Formulation and evaluation of microspheres loaded with methotrexate drug

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ABSTRACT

The main aim of the research work was the formulation and evaluation of controlled release microspheres loaded with methotrexate drug. Literature review showed that suspensions of degradable microspheres can be employed for sustained drug release at desirable doses. They used as carriers for anticancer drugs. These spheres manufactured in micron size. For this preparation used ethyl cellulose and other excipients. Hence it was investigated and microspheres were prepared with anticancer drug like methotrexate. These are tested furthermore with scanning electron microscopy studies, Fourier transform infra red spectroscopy studies and dissolution studies.

Key words: formulation, microspheres, methotrexate, controlled release

1. INTRODUCTION

It takes long time to discovery of the active drug molecule to the production of an effective drug product. For effectiveness of drug product started with selection of its physical and chemical form. The drug product is formulated in a required dosage form for respective route of administration. The main reason behind to prepare this dosage forms are to reduce the frequency of dosing, local and systemic side effects.

Microspheres[1] are more advantageous because it can be given by oral route or parenteral route. These are used for targeted drug delivery system also. So these can be used for cancer treatment also. The drug loaded microspheres show many advantageous because it shows the pharmacological effects at the target tissue after degradation of polymer.

Drug Profile

Methotrexate

Folic acid reductase[2] inhibited by Methotrexate, it is liable for the transfer of folic acid to tetrahydrofolic acid. Tetrahydrofolic acid is used for the production of purines and also for the production of pyrimidines. From tetrahydrofolic acid, Single carbon transport reactions take place which necessitate definite coenzymes prepared in the cell.

Folic acid is used to synthesize Tetrahydrofolic acid in the cell with an enzyme called folic acid reductase. So that, DNA synthesis cannot proceed further. Because single carbon transfer reactions coenzymes are needed. Purines and pyrimidines are not produced from tetrahydrofolic acid because tetrahydrofolic acid is not synthesized. Finally, without DNA there is no cell division.

Aim and Plan of work

The main aim and plan of the research work was the formulation and evaluation of microspheres loaded with Methotrexate drug.

2. METHOD

Standard curve by UV spectrophotometer

A stock solution of (100mg/100ml) standard drug was prepared, required dilutions (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml) were made with phosphate buffer pH 7.2 in 10 ml volumetric flasks. The absorbance of these solutions was measured at 303 nm using 1 cm quartz cuvette in UV-Visible double beam spectrophotometer.

Formulation of microspheres: Method of preparation of microspheres - Emulsification method:

The weighed amounts of methotrexate and ethyl cellulose were dissolved in 40 ml solvent mixture containing dichloromethane and acetone (1:1). The initial w/o emulsion was formed by adding 2 ml of distilled water to the drug polymer solution with constant stirring at 500 rpm for 5 min. Then prepare a solution with liquid paraffin and tween eighty. Then add emulsion to the above solution, stir for one hr with twelve hundred revolutions per minute. Then filter the slurry and wash it with water for several times and dried it. Prepared different formulations with specific ratios.

Evaluation of microspheres: Evaluation and optimization

The prepared microspheres were evaluated for surface morphology using SEM, compatibility studies by FT-IR and *in-vitro* drug release studies.

Scanning electron microscopy (SEM) of microspheres

The particle surface morphology, texture was determined by SEM analysis.

***In-vitro* studies**

In-vitro evaluation of the prepared microspheres was done by using dissolution test apparatus (paddle method). 1000 ml of pH 7.2 phosphate buffer was used as dissolution medium. Equivalent wt was calculated, measured and used for *in- vitro* studies. Specified temperature and speed are maintained in the apparatus. 5 ml samples were withdrawn at regular time intervals and with a syringe fitted with prefilter and replaced the same volume with dissolution medium maintained at 37°C. The samples were diluted suitably and assayed for drug content at 303nm using UV spectrophotometer at 303nm.

3. RESULTS & DISCUSSION

Table 1: Standard Curve of Methotrexate pure sample

S.No	Concentration (µg/ml)	Absorbance
1	0	0.000
2	10	0.136

3	20	0.243
4	30	0.322
5	40	0.416
6	50	0.516
7	60	0.605
8	70	0.687
9	80	0.748
10	90	0.813
11	100	0.889

