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Research Article

Comparative Study of Sulfasalazine Loaded Microcapsules and Microspheres

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ABSTRACT

OBJECTIVE: The objective of the investigation was to prepare sulfasalazine microcapsules and microspheres and comparative study was done among the best formulations of microcapsules and microspheres**.** The sulfasalazine microcapsules were prepared by solvent evaporation method and microspheres by emulsion solvent evaporation technique. Ethyl cellulose was used as a polymer for the preparation of microcapsules and microspheres. Different parameters were optimized for the preparation of microcapsules and microspheres such as drug: polymer concentration, various organic solvent, organic: aqueous phase ratios, RPM**.** The prepared formulations were subjected to drug content analysis, entrapment efficiency, and size analysis and *in vitro* drug release studies. The microcapsules showed drug content of 91.2%, entrapment efficiency of 87.5%, and *in vitro* dug release of 89.26% for 12hrs.Whereas microspheres have shown drug content of 94.2%, entrapment efficiency of 84.6% and *in vitro* drug release of 90.7% for 12hrs. Drug: polymer concentration showed significant increase on entrapment efficiency. On comparison of the best formulations of sulfasalazine microcapsules and microspheres the microcapsules were found to be the best formulation with the highest entrapment efficiency (87.5%), drug content (91.2%), and *in vitro* drug release (89.26%) up to 12 hrs.

INTRODUCTION

Controlled drug delivery systems are designed to deliver the active drug in predetermined rate at the specific site thereby reducing the dose, dosing frequency and side effects of the drug [1, 2]. Controlled drug delivery system holds a promising system in site specific targeting. Microcapsules are a small sphere with a uniform wall around it [3]. The material inside the microcapsule is referred to as the core/ internal phase, whereas the wall is sometimes called a shell/coating. The size of microcapsules ranges from 1µm-5000µm in diameter [4]. Microspheres are small spherical particles, with 1 µm to 1000 µm in size [5-7]. Sulfasalazine is an anti-inflammatory agent and is used in the treatment of inflammatory bowel disease including ulcerative colitis and crohn's disease. Sulfasalazine is a Disease modifying anti-rheumatoid drug used in second line treatment of rheumatoid arthritis when patients do not respond to the NSAID's treatment [8]. DMARDs not only reduces the pain and swelling of arthritis, but also prevent damage to joints, reducing risk of long term loss of function. The dose of sulfasalazine at initial treatment is 1gm per day and increased up to 2-3 grams per day in a twice daily dosing regimen. And dosing frequency is a drawback of conventional dosage form. Formulating such drug into a novel drug delivery system such as microcapsules and microspheres is expected to increase the sustain release action thereby reducing the dose and dosing frequency of the drug, side effects and improving patient compliance.

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MATERIALS AND METHODOLOGY

Materials

Sulfasalazine was obtained as gift sample from posh chemicals. Ethyl cellulose, dichloromethane, acetone and tween 80 were purchased from SD Fine Chem. Limited, Mumbai.

Preparation of sulfasalazine microcapsules by solvent evaporation method

Various parameters were optimised for the preparation of microcapsules such as organic

solvent (DCM, acetone), organic: aqueous phase ratio= 1:10, stirring speed at 1000rpm. Sulfasalazine was accurately weighed and dissolved in dichloromethane. Ethyl cellulose was weighed and dissolved in acetone [9].The drug solution was added to the polymeric solution and mixed thoroughly to form a fine dispersion. This dispersion was then added to 50ml of aqueous phase containing 0.5% sodium carboxy methyl cellulose. It was stirred at 1000 rpm for the evaporation of solvent and formation of microcapsules. Stirring was continued for three hours to produce microcapsules. The microcapsules formed were collected by filtration and washed with distilled water. The product was then air dried. Five formulations of microcapsules were prepared by varying the concentration of drug: polymer ratios [10].

