WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 7, 647-655 Research Article ISSN 2278 – 4357

FORMULATION AND EVALUATION OF METHOTREXATE POLYMERIC NANOPARTICLES

Shanti Sagar*[1], Dr. Manichandrika, M. Srivani, M. Reethika, M. Sugandhi Reddy, M. Ruchitha, Usha Sree Rathod

Bojjam Narasimhulu Pharmacy College for Women, Vinaynagar, Saidabad, Hyderabad-500059.

Article Received on
30 April 2018,
Revised on 20 May 2018,
Accepted on 10 June 2018
DOI: 10.20959/wjpps20187-11841

***Corresponding Author**
Shanti Sagar
Bojjam Narasimhulu Pharmacy College for Women, Vinaynagar, Saidabad. Hyderabad-500059.

ABSTRACT

Nanoparticles are formulated to target the drug to the specific organ site and to control the rate of delivery of drug. By encapsulating a drug into nanostructures, the being of the drug in the systemic circulation can be prolonged and decrease the toxicity. The main aim of this study is to achieve prolonged release of Methotrexate such that the dosing frequency of the drug can be reduced by which we may decrease the side effects and improve the patient compliance. Investigation of the preparation, characterization and in-vitro delivery of the nanoparticles was carried out. The different formulations of with different concentration of drug-polymer and surfactant were examined and finalized. Encapsulation efficiency of nanoparticles ranged between 68%-81%. The prepared particles showed good drug-loading capacity. The *in vitro* release studies showed that after the initial burst, all of the drug-loaded batches provided a continuous and slow release of the drug. The present study revealed that solvent evaporation followed by homogenization can be used as an effective tool for preparation of Methotrexate nanoparticles.

KEY WORDS: Methotrexate, chitosan, sodium alginate, poly vinyl alcohol, Ionotropic gelation technique, franz diffusion cell.

1. INTRODUCTION

Colloidal drug delivery systems offer a number of advantages over conventional dosage forms. Due to their small particle size, colloidal preparations lend themselves to parentral preparations and may be useful as sustained release injections for the delivery to a specific

organ or target site.[1,2] Chitosan exhibits many advantages in developing nanoparticles, including biocompatibility, biodegradability, and low-immunogenicity. The high positive charge density also confers its mucoadhesive properties and makes it an ideal candidate for the delivery of drugs to mucosal tissues.[3] Chitosan also has a very low toxicity. Methotrexate is a drug of choice in the treatment of colon cancer and now a day's rheumatic disease. MTX is a folate antimetabolite.[4] It is an analogue of aminopterin, which is also derived from folic acid. MTX has a half-life of 3 to 10 hours (for low doses), 8 to 15 hours (for high doses) and insoluble in water, ethanol, and soluble in dilute solutions of alkaline hydroxides, carbonates and in dilute HCl.[5] The oral bioavailability of methotrexate is relatively very small i.e. 33%.[6] The present study is to prepare Methotrexate nanoparticles to extend the release so as to reduce the adverse effects and to evaluate their particle size, drug loading capacity and drug release.[7] Methotrexate drug to ensure satisfactory drug release with the help of polymers, to prevent drug toxicity due to accumulation, and prolong duration of action.[8]

2. MATERIALS AND METHODS

2.1 Materials

Methotrexate obtained as gift sample from Aurobindo Laboratories Ltd, chitosan, sodium alginate were purchased from A. R chemicals. All other ingredients used throughout the study were of analytical grade and were used as received.

2.2 Methodology[9,10]

Drug excipient compatability

The pure OST (drug) and its physical mixture were subjected to IR spectral studies by employing the KBr pellet method using FTIR spectrophotometer. One to two milligram of fine solid powder of MTX and 200-300 mg of dry powder of KBr (IR grade) were taken in a mortar and mixed well with the help of the spatula. Spectrum measurement was carried out using KBr disk method in the wavelength region of 4000-400 cm^{-1} by FTIR spectrophotometer. The spectra obtained for MTX, and the physical mixture was compared.

