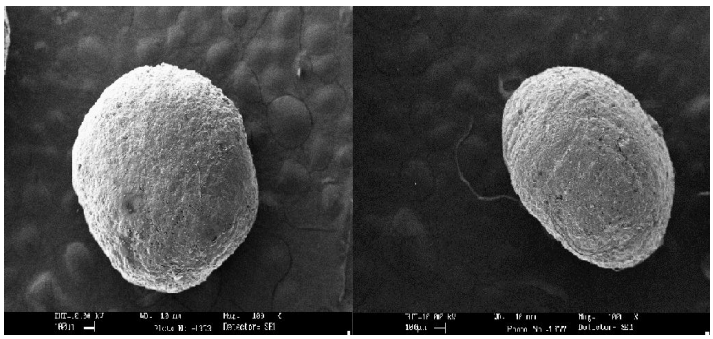

**Fig 6: SEM of SLM gel beads using Sodium Alginate and White Bess Wax**

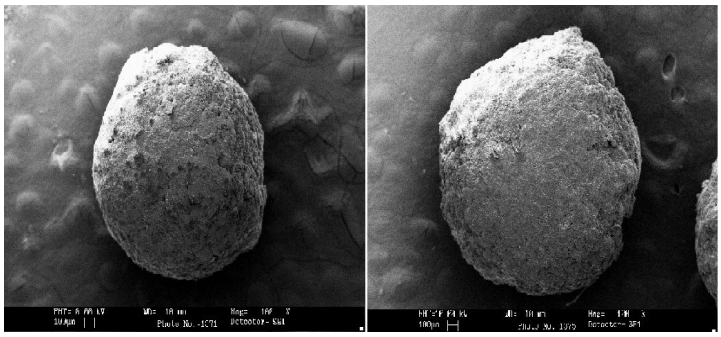

**Fig 7: SEM of SLM gel beads using Sodium Alginate and Carnauba Wax**

**Buoyancy Character:**

The floating ability of prepared beads was evaluated. The beads sank immediately in 0.1 N HCl (pH 1.2) showed excellent floating ability. The beads remained afloat throughout the study period (12hrs). It was observed that varying the wax concentrations in the bead formulations did not affect the floating lag time or the floating duration of the beads.

**Percentage Drug Entrapment Efficiency:**

The percent of drug content in the formulations were found to be in the range of 16.17% to 25.06% (Fig 9). The percentage entrapment efficiency was found to be 84.80% to 94.35%. A maximum of 94.35% drug entrapment efficiency was obtained in SLM beads which were prepared using sodium alginate and beeswax. It was further observed that the drug entrapment was proportional to the SLM **:** Wax ratio and size of the SLM beads. By increasing the wax concentration, the encapsulation efficiency was increased. The entrapment efficiency of bees wax gel beads was better than carnauba wax gel beads.