Table 1: List of formulations of microcapsules

S.No	Formulations	Drug: polymer ratio
1	C1	1:1
2	C2	1:2
3	C3	1:3
4	C4	2:1
5	C5	3:1

Preparation of sulfasalazine microspheres by solvent evaporation method

Various parameters were optimised for the preparation of microspheres such as solvent (dichloromethane) Organic: aqueous phase ratio (1:10), stirring speed (800rpm), emulsifying agent (Tween 80 0.1%). Ethyl cellulose was weighed and dissolved in dichloromethane to form a homogenous solution. Sulfasalazine was accurately weighed and added to the homogenous solution and mixed thoroughly. This solution was then added to 50ml of aqueous phase containing 0.5% sodium carboxymethylcellulose and tween 80 1% as an emulsifying agent stirred at 800 rpm to emulsify the added dispersion as fine droplets. The solvent removal was achieved by continuous stirring at room temperature for three hours to produce spherical microspheres. The microspheres formed were collected by filtration and washed repeatedly with distilled water. The product was then air dried. Five formulations of microspheres were prepared by varying the concentration of drug: polymer ratios [11, 12].

Table 2: List of formulations of Microspheres

S.No	Formulations	Drug: polymer ratio
1	S1	1:1
2	S2	1:2
3	S3	1:3
4	S4	2:1
5	S5	3:1

Characterisation and Evaluation of Microcapsules and Microspheres

Compatibility studies by Fourier transform infrared (FTIR analysis)

The FT-IR 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 a FT-IR spectrophotometer in the wave number region of 4000-400 cm-1.The IR spectra of drug, polymers and their combination are shown in spectra [13, 14].

Study of surface morphology by scanning electron microscope (SEM)

The prepared formulations were dispersed in deionised water and sonicated for30 minutes. A circular metal plate is taken on to which carbon double tape (1mm×1mm) is stickered; a drop of the resultant dispersion is placed on to the tape and allowed to dry for a while. Then it is scanned under SEM for morphology [15, 16].

Production 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 [17, 18]. Production yield is calculated by using the following Equation

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

Drug content

The prepared 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 hours then filter. The UV

absorbance of the filtrate was measured using a UV spectrometer at 359nm [19].
The drug content for the formulations was determined by calculating:

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

Entrapment efficiency
The prepared 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 ultra-centrifuge at 17240rpm at - 4°C for 40 minutes. The free drug concentration was determined spectrophotometrically at 359nm. The entrapment efficiency for all the formulations was calculated by:

$$\text{Entrapment efficiency} = \frac{\text{Total amount of drug - Free drug in supernatant}}{\text{Total amount of drug}} \text{ X } 100$$

***In vitro* drug release studies**
The in-vitro drug release studies for sulfasalazine formulations were carried out for 12hrs using USP type II dissolution apparatus(Paddle type).Dissolution medium used was phosphate buffer (pH 7.4),each 900ml and temperature was maintained at 37 ± 2 °C at 50 rpm [20]. Sulfasalazine formulations equivalent to 50mg was used for each dissolution study.5ml Samples were collected periodically and replaced with a fresh 5ml of pH7.4 phosphate buffer. The concentration of sulfasalazine was determined spectrophotometrically at 359 nm by suitable dilutions.

RESULTS AND DISCUSSION
The prepared microcapsules five formulations C1, C2, C3, C4 and C5 were evaluated for product yield, drug content, entrapment efficiency, surface morphology & size and *in vitro* drug release. The results were given in tabular form.

The drug content of C1, C2, C3, C4, C5 was found to be 83.3%, 86.5%, 91.2%, 82.6% and 73.7% respectively. Entrapment efficiency of C1, C2, C3, C4 and C5 formulation was found to be 79%, 81.8%, 87.5%, 63.2 and 61.1 respectively. With increase in polymer concentration the percentage of drug entrapment efficiency was increased. The drug release from C1 formulation was 93.16% within 6 hrs, C2 showed 95.26% drug release for 8hrs, C3 showed a sustained release of 89.26 for 12hrs, C4 showed 96.92 % for 4 hrs and C5 showed 95.04 % for 3 hrs. With increase in polymer concentration the sustain release profile of the formulation was found to be increased. Among all the formulations of microcapsules C3 was found to be best with drug content of 91.2%, entrapment efficiency of 87.5% . The size of obtained microcapsules was found to be in the range of 99.6μm to 230 μm and the drug release in a time period of 12 hrs was found to be 89.2 %. The drug release followed zero order kinetics following non Fickian diffusion. FTIR studies revealed no drug polymer interactions.

The five formulations of microspheres S1, S2, S3, S4, and S5 were evaluated for product yield, drug content, entrapment efficiency, surface morphology & size and *in vitro* drug release. The results were given in the tabular form.