Method of preparation of Methotrexate loaded nanoparticles

Nanoparticles formulations were prepared by solvent evaporation method. The various different amount of polymers was dissolved in solvent mixture of methanol (2 ml) and dichloromethane (8 ml) very slowly on a magnetic stirrer and methotrexate (20mg) was added to it and the contents were allowed to stand at room temperature for 30 to 45 minutes

with occasional vortexing to allow complete solubilisation of drug and polymer. This solution was poured into 5 ml of each different concentration aqueous polyvinyl alcohol solution. The resulting solution was homogenized by using high pressure homogenizer for 3 minutes to form o/w emulsion. This emulsion was immediately added drop wise to 125 ml of aqueous PVA solution. The contents were stirred for 6 hours at room temperature with a magnetic stirrer to evaporate organic volatile solvent, allowing the formation of a turbid nanoparticulate suspension. The suspension was filtered through membrane filter. The filtrate was centrifuged (1000 rpm for 10 minutes) and supernatant was collected. Further the ultracentrifugation (35000 rpm for 1 hour) was carried for supernatants. Following ultracentrifugation, the pellet was washed and collected two times with deionized water to remove adsorbed drug and was suspended in deionized water to prevent clumping on storage.

Table-1: Composition of the Nanoparticles.

Ingredients	Batch no				
	F1	F2	F3	F4	F5
Chitosan	1000	-	500	250	750
Sodium alginate	-	1000	500	750	250
Methotrexate (mg)	20	20	20	20	20

Evaluation of Methotrexate loaded nanoparticles[11,12,13,14]

Particlesize

All the prepared batches of nanoparticleswere viewed under microscope to study their size. Size of liposomal vesicles from each batch was measured at different location on slide by taking a small drop of nanoparticle dispersion on it and average size of nanoparticles were determined.

SEM analysis

The morphology of NPs was studied by a scanning electron microscope. For this purpose, the nanoparticles are placed on aluminum stubs and the surface was coated with a layer of gold particles using a sputter coater. The shape of the NPs was determined by scanning electron microscopy (SEM) (XL30, Philips, the Netherlands) at 15 kV and 750 mA.

Drug encapsulation efficiency

Nanoparticles 50mg were dissolved in 100ml of phosphate buffer and the drug amount was determined by UV analysis. The encapsulation efficiency was determined as the mass ratio of

entrapped Methotrexate in nanoparticles to the theoretical amount of the drug used in the preparation .The entrapment of the Methotrexate nanoparticles was expressed as loading capacity.

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount entrapped}}{\text{Total drug loaded}} \times 100$$

In-vitro drug release studies

The release studies were carried out by franz diffusion cell. It containing 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (100 ml) was placed in a 10 ml of beaker. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37±5^{0}C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non entrapped methotrexate dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. 1ml of aliquots were withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium.

Percentage of drug release was determined using the following formula.

$$\text{Perentage drug release} = \frac{Da}{Dt} \times 100$$

Where, Dt = Total amount of the drug in the patch

Da = The amount of drug released

Stability studies

Selected Formulation was subjected to stability studies as per ICH guidelines.

Following conditions were used for Stability Testing.

1. 25^{0}C/60% RH analyzed every month for period of one month.
2. 30^{0}C/75% RH analyzed every month for period of one month.
3. 40^{0}C/75% RH analyzed every month for period of one month.

3. RESULTS AND DISCUSSION

Drug - excipient compatibility studies (FT-IR)

The compatibility between the drug and other excipients was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-excipient mixture, which confirmed the absence of any chemical interaction between the drug, excipient and other chemicals.