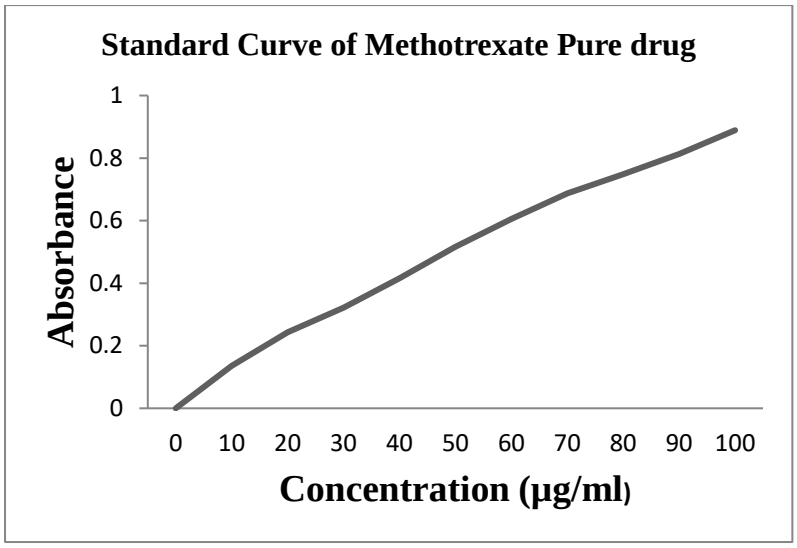

Fig 1: Standaard curve of Methotrexate

FT-IR spectra

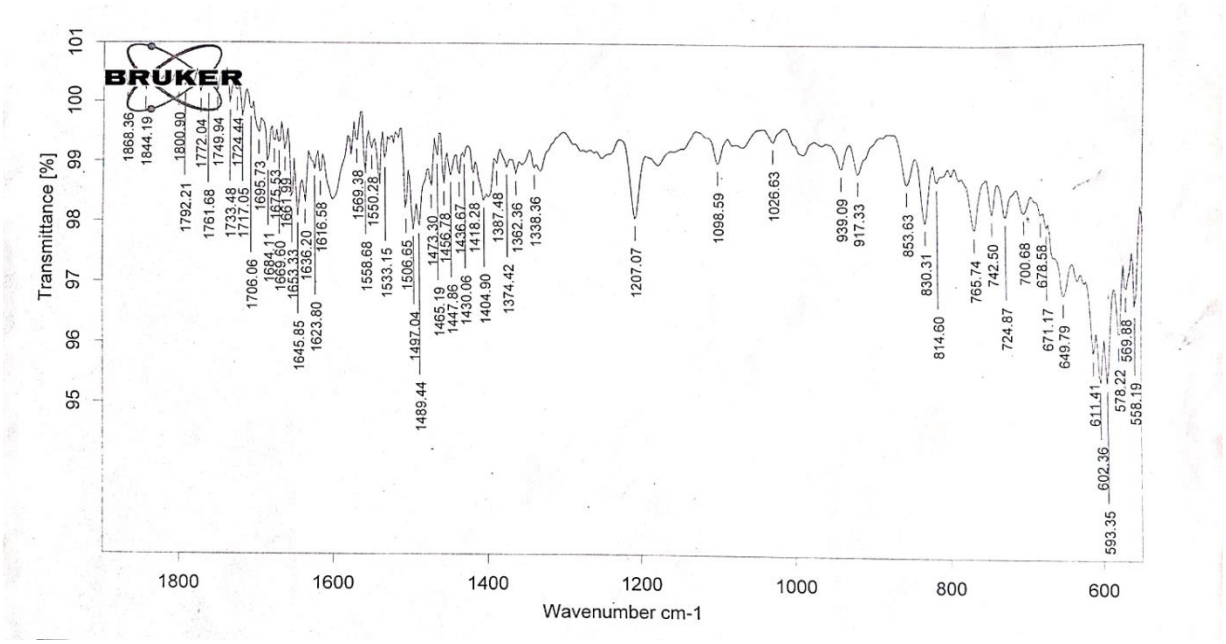

Fig 2: FT-IR Spectra of Methotrexate pure drug.

