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Research Article

PREPARATION AND EVALUATION OF SULFASALAZINE LOADED SODIUM ALGINATE MICROBEADS FOR SUSTAINED DELIVERY

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ABSTRACT

Objective: The main objective of the work was to prepare and evaluate sulfasalazine loaded sodium alginate microbeads for sustained delivery for the treatment of inflammatory bowel disease and rheumatoid arthritis. Sulfasalazine has crystalluria, thrombocytopenia, and megaloblastic anemia as side effects, so to reduce side effect microbeads were prepared.

Methods: The sulfasalazine microbeads were prepared by inotropic gelation method by optimizing process parameters such as concentration of calcium chloride, agitation speed, and time of agitation. The concentration of polymer sodium alginate was varied.

Result: Among the five formulations, the best formulation was considered by comparing process parameters such as the entrapment efficiency, drug content, *in vitro* drug release studies, scanning electron microscope analysis, and zeta potential.

Conclusion: On comparison, B3 formulation was considered as best formulation with a mean particle size ranging from 40.9 to 244 µm, drug content of 94.7%, entrapment efficiency of 87.7%, and the drug release was found to be 97.1% for 12 hrs and followed zero order kinetics and non-Fickian diffusional pathway, with a zeta potential value of −56.8 mV indicating higher stability.

Keywords: Inotropic gelation method, Sodium alginate, Microbeads, Rheumatoid arthritis, Side effects.

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INTRODUCTION

Controlled drug delivery systems are designed to deliver the drug at predetermined rate at a specific site thereby reducing the dose, dosing frequency, and side effects of the drug [1,2]. Controlled drug delivery system holds promising results in site-specific targeting. One of such approach is the formulation of microbeads as drug carriers. Microbeads are defined as the uniform polymeric particles, typically ranging in size from 0.5 µ to 500 µm in diameter [3]. Inotropic gelation method is based on the electrostatic interaction between amine groups of the polymer and negatively charged group of polyanion. Naturally occurring polysaccharides are used as biopolymers in novel drug delivery system, thus providing ecofriendly pharmaceutical process. Among the natural polymers, sodium alginate was considered to be the best polymer and forms a reticulated structure when cross-linked with polyvalent or divalent ions. Sulfasalazine was selected as a drug of choice for the preparation of sodium alginate microbeads. It belongs to a class of disease-modifying antirheumatic drugs used in the treatment of rheumatoid arthritis to slow down disease progression. Sulfasalazine is a sulfa drug, a derivative of mesalazine and prodrug of 5 aminosalicylic acid that is covalently linked to the antibiotic sulfapyridine by an azo bond. It has been used in the treatment of inflammatory bowel disease and rheumatoid arthritis because of its ability to induce T-lymphocyte apoptosis modulates inflammatory mediators. Sulfasalazine is available in the dosage form of salazopyrin 500 mg tablets, salazopyrin enteric coated tablets. For treatment of rheumatoid arthritis, 500 mg tablets are taken 2-3 times a day. As the oral route of solid dosage forms have many side effects and chances of missing the doses, sulfasalazine loaded sodium alginate microbeads were prepared, thereby to reduce the dose, dosing frequency, and side effects of the drug.

MATERIALS AND METHODS

Materials

Drug

Sulfasalazine (gift sample from posh chemicals).

Excipients

- Sodium alginate obtained from SD Fine-Chem Limited, Mumbai,
- Ethanol (SD Fine-Chem Limited, Mumbai),
- Calcium chloride (SD Fine-Chem Limited, Mumbai),
- Potassium dihydrogen phosphate (SD Fine-Chem Limited, Mumbai),
- Sodium hydroxide (SD Fine-Chem Limited, Mumbai).

