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DEVELOPMENT AND CHARACTERIZATION OF METHOTREXATE LOADED CHITOSAN NANOPARTICLES

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ABSTRACT

The purpose of the present research work is to develop sustained release nanoparticles with higher encapsulation efficiency of slightly water soluble drug, methotrexate by suitable technique.

Nanoparticles can offer number of advantages over available conventional dosage form, which are described as; their small size and relative narrow size distribution permits site specific drug delivery. Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration. System can use for various routes of administration including oral, parenteral etc.

Controlled and sustained release of active drug. The controlled and sustained release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.

KEYWORDS: Methotrexate, chitosan, nanoparticles.

INTRODUCTION

Defining Cancer

Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues. Cancer cells can spread to other parts of the body through the blood and lymph systems.

Cancer is a disease that begins in the cells of the body. In normal situations, the cells grow and divide as the body needs them. No more, no less. This orderly process is disturbed when new cells form that the body was not needed and old cells don't die when they should. These extra cells lump together to form a growth or tumor. Two types of tumors exist, benign and malignant. Benign tumors are not cancerous. They can usually be removed and generally don't grow back once they're gone. The cells in benign tumors don't spread and it is rare for a benign tumor to be life-threatening. Malignant tumors, on the other hand, are cancerous. The cells in them are abnormal and divide randomly and chaotically. The cells behave aggressively and attack the

tissue around them. They also can jump away from the malignant tumor and enter the bloodstream or lymphatic system to form new tumors in other parts of the body. This type of spread is known as metastasis.[1]

Origins of Cancer

All cancers begin in cells, the body's basic unit of life. To understand cancer, it's helpful to know what happens when normal cells become cancer cells. The body is made up of many types of cells. These cells grow and divide in a controlled way to produce more cells as they are needed to keep the body healthy. When cells become old or damaged, they die and are replaced with new cells. However, sometimes this orderly process goes wrong. The genetic material (DNA) of a cell can become damaged or changed, producing mutations that affect normal cell growth and division. When this happens, cells do not die when they should and new cells form when the body does not need them. The extra cells may form a mass of tissue called a tumor[5].

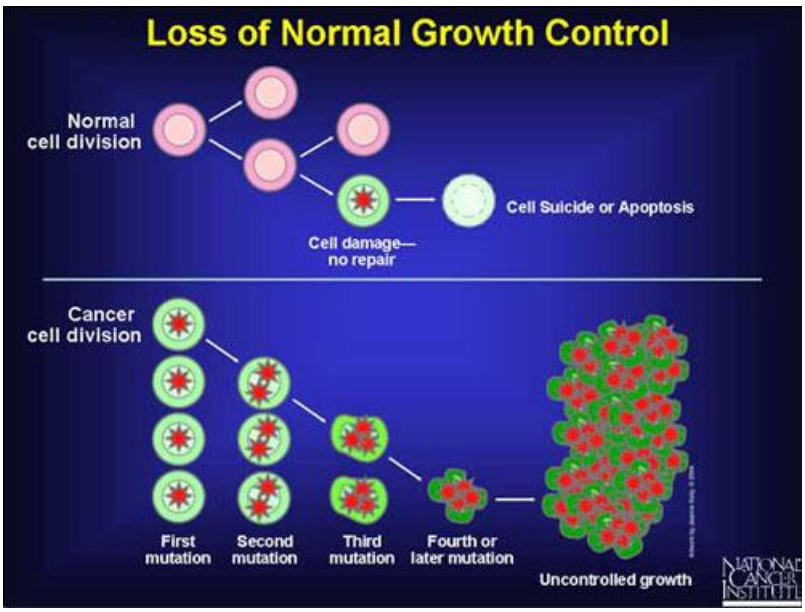


Figure: 1 Image-Loss of normal growth cell.