SEM analysis

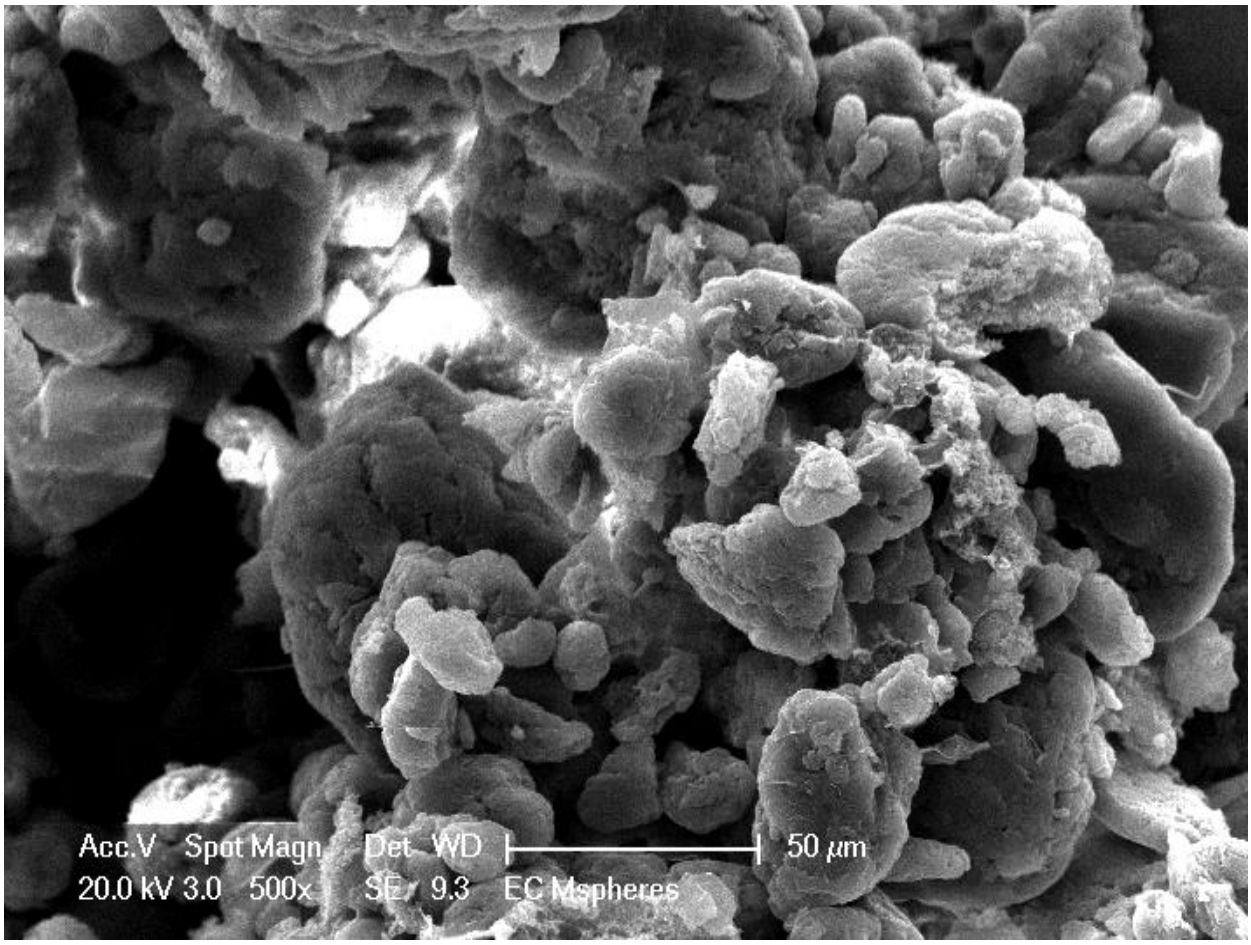

Fig 3: SEM of ethyl cellulose microspheres loaded with Methotrexate

Drug Release

The optimized formulations were evaluated for the drug release studies.

Table 2: *In vitro* dissolution studies of ethyl cellulose microspheres loaded with Methotrexate

Time (hrs)	Cumulative percentage release							
	1:1 ratio	1:2 ratio	1:3 ratio	1:4 ratio	1:5 ratio	1:6 ratio	1:7 ratio	1:8 ratio
0	14.16	13.49	13.18	12.73	11.07	11.49	11.74	10.17
1	20.23	19.43	20.18	20.47	19.45	19.73	19.91	20.73
2	25.53	24.82	23.19	23.46	23.94	24.63	24.47	23.43
3	35.95	34.27	33.06	32.93	32.57	31.14	31.38	30.75
4	40.43	39.34	39.01	37.88	36.52	36.05	36.33	35.48
5	42.44	41.45	42.47	42.27	41.91	41.44	40.72	40.42
6	44.76	43.22	44.05	44.92	43.56	42.09	41.37	41.18
7	47.95	46.43	46.48	46.35	45.99	45.52	44.84	43.14
8	48.48	48.14	47.39	48.26	47.97	46.43	45.71	44.75
9	49.04	48.52	48.56	49.13	48.07	49.61	48.88	47.37
10	50.09	49.75	49.73	49.62	50.24	49.77	49.05	48.43
11	51.49	50.99	51.42	51.29	51.93	50.46	50.74	49.72