Methods

Preparation and evaluation of microbeads

Sulfasalazine loaded microbeads were prepared by dissolving sodium alginate in distilled water at 800 rpm for 30 minutes to get a bubble free clear solution of different concentrations (1%, 2%, 3%, 2:1, and 3:1). Sulfasalazine was accurately weighed and added to the polymeric solution to form a clear solution. The counterion solution was prepared by dissolving 4% calcium chloride solution. The drug loaded polymeric solution was added dropwise using 20-gauge hypodermic needle fitted with a syringe into aqueous solution of polyvalent cations. The cations diffuse into the drug loaded polymeric drops, forming a three dimensional lattice of ionically cross-linked moiety. The formed microbeads were kept in calcium chloride solution for 30 minutes to undergo complete curing. The microbeads were filtered and air dried [4].

Five formulations of microspheres were prepared by varying the concentration of drug:polymer ratios (Table 1).

Characterization of microbeads

Compatibility studies Fourier transform infrared (FTIR) analysis

The FTIR analysis of the sulfasalazine was carried out for qualitative compound identification. To check the compatibility of the drug with various polymers, IR spectra of drug, polymers, and combination of the drug and polymers were taken on an FTIR spectrophotometer in the wave number region of 4000-400/cm. The IR spectra of drug, polymers, and their combination are shown in spectra.

Study of surface morphology by scanning electron microscope (SEM)

The prepared formulations were dispersed in deionized water and sonicated for 30 minutes. A circular metal plate is taken onto which carbon double tape (1 mm×1 mm) is stickered; a drop of the resultant dispersion is placed onto the tape and allowed to dry for a while. Then, it is scanned under SEM for morphology.

Zeta potential measurement

The zeta potential (surface charge), which indicates the stability of the microbeads, can be defined as electrokinetic potential that is determined by electrophoretic mobility. The sample was prepared by diluting with doubled distilled water and corresponding zeta potential measured using Malvern Zetasizer.

Product yield

The yield of the prepared formulations was calculated as the percentage of the weight of the dried product at room temperature compared to the theoretical amount. Production yield is calculated using the following equation:

$$\text{Product yield} = \frac{\text{Weight of the product}}{\text{Weight of raw materials}} \times 100$$

Drug content

The various batches of the formulations were subjected for drug content analysis. Accurately weighed microsphere samples were mechanically powdered. The powdered microspheres were dissolved in adequate quantity of pH 7.4 phosphate buffer in two-necked round bottomed flask. With the help of mechanical stirrer, it was allowed to stir for 3 hrs then filter. The ultraviolet (UV) absorbance of the filtrate was measured using a UV spectrometer at 359 nm.

The drug content for the formulations was determined by calculating:

$$\text{Drug content} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

Entrapment efficiency

The various batches of the formulations were subjected for entrapment efficiency. Accurately weighed microsphere samples were added in adequate quantity of pH 7.4 phosphate buffer and were centrifuged in ultracentrifuge at 17,240 rpm at −4°C for 40 minutes. The free drug concentration was determined spectrophotometrically at 359 nm.

The entrapment efficiency for all the formulations was calculated by:

$$\text{Entrapment efficiency} = \frac{\text{Amount of microcapsules taken} - \text{unentrapped drug}}{\text{Amount of microcapsules taken}} \times 100$$

Table 1: List of formulations of microbeads

Serial number	Formulations	Drug:Polymer ratio
1	B1	1:1
2	B2	1:2
3	B3	1:3
4	B4	2:1
5	B5	3:1

***In vitro* drug release studies**

The *in vitro* drug release studies for sulfasalazine formulations were carried out for 12 hrs using USP Type II dissolution apparatus (paddle type). Dissolution medium used was phosphate buffer (pH 7.4), each 900 ml and temperature was maintained at 37°C±2°C at 50 rpm. Sulfasalazine formulations equivalent to 50 mg was used for each dissolution study. 5 ml samples were collected periodically and replaced with a fresh 5 ml of pH 7.4 phosphate buffer. The concentration of sulfasalazine was determined spectrophotometrically at 359 nm by suitable dilutions.

RESULTS AND DISCUSSION

FTIR spectrum

The prepared five formulations were characterized for drug-polymer interactions using FTIR (Horiba Scientific, Ltd.).