- **Benign tumors** –These are not cancerous. They can often be removed, and, in most cases, they do not come back. Cells in benign tumors do not spread to other parts of the body.
- **Malignant tumors** – These are cancerous. Cells in these tumors can invade nearby tissues and spread to other parts of the body. The spread of cancer from one part of the body to another is called metastasis.

Types of Cancer:

Cancer types can be grouped into broader categories. The main categories of cancer include:

- **Carcinoma** - cancer that begins in the skin or in tissues that line or cover internal organs. There are a number of subtypes of carcinoma, including adenocarcinoma, basal cell carcinoma,squamous cell carcinoma, and transitional cell carcinoma.
- **Sarcoma** - cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue.
- **Leukemia** - cancer that starts in blood-forming tissue such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the blood.
- **Lymphoma and myeloma** - cancers that begin in the cells of the immune system.
- **Central nervous system cancers** - cancers that begin in the tissues of the brain and spinal cord.

Nanoparticles for cancer targeting

Introduction

Nanoparticles are one of the polymer-based colloidal drug delivery systems with the size ranging from 1 nm to 1000 nm. They consist of macromolecular materials in which the active principle (drug or biologically active material) is dissolved, entrapped, or encapsulated, and/or to which the active principle is adsorbed or attached. Nanoparticles have been around since Michael Faraday's time of 1857 when he first developed the gold colloidal particles. Recently, polymer nanoparticles have been widely investigated as a carrier for drug targeting.

Nanoparticles offer an alternative delivery system for cancer therapy that have the potential to control the release rate of drug, improve the drug pharmacokinetics and biodistribution, and reduce drug toxicity. Due to their small size, nanoparticles with entrapped drugs may penetrate tumors due to discontinuous and leaky nature of the microvasculature of tumors. The poor lymphatic drainage of tumors may result in slower clearance of nanoparticles that accumulate in tumors. This effect is referred to as the enhanced permeability and retention (EPR) effect.

MATERIALS AND INSTRUMENTS:

The following materials, chemicals and instruments were used for preparation and evaluation of methotrexate nanoparticulate drug delivery system.

Materials:

Table 1:Materials used in the Present Work

Category	Name of Materials	Name of Suppliers
Drug	Methotrexate	Cadila Health care, Goa
Polymer	Chitosan	Balaji drugs, Surat
Ion gelating agent	TPP	Krishna chem.
Solvent	Acetic acid	Aatur Instru chem. Vadodara

Instruments:

Table 2 : Instruments used in the Present Work

Name of Instruments	Model Name and Manufacturer
Electronic Balance	Ohaus corporation, Pine Brook, NJ,USA
Magnetic Stirrer	Macro Scientific Work PVT LTD, Delhi
Cooling centrifuge	Remi instrument pvt ltd, Mumbai
FT-IR	Shimadzu, Japan
UV Spectrophotometer	UV-1800 Shimadzu, Japan
Centrifuge Machine	Remi Instrument Private Limited , Mumbai
Zeta sizer	Malvern Instrument LTD , UK
Stability chamber	Macro Scientific Work PVT LTD, Delhi

DRUG PROFILE

METHOTREXATE

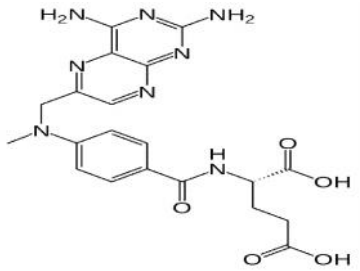
Methotrexate is abbreviated MTX and formerly known as amethopterin, is an antimetabolite and antifolate drug used in treatment of cancer and autoimmune diseases.MTX is also used in Psoriasis & Rheumatoid arthritis. It acts by inhibiting the metabolism of folic acid

Brand Names: - Abitrexate, Antifolan, Arbitrexate, Emtexate, Folex, Ledertrexate, Metatrexan, Methotrate, Mexate, Rheumatrex, Trexall

Chemical Formula:- $C_{20}H_{22}N_8O_5$

Molecular Weight:- 454.46 g/mol

Chemical Structure:-



Chemical IUPAC name:-

N-[4 - {[(2, 4- diamino – 6- pteridinyl) - methyl] methylamine} benzoyl] glutamic acid

Description: - bright yellow-orange, odourless powder

Solubility: - practically insoluble in water, alcohol, chloroform and ether. It is freely soluble in dilute solutions of alkaly and carbonates. It is soluble in dilute hydrochloric acid.