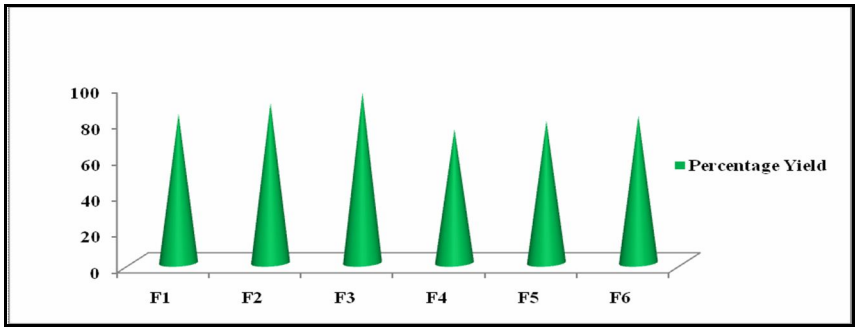


**Fig 8: Percentage yield of SLM gel beads**

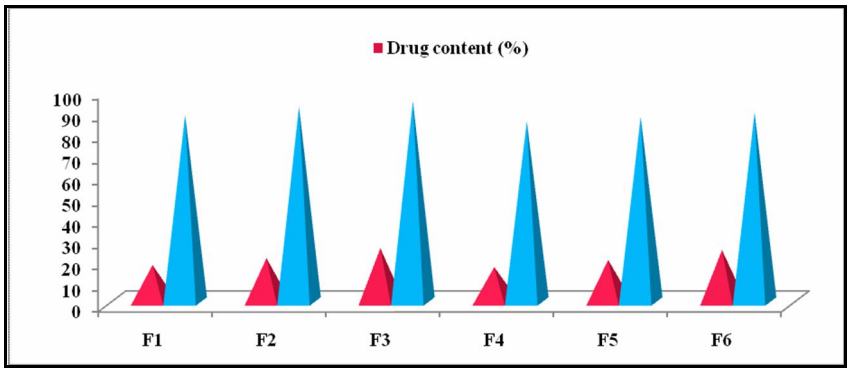


**Fig 9: Drug content & Entrapment Efficiency of SLM gel beads**

***In-vitro* Release Studies:**

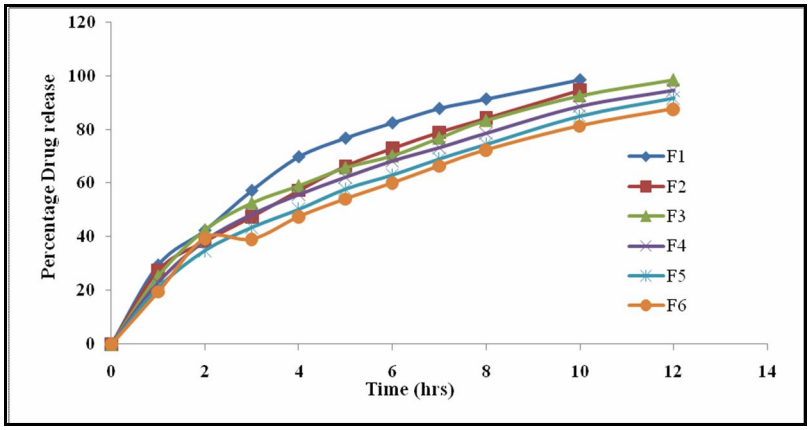


**Fig 10: *In-vitro* dissolution profiles of prepared formulations ($F_1$-$F_6$)**

The *in-vitro* performance of SLM beads showed prolonged and controlled release of SLM (Fig 10). The results of the *in vitro* dissolution studies showed controlled release in a predictable manner. The release showed a biphasic release with an initial burst effect. At the end of first hour drug release was 29.52%, 27.53%, 25.50%, 23.13%, 21.12 & 19.57% for $F_1$ to $F_6$ respectively. As the wax concentration was increased, the drug release from the floating beads was found to decrease. Compared to beeswax, carnauba wax retarded drug release more effectively; however, the bees wax incorporated alginate gel beads had an optimum release at the end of 12$^{th}$ hour.

**Table 2: *In-vitro* Release Profile of Formulations $F_1$-$F_6$**

| Time (hrs) | Percentage Drug Release | | | | | |
|---|---|---|---|---|---|---|
| | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | $F_6$ |
| 1 | 29.52 | 27.53 | 25.50 | 23.13 | 21.12 | 19.57 |
| 2 | 42.40 | 38.35 | 42.67 | 38.67 | 34.96 | 39.54 |
| 3 | 57.22 | 47.50 | 52.69 | 48.43 | 43.53 | 39.15 |
| 4 | 69.72 | 57.22 | 59.09 | 55.78 | 50.28 | 47.54 |
| 5 | 76.68 | 66.34 | 65.82 | 62.27 | 57.83 | 54.18 |
| 6 | 82.33 | 72.92 | 70.26 | 68.30 | 63.06 | 60.07 |
| 7 | 87.69 | 78.91 | 76.91 | 73.24 | 69.03 | 66.46 |
| 8 | 91.20 | 84.14 | 83.42 | 78.61 | 74.46 | 72.36 |
| 10 | 98.34 | 94.57 | 92.61 | 88.61 | 84.94 | 81.33 |
| 12 | - | - | 98.55 | 94.58 | 91.66 | 87.66 |
| hrs : Hours; $F_1$: Formulation 1; $F_2$: Formulation 2; $F_3$: Formulation 3; $F_4$: Formulation 4; $F_5$: Formulation 5; $F_6$: Formulation 6 | | | | | | |

## Conclusion

After observing all the experimental results it was conclusively demonstrated that floating gel beads loaded with silymarin were formulated by hot melt extrusion along with Ionotropic gellation method using sodium alginate as biodegradable polymer and bees wax and carnauba wax as rate controlling polymers. *In-vitro* dissolution profiles showed that the release was sustained for a period of 12 hrs. Formulation with higher concentration of wax showed optimum results within all the evaluated parameters and hence considered as the ideal formulations. However, a more promising controlled release was observed in formulation with highest amount of bees wax which showed the best results with other parameters within the specified limits.

## Acknowledgement

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