The peaks obtained in the spectra correlated with the peaks of drug spectrum. 1676/cm indicating the presence of carboxyl group. 1200-1120/cm indicating the presence of sulfonyl group. There

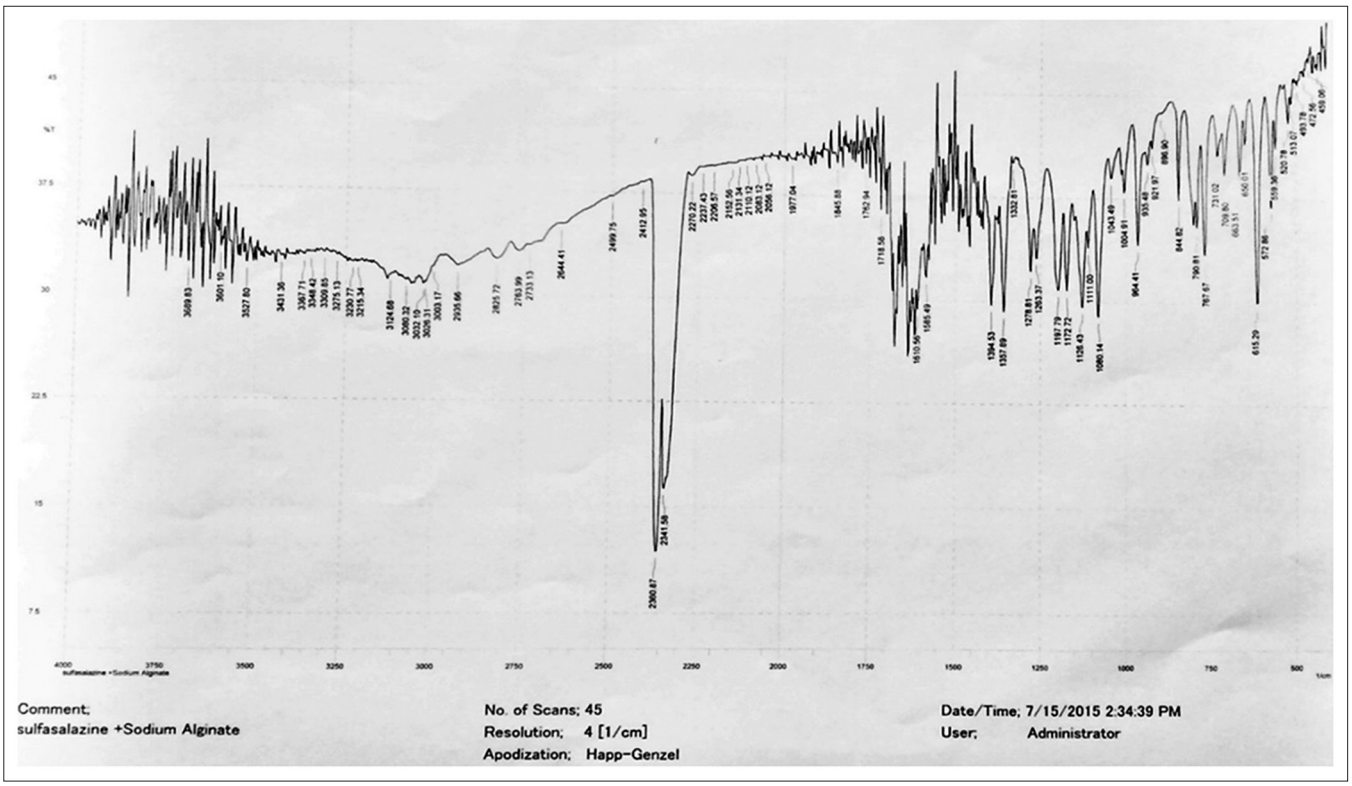

Fig. 1: Fourier transform infrared spectrum of best formulation of microbeads

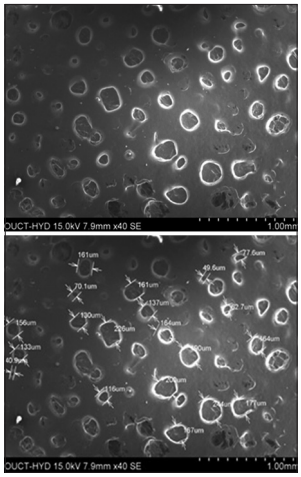

Fig. 2: Scanning electron microscope images of best formulation of microbeads

were no physical or chemical interactions and the peaks obtained in the spectra's of each formulation correlated with the peaks of drug spectrum (Fig. 1).