The drug content of the five formulations was found to be 76.8%, 85.9%, 94.2%, 71.5% and 63% respectively. Entrapment efficiency was noted as 72.3%, 78%, 84.6%, 62.07 and 63.3 respectively. As the polymer concentration increases entrapment efficiency was found to be increased. The drug release from S1 formulation was 83.8% within 6hrs, S2 showed 86.5% for 8hrs, S3 showed a sustained release of 90.7% for 12 hrs, S4 showed 92.04 % for 4 hrs and S5 showed 93.04 % for 3 hrs. Increased polymer concentrations have shown sustained release. The size of microspheres was found to be in the range of 92.6μm-294 μm. Among all the formulations of microspheres S3 was found to be best with drug content of 94.2%, entrapment efficiency of 84.6%, and was able to sustain the drug release for more than 12 hrs with a release rate of 90.7%. The release followed first order kinetics with non fickian diffusion mechanism, FTIR studies revealed no drug polymer interactions. The best formulations of microcapsules and microspheres were found to be C3 and S3 respectively. A comparative study was done among the best formulations.

Comparative study between the best formulations of microcapsules and microspheres of sulfasalazine
A Comparative study was performed for the best formulations of sulfasalazine loaded microcapsules and microspheres for size & surface morphology, product yield, drug content, entrapment efficiency, *in vitro* drug release studies.

Table 3: Results of sulfasalazine microcapsules

Formulations	Drug content	Entrapment efficiency	*In vitro* drug release
C1	83.3%	79%	93.16% for 6hrs
C2	86.5%	81.8%	95.26% for 8hrs
C3	91.2%	87.5%	89.26% for 12hrs
C4	82.6%	63.2%	96.92% for 4hrs
C5	73.7%	61.1%	95.04% for 3hrs

Table 4: Results of sulfasalazine microspheres

Formulations	Drug content	Entrapment efficiency	*In vitro* drug release
S1	76.8%	72.3%	83.8% for 6hrs
S2	85.9%	78%	86.5% for 8hrs
S3	**94.2%**	**84.6%**	**90.7% for 12hrs**
S4	71.5%	62.07%	92.04% for 4hrs
S5	63%	63.3%	93.04% for 3hrs

