International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 4 July-Aug 2021, pp: 1636-1639 www.ijprajournal.com ISSN: 2249-7781

Formulation and Evaluation of Porous Microspheres of Methotrexate

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Date of Submission: 10-08-2021 Date of Acceptance: 29-08-2021

ABSTRACT
This research paper is about formulation and evaluation of porous microspheres of methotrexate. In the current work an endeavour was made up to set up a to prepare a stomach specific drug delivery system for anticancer drugs - porous microspheres loaded with methotrexate, doxorubicin, and 5-fluoro uracil. Microspheres loaded with drug were prepared using biodegradable polymers like pectin and casein as emulsifying agent by using emulsification extraction method. Microspheres with high drug entrapment efficiency, desired particle size, percentage buoyancy and controlled drug release rate were successfully prepared. The ultimate goal of formulating microsphere with enhanced pharmacokinetic with ideal discharge of drug was prepared.

I. INTRODUCTION

In this paper i.e., microspheres of methotrexate were prepared by using emulsification extraction method. It has normal features and benefits over conventional dosage in better form.

Porous Microspheres is a type of FDDS. Multiple unit dosage forms such as microspheres provide the possibility of achieving long lasting and more reliable release of drugs. The multiple unit dosage forms have a specific surface that is about thousand times greater than the single unit dosage form in equivalent dosage.

Conventionally, the microspheres include microparticles, which are substantially spherical and of micron size. In this the drug is dispersed homogeneously in the polymer matrix. The microsphere may have a size range less than 200µm give good retention in the stomach. Gastric retention of porous microspheres is increased because of buoyancy.

Gastric cancer is the second most common cancer and cause of cancer related death worldwide. Gastric cancer was the leading cause of cancer death in men and third leading cause of cancer of women. India is a developing country with one of the most diverse populations and diets in the world. Cancer rates in India are lower than those seen in the western countries, but are rising with increasing migration of rural population to the cities, increase in life expectancy and changes in the life style.

Cancer of stomach is a major subject of the study and research in medical sciences. Cancer of the major organ of the body that holds food for digestion is referred to as gastric cancer. It can develop in any part of the stomach and spread to the other organ. Infection with a bacterium called **Helicobacter pylori** is associated with gastric cancer. The risk of gastric cancer is also increased with Down syndrome.

Combination of active agents has been used since the late 1970's, aiming to improve the results of single agent chemotherapy. 5-FU has almost been universally used as the basis in the designing of combination treatment. Advances in basic research resulted in better understanding of the mechanism of action of many chemotherapeutic agents, including 5-FU, the main drug used in advanced gastric cancer.

In vitro studies have shown that methotrexate can enhance the activity of 5-FU by blocking the pyrimidine salvage pathway, thus leading the increased intracellular phosphoribosyl pyrophosphate. This shifts 5-Fu into the RNA pathway, increasing destruction of cancer cells. Based on these data several second-generation regimens were developed in the late 1980s.

II. METHODOLOGY

PROCEDURE FOR PREPARATION OF MICROSPHERES

10 ml of 15% w/v casein and pectin solution + 60ml Soya oil.

Methotrexate + polymer emulsifier solution in two different quantities (50mg and 100mg)

The mixture was mechanically stirred at 1000rpm to form o/w emulsion, After 5 min the solution was rapidly cooled at 15°C.

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250ml of acetone was added to dehydrate & flocculate coacervate droplets.
The microspheres were isolated by filtration through sintered glass filter.

Preparation of calibration curve

- 100mg of methotrexate was dissolved in different buffers (HCl buffer pH - 2.0 and acetate buffer pH – 3.5) in a 100ml volumetric flask, diluted with buffer to volume & mixed.
- 20 ml of this solution was pipetted out into a second 100ml volumetric flask, buffer was added to make volume & mixed.
- A set of standard dilutions of 2, 4, 6, 8, and 10µg/ml of drug were prepared by transferring aliquots of 0.1, 0.2, 0.3, 0.4, and 0.5ml of stock solution (200µg/ml) in 10ml volumetric flask and volume make up to the mark with medium.
- The optical density values of resulting solutions were measured using 1cm cells at the wavelength of maximum absorbance using hydrochloric acid and acetate buffer as a blank.