SEM

Surface morphology was determined for all five formulations using SEM (S-3700N, Hitachi, Japan).

The SEM images were found to be spherical with size ranging from 40.9 to 244 µm (Fig. 2).

Zeta potential

The best formulation of microbeads was characterized for zeta size to determine the stability of the formulation.

From Fig. 3, the zeta potential was found to be −56.8 mV indicating higher stability.

Product yield

Sulfasalazine microbeads were prepared by inotropic gelation method. After filtration, the obtained microbeads were evaluated for product yield.

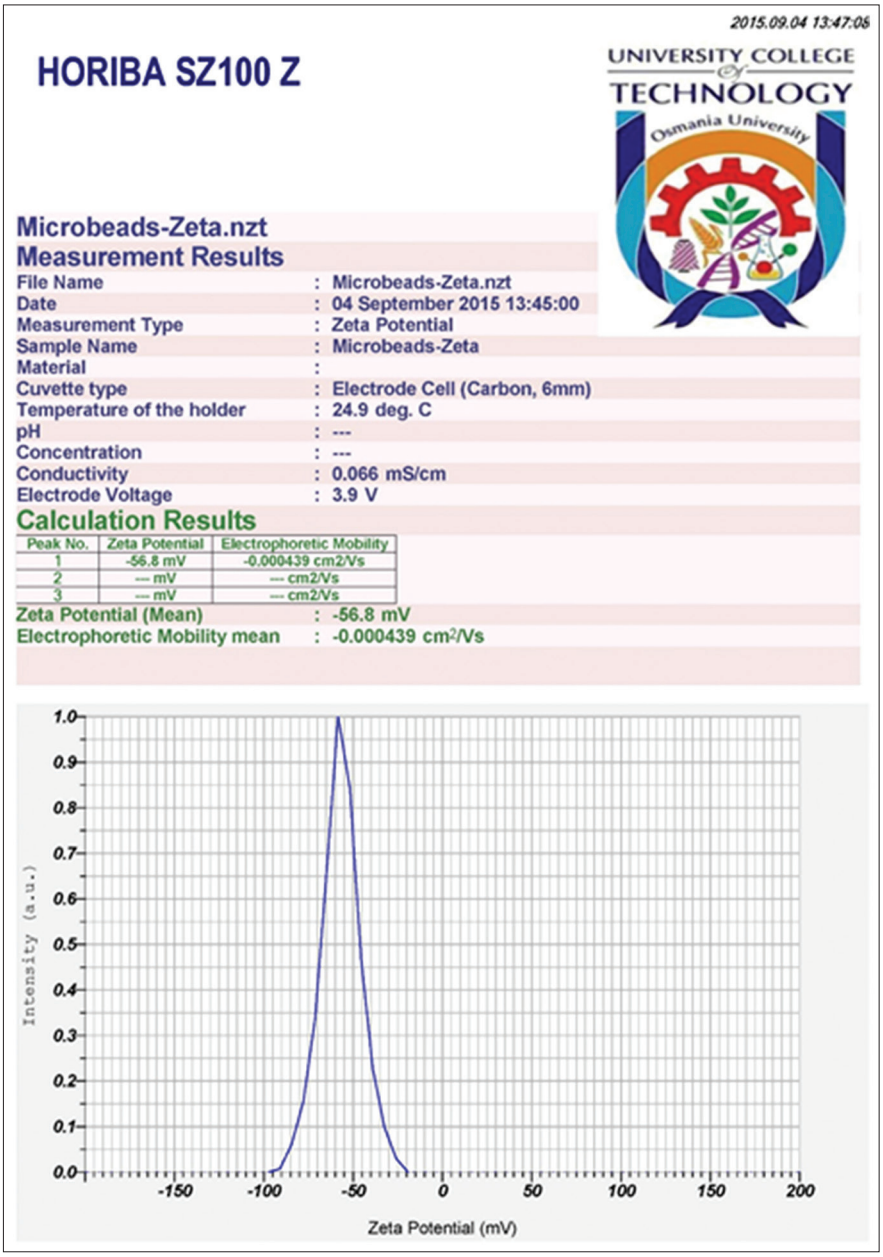

Fig. 3: Zeta potential report of best formulation B3 microbeads

The product yield of prepared five formulations B1, B2, B3, B4, and B5 was found to be 90%, 91%, 97%, 86%, and 84%, respectively. Out of the five formulations, the highest product yield was observed for B3 formulation (Fig. 4).

Drug content

The prepared microbeads formulations were evaluated for drug content. Drug content of B1, B2, B3, B4, and B5 was found to be 82%, 90.8%, 94.7%, 79.3%, and 75%, respectively. Out of the five formulations, the higher drug content was observed for B3 formulation (Fig. 5).

Entrapment efficiency

The entrapment efficiency of all the formulations of microbeads was calculated.

Entrapment efficiency of B1, B2, B3, B4, and B5 was found to be 77.6%, 84.4%, 87.7%, 68%, and 66.6%, respectively. Out of the five formulations, the highest entrapment efficiency was observed for B3 formulation (Fig. 6).