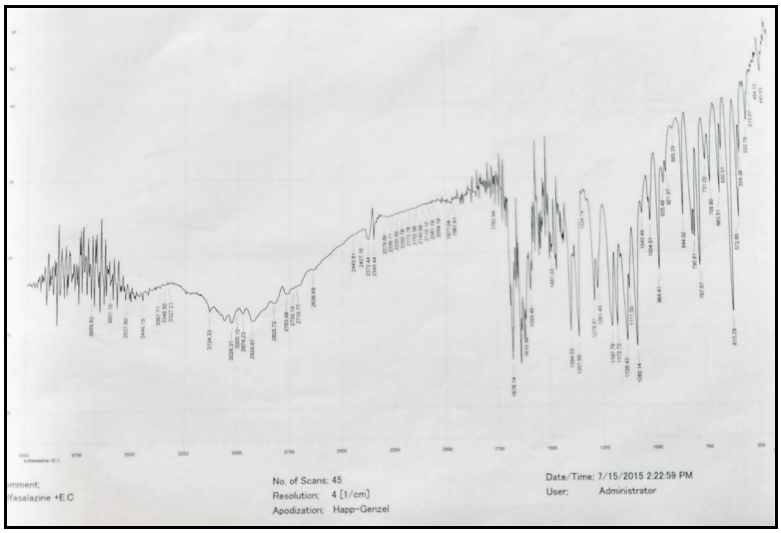


Figure 1: FTIR spectrum of Microcapsules

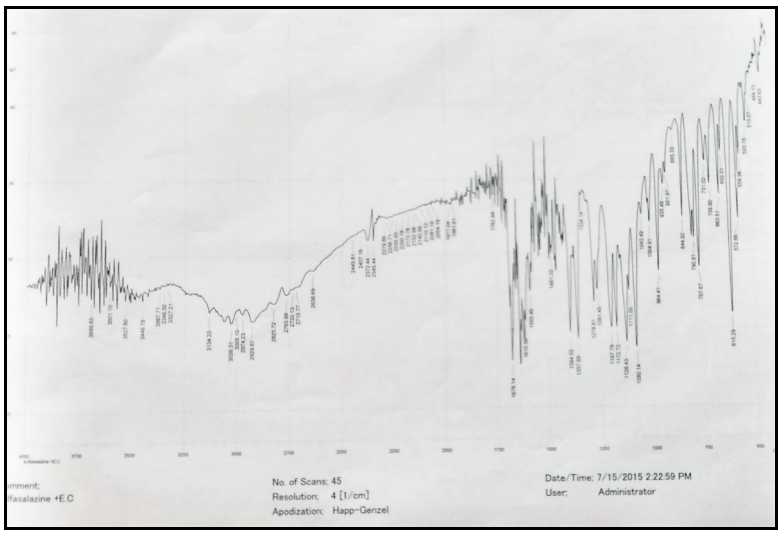


Figure 2: FTIR spectrum of microspheres formulation

FTIR Spectrum

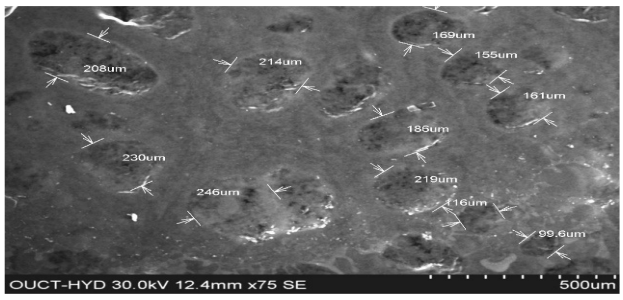
The prepared five formulations were characterized for drug-polymer interactions using FT-IR (Horiba scientific, Ltd).

The peaks obtained in the spectra correlated with the peaks of drug spectrum.1676 cm^{-1} indicating the presence of carboxyl group.1200-1120 cm^{-1} indicating the presence of sulphonyl group. There was no physical or chemical interaction and the peaks obtained in the spectra's of each formulation correlated with the peaks of drug spectrum.

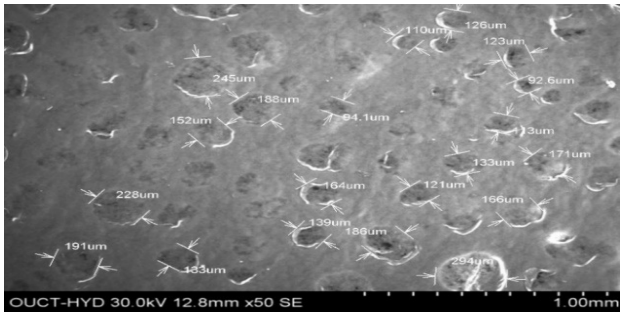
From the figure, the characteristic peaks of sulfasalazine show that acid exist as hydrogen bonded that have characteristically broad hydrogen-bonded stretching bands around 2860cm^{-1} This broad band superimposed on C-H stretching with a strong carbonyl band at1612cm^{-1} .So_2 group can be identified by the appearance of the strong band peak in the 1200-1120 cm^{-1} regions due to symmetric vibration. The peaks for ethyl cellulose polymer were at 1150cm^{-1}, 963cm^{-1}, 1540cm^{-1} for O-H stretch, O-CH_3 and N-H stretch respectively. There were no additional peaks. This indicates that the drug was compatible with the formulation components.

Size and Surface Morphology

The best formulations of microcapsules and microspheres were compared for the size and surface morphology.



(A)



(B)

Figure 3: SEM images of best formulations of (a) microcapsules and (b) microspheres.

Product yield

The best formulations of microcapsules and microspheres were compared for the product yield.

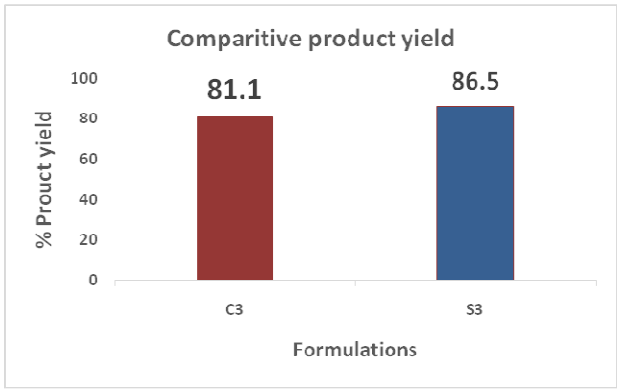


Figure 4: Comparative product yield of best formulations of microcapsules and microspheres.