Log P or Hydrophobicity:- -1.8

Dissociation constant (Pka):- 4.7

Melting point:- 185°C - 195°C

Mechanism of action:-Folic acid analogue that inhibits the enzyme dihydrofolate reductase and blocks conversion of dihydrofolate to tetrahydrofolate so disrupting synthesis of DNA & RNA components.

EXPERIMENTAL WORK

Organoleptic properties:

Colour, odour and appearance of the drug was observed.

Melting point:

Melting point was by determined by filling the drug at the one end of sealed capillary, than the filled capillary was put on the apparatus with thermometer. The temperature at which the drug melts is noted down and considered as melting point of the drug.

Solubility studies:

The solubility of Methotrexate was determined by adding excess amount of drug in the solvent 3ml and kept for 72 hrs for equilibrium with occasional shaking. Equilibrium solubility was determined by taking supernatant and analyzing it using U.V spectrophotometer.

Determination of λmax for MTX:

Wavelength (λ) at which MTX gave maximum absorbance was found out in 0.1N HCL & in Phosphate buffer pH 7.4. Here in present study, dilute solution of MTX in 0.1 N HCl and in Phosphate buffer pH 7.4 were prepared and screened in UV Spectrophotometer.

Preparation of calibration curves:

Spectrophotometric analysis of MTX was carried out on double beam UV-spectrophotometer (UV-1800, Shimadzu, Japan).

Preparation of calibration curve of MTX in pH 7.4 Phosphate Buffer

Accurately weighed 25 mg of MTX was transferred to 25 ml volumetric flask. Then 10 ml of phosphate buffer was added to the above volumetric flask, the drug was dissolved properly and the final volume of the flask was made up to 25 ml with phosphate buffer to produce 1000 µg per ml of MTX solution.

In 100 ml volumetric flask; 10 ml of the above solution was transferred by graduated pipette. The final volume was made up to 100 ml with phosphate buffer to prepare stock solution of 100 µg per ml of MTX. By appropriate dilution, standard solution of 10 µg/ml to 30 µg/ml were prepared. The solutions were shaken well and the absorbance of individual solutions was measured at 336 nm using UV-visible spectrophotometer, using phosphate buffer as a blank.

Preparation of calibration curve of MTX in 0.1N HCl

Accurately weighed 25 mg of MTXwas transferred to 25 ml volumetric flask. Then 10 ml of 0.1N HCl was added to the above volumetric flask, the drug was dissolved properly and the final volume of the flask was made up to 25 ml with 0.1N HCl to produce 1000 µg/ml of MTX solution.

In 100 ml volumetric flask; 10 ml of the above solution was transferred by graduated pipette. The final volume was made up to 100 ml with 0.1N HCl to prepare stock solution of 100 µg per ml of MTX. By appropriate dilution, standard solution of 10 µg/ml to 30 µg/ml were prepared. The solutions were shaken well and the absorbance of individual solutions was measured at 336 nm using UV-visible spectrophotometer, using 0.1N HCl as a blank.

Preparation of Nanoparticles:

Factorial Designs:

Various batches of methotrexate loaded nanoparticles were prepared according to 3^2 full factorial designs.

Table 3: Factorial Designs.

Factor	*Level*		
	Low	**Medium**	**High**
X_1	3 mg/ml	5 mg/ml	7 mg/ml
X_2	2 mg/ml	4 mg/ml	6 mg/ml
Factorial levels	-1	0	+1

Where;

X_1: Concentration of Chitosan in 1% Aq. Acetic Acid.