***In vitro* release studies**

In vitro drug release studies were performed using USP dissolution apparatus Type 2 paddle apparatus. The study was conducted for 12 hrs.

From the data, it was observed that B1 formulation showed 87.4% of drug release within 6 hrs and from B2 formulation 98.07% of drug release was observed within 8 hrs. From B3 formulation, 97.1% of drug release was observed within a period of 12 hrs. B4 formulation showed 91.16% of drug release in 4 hrs and B5 showed 94.4% in 4 hrs (Fig. 7).

With increase in polymer concentration, the sustain release profile of the formulation was found to be increased. The burst release was observed due to the drug present on the surrounding wall of the microbeads.

Comparison of best formulation with various kinetic models

Several plots (zero order plot, first order plot, Higuchi plot, and Peppas plots) were drawn to know the release kinetics and drug release mechanism.

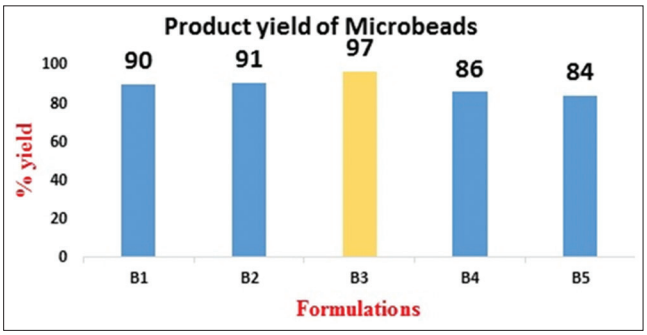

Fig. 4: Comparison of product yield among the five formulations of sulfasalazine microbeads

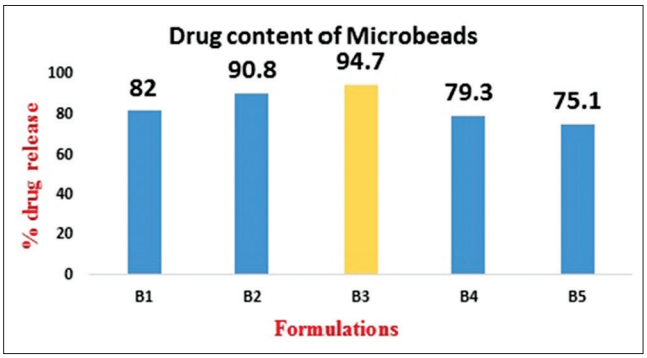

Fig. 5: Comparison of drug content among the five formulations of sulfasalazine microbeads

From the results, it was concluded that the drug release was following zero order kinetics with non-Fickian diffusional pathway (Fig. 8).