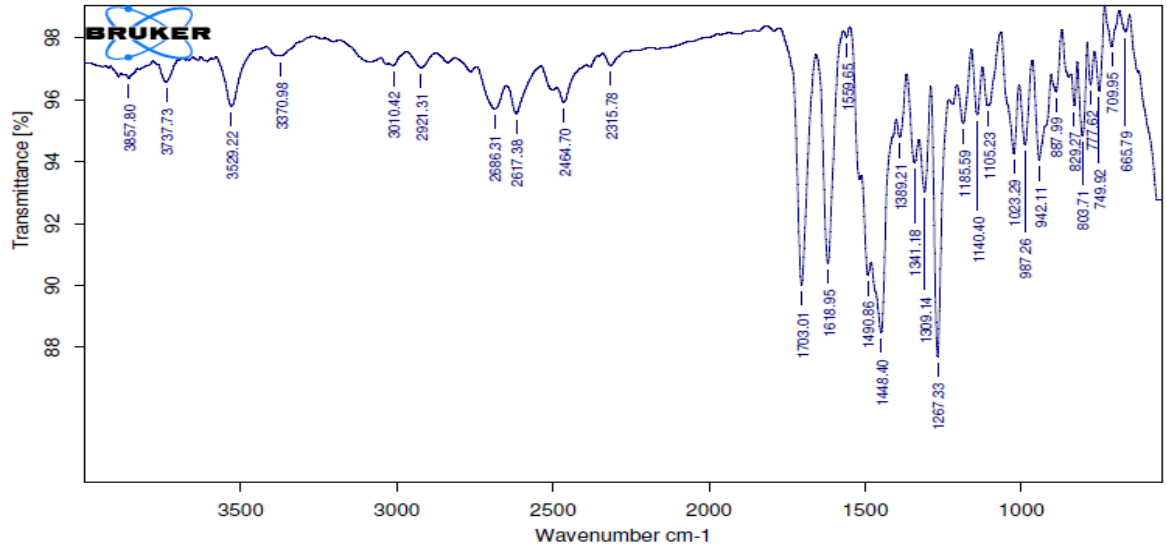

Fig-1: FT-IR Sample for Methotrexate.

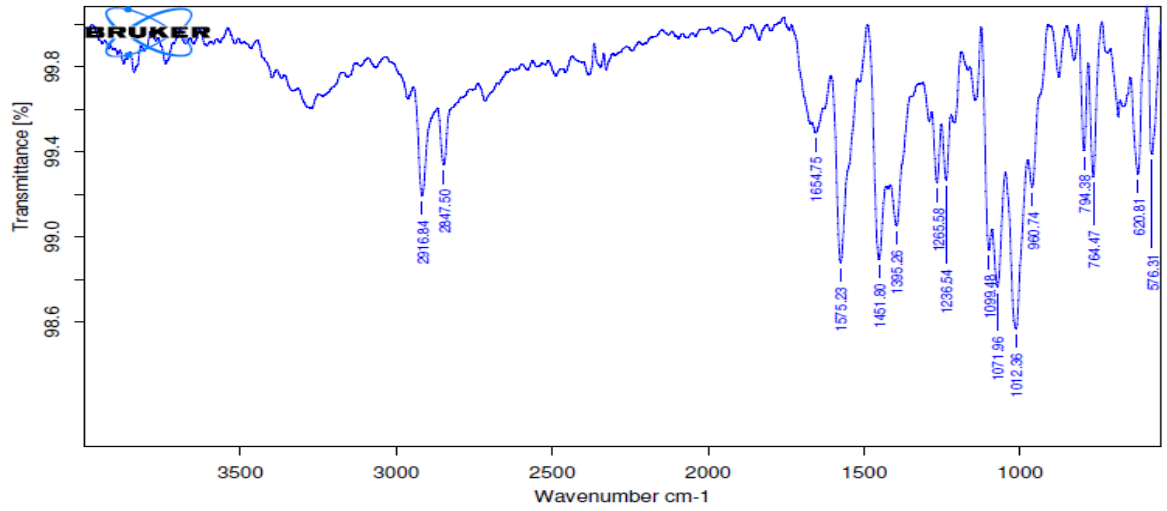

Fig-2: FT-IR Sample for Optimaized Formulation.

Evaluation parameters

The nanoparticles prepared were evaluated as per the following parameters-

- Particle size and SEM analysis
- Entrapment efficiency
- In vitro release study
- Stability studies

Particle size

The particle size increased with increasing surfactant concentration although the increase was not significant. Entrapment efficiency decreased with increasing PVA concentration. Based

on particle size distribution and entrapment efficiency, PVA concentration was selected for further studies.

Surface morphology

Scanning electron microscopy (SEM) SEM revealed that the MTX nanoparticles were smooth and spherical without any aggregation.