The product yield of the C3 formulation of microcapsules was found to be 81.1%. The product yield of the S3 formulation of microspheres was found to be 86.5%.The S3 formulation of microspheres revealed better % product yield compared to the C3 formulation of microcapsules.

Drug content

The three best formulations of microcapsules and microspheres were compared for drug content.

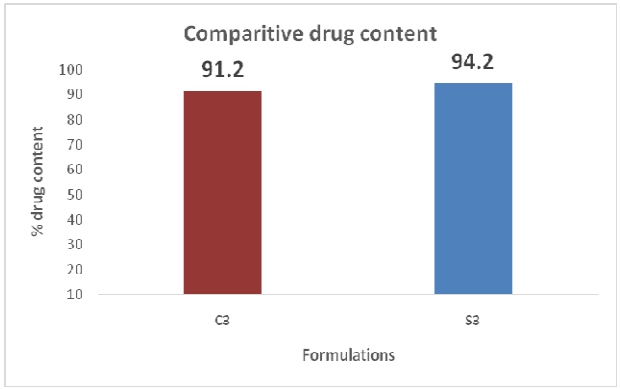


Figure 5: Comparative drug content of best formulations of microcapsules and microspheres.

The drug content of the C3 formulation of microcapsules was found to be 91.1%. The drug content of the S3 formulation of microspheres was found to be 94.2% The S3 formulation of microspheres revealed better % drug content compared to the C3 formulation of microcapsules.

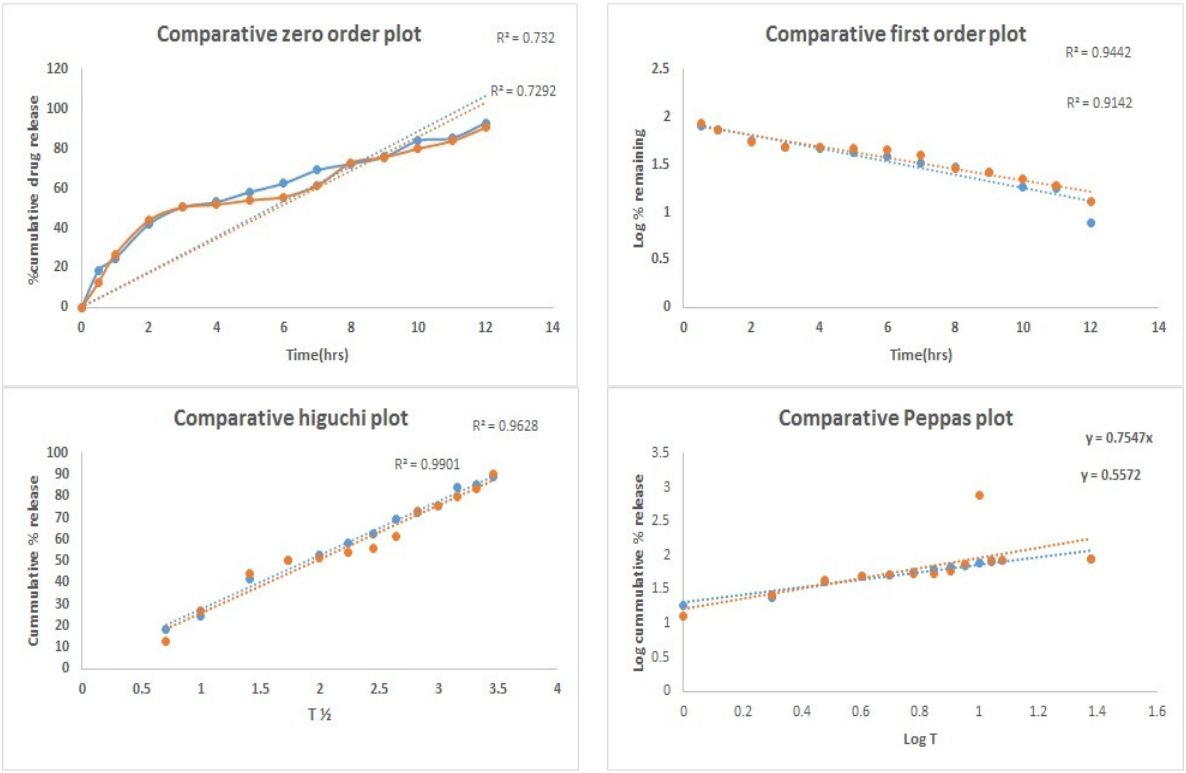


Figure 8: Comparative plots of best formulations

Entrapment Efficiency

The three best formulations of microcapsules, microspheres and microbeads were compared for entrapment efficiency.