DISCUSSION

The formulation of sulfasalazine loaded microbeads for the treatment of rheumatoid arthritis was prepared using sodium alginate as the sustained release polymer and calcium chloride as a cross-linking agent [5]. The inotropic gelation technique was used to prepare microbeads, in which the gelation of anionic polysaccharide (sodium alginate) was achieved with oppositely charged counterion, i.e., calcium (Ca^{2+}) ions to form microbeads [6]. The gelation of alginate was caused by the formation of egg box junction to associate divalent metal ions of alginate polymer chain. The particle size of microbeads was found to be 40.9-244 µm, respectively. It was observed that the particle size increases with increase in polymer sodium alginate concentration or by varying the exposure time to calcium chloride. A significant increase in the percentage entrapment efficiency was observed with increase in polymer concentration and calcium chloride concentration, but polymer concentration was more effective. *In vitro* dissolution studies were carried out by USP Type II apparatus in phosphate buffer of pH 7.4 for 12 hrs. The sulfasalazine loaded sodium alginate microbeads demonstrated a drug release of 97.1% for 12 hrs for B3 formulation. The release of sulfasalazine from microbeads reveals more sustained release nature with an increase in sodium alginate concentration. The results clearly indicate that the increase in apparent cross-linking density delays the alginate gel disintegration in phosphate buffer (pH 7.4) due to retardation of calcium (Ca^{2+}) ions exchange with sodium (Na^{+}) and eventually lead to increase in lag time. The *in vitro* release data of sulfasalazine was processed to understand the linear relationship which follows zero order of kinetics because it shows highest regression value of 0.9354 [7].

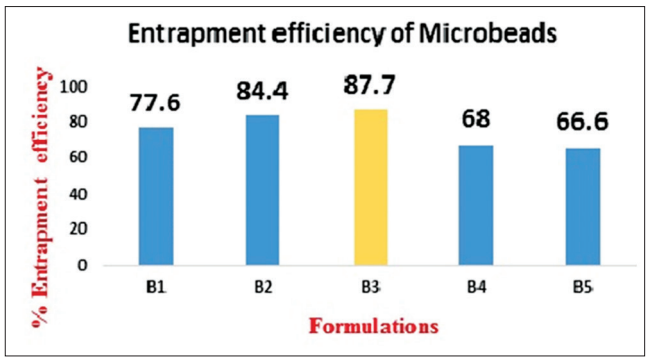

Fig. 6: Comparison of entrapment efficiency among the five formulations of sulfasalazine microbeads