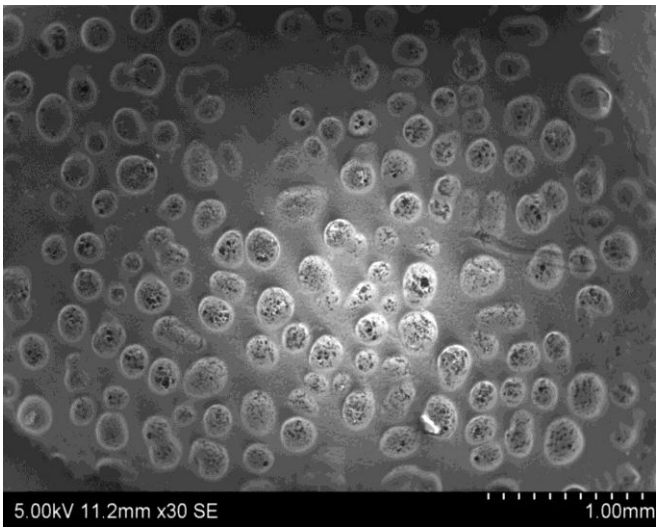

Fig-3: SEM analysis of Optimaized Nanoparticle.

Drug entrapment efficiency

The first part of the plan of work was to optimize the concentration of polymers to be used in the formulation of nanoparticles. The optimization of polymer concentration was done on the basis of particle size and entrapment efficiency of nanoparticles obtained.

Table-2: Evaluation Studies of Prepared Nanoparticles: Entrapment Efficiency and Particle size.

Batch No	Particle size (nm)	Entrapment Efficiency (%)
F1	256	68
F2	248	71
F3	312	69
F4	328	75
F5	398	81

In vitro drug release studies

Results indicate that the formulation showed initial burst release followed by sustained release of the drug for a prolonged period of time. The rapid initial release may be attributed

to the fraction of MTX on the surface of nanoparticles. The in vitro drug release results revealed that the prepared polymeric nanoparticles would be able to control drug release for extended period of time.

Table-3: Diffusion study profiles for all formulations.

Time (hrs)	F_1	F_2	F_3	F_4	F_5
0	0	0	0	0	0
1	28.55	26.45	25.32	26.55	29.55
2	35.25	34.26	32.82	35.60	38.50
3	43.82	34.70	41.77	44.55	48.12
4	52.65	53.54	50.25	52.55	55.65
5	61.28	62.85	60.52	63.58	65.55
6	69.25	72.80	71.56	70.88	77.20
7	78.85	81.63	82.50	85.15	83.85
8	88.56	90.55	91.52	93.20	95.55

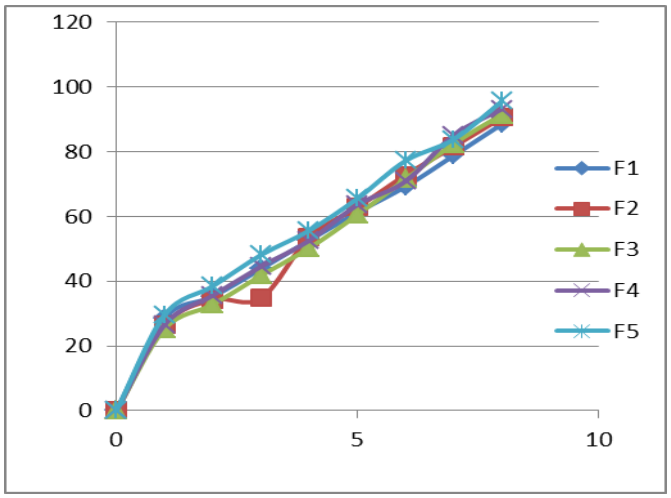

Fig-4: In vitro drug release studies for all formulations.

The in vitro diffusion studies were performed in pH 7.4 buffer using Dialysis membrane for 8 hours. Initially the release of drug from all the three batches was found to be about 25-30% in 8 hours. This was due to the release of adsorbed drug from the surface of Nanoparticles. Later on a constant and slow drug release was observed for 8hrs. F5 formulation which had drug polymer ratio of chitosan and sodium alginate was decided to be the optimized formulation.

Stability studies

There was no significant change in physical and chemical properties of the tablets of formulation F-5 after 30 days. Parameters quantified at various time intervals were shown.

Table-4: Results of stability studies of optimized formulation F-5.