Calibration Curve Data and Regression Values of Methotrexate at pH 2.0

Concentration (µg/ml)	Absorbance (nm)
2	0.154±0.22
4	0.248±0.31
6	0.342±0.20
8	0.436±0.14
10	0.530±0.21

Physical and Chemical Properties of Drug.

S No	Description	Methotrexate
1.	Color	Yellow to orange brown
2.	Nature	Hydrated 8-10%
3.	Crystalline/amorphous	Crystalline powder
4.	Melting Point	180˚ **Physical and Chemical Properties of Drug.** C to 189˚C
5.	pH	pH 7.0-7.9 in sterile solution in water for injection
6.	Odor	Odorless
7.	Stability	It is stable if solution is not strongly acidic or basic

EVALUATION OF MICROSPHERE

- Friability: - The research facility friability analyzer is known as the Roche friabilator. It was operated at 25 rpm for 5 min and then reweighed.
- Hardness: Monsanto hardness tester was employed to measure the hardness of Microsphere. It was expressed in kg/cm2.
- Wetting time: -the water entrance rate into the powder bed is identifying with pore range & is influenced by hydrophilicity of the powders.

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- Weight Variation: This test is performed to keep up with the consistency of weight of each microsphere, which ought to be in the recommended range.

III. RESULT AND DISCUSSION

Drug Content of Methotrexate Loaded Microsphere

S. No	Amount of Drug incorporated (mg)	Amount of drug recovered (mg)	% Yield
M1	100	95.40±0.45	83.21
M2	100	95.45±0.34	83.43
M3	100	95.89±0.37	84.00
M4	100	96.68±0.63	82.43
M5	100	96.64±0.50	82.19
M6	100	96.66±0.59	84.54
M7	100	96.96±0.30	83.65
M8	100	97.16±0.78	83.02
M9	100	97.54±0.48	84.21

n = 3, all values ± standard deviation, statistically significant at 0.05 level

Results of preliminary trial batches of Methotrexate loaded Microsphere.

Batch Code	Polymer to Emulsifier Ratio (mg)	Drug Quantity (mg)	Drug Entrapment (%)	Buoyancy (%)	Particle Size and Sphericity
MP1	1250:250	50	95.32±0.89	50.0±0.79	Small
MP2	1000:500	50	94.29±0.57	59.0±0.63	Small
MP3	750:750	50	94.01±1.34	74.0±0.95	Small/Spherical/Free flowing
MP4	500:1000	50	78.94±2.01	75.0±0.88	Large/Irregular
MP5	250:1250	50	72.38±0.99	76.0±0.92	Large/Irregular
MP6	1250:250	100	97.75±1.24	59.0±0.80	Small
MP7	1000:500	100	96.26±0.73	67.0±0.69	Small
MP8	750:750	100	96.10±1.10	83.0±1.22	Small/Spherical/Free flowing
MP9	500:1000	100	82.01±0.83	84.0±0.96	Large
MP10	250:1250	100	75.33±1.39	86.0±0.87	Large/Irregular

* n = 3, all values ± standard deviation, statistically significant at 0.05 level

IV. CONCLUSION

In the present investigation an attempt was made to prepare a stomach specific drug delivery system for anticancer drugs - porous microspheres loaded with methotrexate. Microspheres loaded with drug were prepared using biodegradable polymers like pectin and casein as emulsifying agent by using emulsification extraction method. Microspheres with high drug entrapment efficiency, desired particle size, percentage buoyancy and controlled drug release rate were successfully prepared.

It may be concluded that the performance of developed stomach specific drug delivery system for methotrexate is promising for the treatment of gastric adenocarcinoma. The formulation provides stomach targeted release of drugs in gastric environment for effective treatment and management of gastric adenocarcinoma. Oral administration of porous microspheres of pectin may significantly improve patient compliance by reducing dosing frequency and help in better management of cancer chemotherapy due to site specific delivery of cytotoxic drug.

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