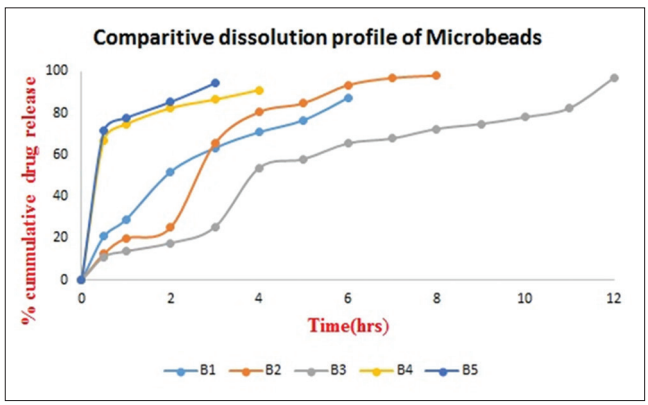

Fig. 7: Comparative *in vitro* drug release of sulfasalazine microcapsules

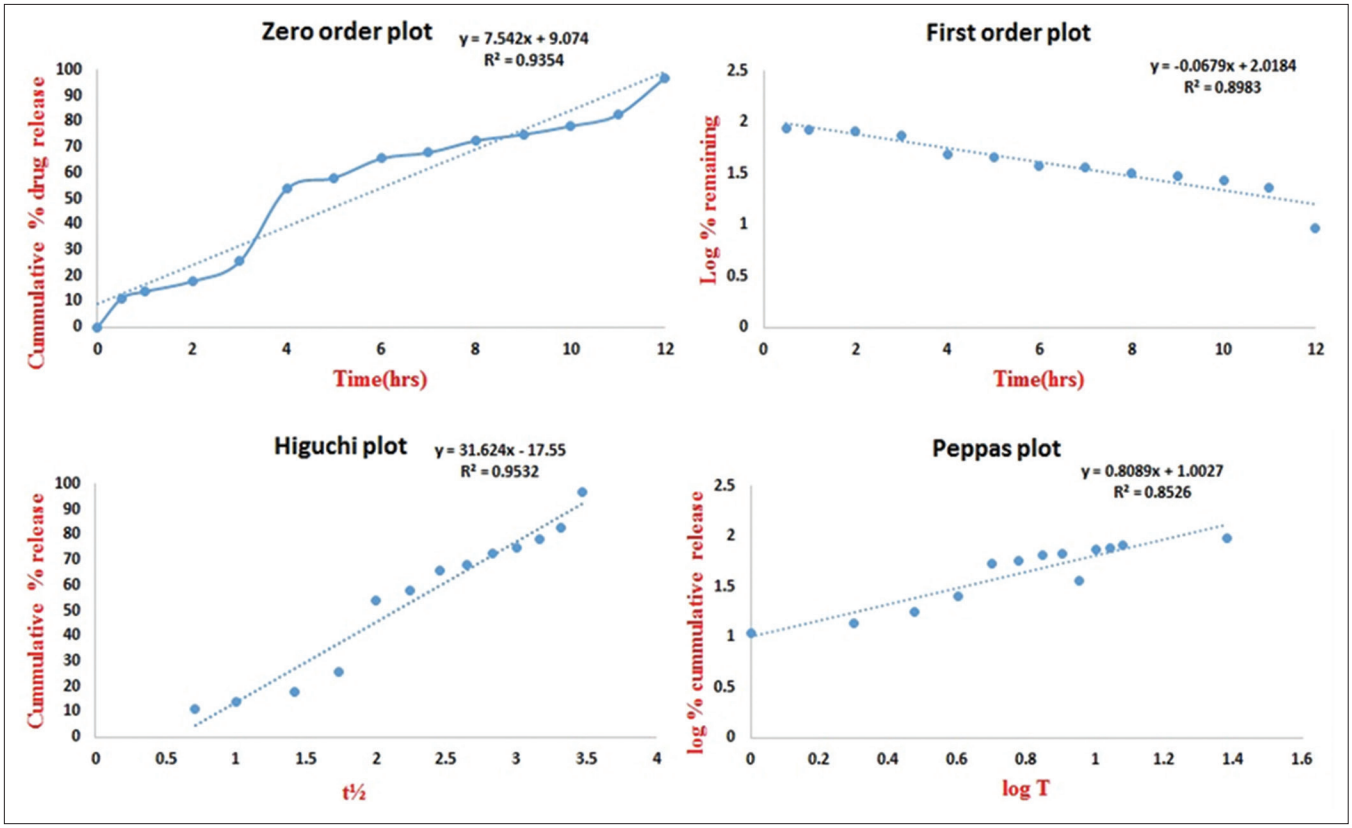

Fig. 8: *In vitro* drug release plots of best formulation of sulfasalazine microbeads

According to Korsmeyer–Peppas equation, the value of n is 0.8089 which is more than 0.5 which indicates that it follows non-Fickian diffusion. Zeta potential value of −56.8 mV indicated high negative surface charge on microbeads which, in turn, indicates higher stability [8].

CONCLUSION

Inotropic gelation technique can be used for the preparation of sulfasalazine loaded sodium alginate microbeads using calcium chloride as cross-linking agent. The prepared microbeads showed higher drug entrapment and drug content. Sulfasalazine release from microbeads was influenced by sodium alginate (sustained release polymer).The microbeads were prepared without the use of organic solvents. Data for *in vitro* drug release indicated sustained release behavior. By fitting the data in various kinetic models, it was found that it exhibit first order kinetics followed by non-Fickian diffusion. Microbeads of sulfasalazine decrease the incidence of side effects and improve patient compliance thereby reducing the dose and dosing frequency.

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