Formulation Code	Parameters	Initial	1st Month	Limits as per Specifications
F-5	25^0C/60%RH % Release	95.55	95.41	Not less than 85 %
F-5	30^0C/75% RH % Release	95.55	95.45	Not less than 85 %
F-5	40^0C/75% RH % Release	95.55	95.50	Not less than 85 %

4. CONCLUSION

The present research proposed a novel formulation Methotrexate Nanoparticles for controlled release. Investigation of the preparation, characterization and in-vitro release of the Nanoparticles was carried out. The different formulations of with various ratios of drug-polymer and surfactant were evaluated and optimised. In this research, a drug encapsulation efficiency as high as 81% has been achieved. The method used for the formulation of Methotrexate containing chitosan and sodium alginate nanoparticles was ionic gelation method followed by homogenization to reduce the particle size. Nanoparticles formulations showed good results in terms of the assayed drug content and encapsulation efficiency. This indicates that the method used for the formulation produced good yield and it was suitable and reproducible in nature. Formulation (F-5) showed the highest encapsulation efficiency. It was found that as the concentration of chitosan increased, the % of encapsulation efficiency was also increased. Permeation studies with dialysis membrane were carried out as per the method reported. The formulations showed good drug release from the polymer, the *in vitro* drug release profiles of all the formulations showed an initial burst effect, and followed by a slow drug release. The burst release of drug is associated with those drug molecules dispersing close to the nanoparticle surface, which easily diffuse in the initial incubation time. The Methotrexate release was faster for those nanoparticles with higher drug content.

5. REFERENCES

1. Pharmaceutical Biotechnology, 1st Edition Edited by Vyas SP and Dixit VK, Published by CBS Publishers & Distributors, New Delhi, Page No. 395; 1998.
2. Encyclopedia of Pharmaceutical Technology, 2nd Edition edited by Elias Fattal, Christine Vauhier. Published by, Marcel Dekker, Inc. New York, 2002; pg (1864).
3. L.S. Nair, C.T. Laurencin. Prog. Polym. Sci., 2007; 32: 762–798.

4. VJ Mohanraj and Y Chen, Nanoparticles – A Review**,** Tropical Journal of Pharmaceutical Research, June 2006; 5(1): 561-573.
5. R. Singh, J.W. Lillard Jr. / Experimental and Molecular Pathology 2009; 86: 215–223.
6. Peltonen L, Aitta J, Hyvönen S, Karjalainen M, Hirvonen J. Improved Entrapment Efficiency of Hydrophilic Drug Substance During Nanoprecipitation of Poly(l)lactide Nanoparticles. AAPS Pharm Sci Tech. 2004; 5(1): article 16.
7. T. Musumeci, C.A. Ventura, I. Giannone, B. Ruozi, L. Montenegro, R. Pignatello and G. Puglisi. PLA/PLGA nanoparticles for sustained release of Docetaxel. International Journal of Pharmaceutics Volume 325, Issues 1-2, 15 November 2006, Pages 172-179.
8. Lim Liancy, Feng Si-Shen and Seow Pei Hsing. Nanoparticles of Biodegradable polymers Applied for clinical Administration of Anti cancer Drugs. www3.ntu.edu. UROP Report
9. L.Mu and S.S.Feng worked on A novel controlled release formulation for the anticancer drug Paclitaxel(taxol);PLGA nanoparticles containing Vit E TPGS. Journal of controlled Release 2003; 86: 33-48.
10. N.Jawahar, T.Eagappanath, Nagasamy Venkatesh, Jubie.S, Samanta M.K, worked on preparation and characterization of PLGA-Nanoparticles containing Anti-hypertensive agents. International Journal of PharmTech Research, April-June 2009; 1(2): 390-393.
11. Raghavan Pillai, Shankari N Somayaji, Monica Rabinovich, Michael C Hudson and Kenneth E Gonsalves. worked on Naficillin loaded PLGA for treatment of osteomyelitis. Biomed. Mater. 3 034114 (7pp) doi: 10.1088/1748-6041/3/3/034114.
12. Vihola H, Laukkanen A, Hirvonen J, Tenhu H. Binding and release of drugs into and from ther-mosensitive poly (N-vinylcaprolactam) nanoparticles. Eur J Pharm Sci., 2002; 16: 69-74.
13. Dustgani A, Vasheghani Farahani E, Imani M. Preparation of chitosan nanoparticles loaded by dexamethasone sodium phosphate. Iranian J Pharmaceutical Sci., 2008: 4: 111.
14. Wilson B, Samanta MK, Santhi K, Sampath Kumar KP, Ramasamy M, Suresh B. Chitosan nanoparticles as a new delivery system for the anti-Alzheimer drug tacrine. Nanomedicine. Nanotechnol Biol Med, 2010; 6: 144-52.