12	52.08	51.92	52.59	53.46	52.14	53.63	51.91	50.29
13	55.36	54.03	55.41	55.78	54.92	55.45	54.73	53.43
14	56.27	56.15	55.84	56.71	56.35	55.88	55.16	54.27
15	58.19	57.79	58.36	59.23	58.87	57.47	56.68	55.49
16	60.73	59.11	60.18	60.05	59.69	59.22	58.58	57.39
17	62.49	61.94	61.35	62.22	60.86	59.39	58.67	57.35
18	64.72	63.04	64.04	63.91	64.55	63.08	63.36	62.16
19	67.48	66.73	67.21	67.08	66.72	66.25	65.53	64.46
20	70.43	71.95	70.64	70.51	70.15	69.68	69.96	69.72
21	75.16	74.47	75.42	74.29	73.93	70.46	70.74	70.49
22	77.18	76.85	76.37	75.24	74.88	71.41	71.69	71.16
23	80.48	79.78	77.41	77.28	76.92	75.45	74.23	72.28
24	85.82	83.03	78.32	79.19	78.83	79.36	75.64	73.92

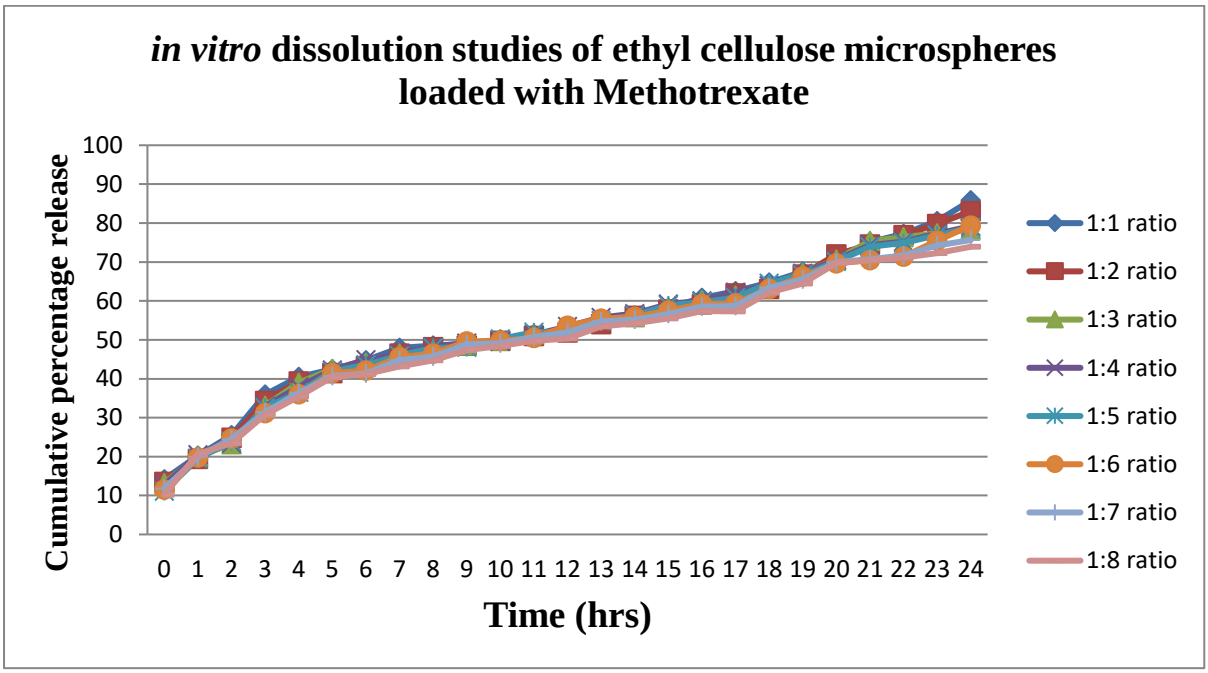

Fig 4: Dissolution studies of ethyl cellulose microspheres loaded with Methotrexate

4. CONCLUSION

Microspheres were prepared with polymer ethyl cellulose. Hence it was investigated and microspheres were prepared with anticancer drug like methotrexate. These are tested furthermore with scanning electron microscopy studies, Fourier transform infra-red spectroscopy studies and dissolution studies. From *in vitro* drug release studies reveals that 1:8 ratio formulation was showed best release than the other formulations.

Peer-review

External peer-review was done through double-blind method.

Funding

This study has not received any external funding.

Conflict of Interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES & NOTES

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