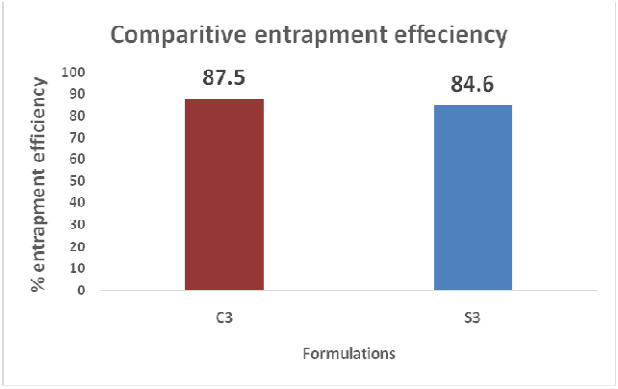


Figure 6: Comparative entrapment efficiency of best formulations of microcapsules and microspheres.

The entrapment efficiency of the C3 formulation of microcapsules was found to be 87.5%. The entrapment efficiency of the S3 formulation of microspheres was found to be 84.6%.The C3 formulation of microcapsules revealed better % entrapment efficiency compared to the C3 of microcapsules.

Comparative *In vitro* Drug Release

The two best formulations of microcapsules and microspheres were compared for *in vitro* drug release studies.

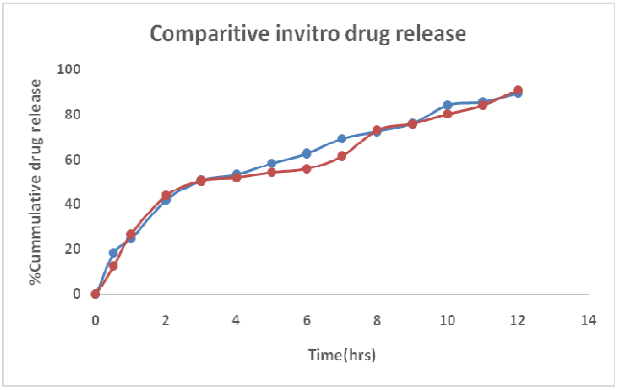


Figure 7: Comparison of *in vitro* drug release profiles of the best formulations of microcapsules and microspheres.

The percentage of drug release of the C3 formulation of microcapsules was found to be 89.26%. The percentage drug release of the S3 formulation of microspheres was found to be 90.7%. The C3 formulation of microcapsules revealed better percentage drug release compared to the S3 formulation of microcapsules.

Finally the C3 formulation of microcapsules and S3 formulation of microspheres were compared. Among the two formulations with respect to the all evaluation parameters statistically there is no significant difference.

Comparison of Best Formulation with Various Kinetic Models

Several plots (Zero order plot, first order plot, higuchi plot and peppas plots) were drawn in order to know the release kinetics and drug release mechanism.

Table 5: Kinetic data of best formulations

Formulation	Zero order plot (R^2)	First order plot (R^2)	Higuchi plot (R^2)	Peppas plot (n)
C3	0.9165	0.9142	0.9882	0.5572
S3	0.9044	0.9442	0.9626	0.7547

C3 formulation of microcapsule followed zero order with non fickian diffusional pathway.S3 formulation of microsphere followed first order with non fickian diffusion.

CONCLUSION

In the present study, a comparative study of Sulfasalazine loaded microcapsule and microsphere was done. Sulfasalazine is a hydrophobic drug can be better entrapped with ethyl cellulose. Sulfasalazine was successfully entrapped within the polymer with high entrapment efficiency showed all the physical characteristics within acceptable limits with significant in vitro release pattern and good stability also. The kinetic data analysis revealed that the formulation follows zero order release kinetics with non fickian diffusion in case of microcapsules. The drug release followed first order kinetics in case of microspheres. The above results lead us to the conclusion that the microcapsules and microspheres can be a promising carrier for sulfasalazine because of highest drug content, entrapment efficiency and sustain release property.

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