X_2: Concentration of TPP in water.

Whereas amount of Drug was kept constant (Methotrexate-120mg)

Formulation table:

Table 4:- Table of formulation.

BATCH	CHITOSAN CONCENTRATION	TPP CONCENTRATION
MTX-1	90	24
MTX-2	90	24
MTX-3	90	24
MTX-4	150	36
MTX-5	150	36
MTX-6	150	36
MTX-7	210	48
MTX-8	210	48
MTX-9	210	48

Preparation of Cs-NPs By Ionotropic Gelation Method:

Chitosan was dissolved in aqueous acetic acid (1%v/v) to obtain different concentrations (3mg/ml, 5mg/ml, 7mg/ml). Under magnetic stirring at room temperature, 12ml of TPP solution was added dropwise using syringe needle to 30ml of chitosan solution containing drug 120 mg. The stirring was continued for about 30 minutes. The resultant nanosuspension was centrifuged

till particles get settled down and clear supernanent solution appears. The particles obtained after centrifugation are finally freeze dried and store in air tight close container.

Characterization of Nanoparticles:

%Drug Entrapment:

Separation of free drug

Analysis of MTX from CS-NPs was done by separating free drug from the nanoparticles dispersion. The separation was done by centrifugation of nanoparticles at 15000 rpm for 30 minutes at 4 ^{0}C (cooling centrifuge). Then, the nanoparticles and supernatant were separated.

Indirect method

In this method, analysis of MTX from CS-NPs was done by appropriately diluting supernatant in 0.1N HCl and absorbance was taken against 0.1N HCl as a blank on UV-Visible Spectrophotometer (at 336nm).

Percent Drug Loading:

The drug content in the NP was determined by dissolving 10 mg of lyophilized NP in 10 ml of 0.1N HCl. Absorbance of the solution was then measured spectrophotometrically at 336 nm after filtration and appropriate dilution with 0.1N HCl and drug content in the NP was determined.

The separation of free drug from NPs was done.

Then, %Drug loading was calculated using following equation

$$\% \text{ Drug Loaded} = \frac{\text{Drug Entrapment}}{\text{Total Weight of NPs}} \quad 100$$

In-Vitro Drug Diffusion:

A 4–5 cm long portion of the dialysis tubing was made into a dialysis sac by folding and tying up one end of the tubing with thread. It was then filled up with phosphate-buffered pH 7.4 and examined for the leaks. The sac was then emptied and 2 ml of methotrexate nanoparticle dispersion were accurately transferred into sacs which served as the donor compartments. The sacs were once again examined for leak and then suspended in the glass beakers containing 50 ml phosphate-buffer, which become the receptor compartment. At predetermined time intervals, 5 ml samples was withdrawn from the receptor compartment and analyzed spectrophotometrically. Fresh buffer was used to replenish the receptor compartment at each time point. The samples were withdrawn till 74 hour. Absorbance was measure at 336 nm

Particle Size and Zeta Potential:

The average diameter (Z-AVE),and zeta potential of optimized methotrexate nanoparticle was determined by photon correlation spectroscopy (PCS) (Zeta- sizer Nano ZS, Malvern Instruments, UK) at room temperature. Nanosuspension was added to the sample dispersion unit (deionized water) and stirred at 2000 rpm with magnet in order to reduce the inter-particulate aggregation. The samples were adequately diluted with deionized water and placed in an electrophoretic cell. The average particle size was measured after performing the experiment in triplicates.

Scanning Electron Microscopy (SEM):

The morphology of optimized batch MTX6 methotrexate nanoparticle was determined using scanning electron microscopy (SEM). Prior to examination, the samples were mounted on to metal stubs using a double sided adhesive tape under vacuum. The scanning electron microscope was operated at an acceleration voltage of 15kV.

Stability Studies:

The optimize batch viz **MTX-6** was subjected to the accelerated stability testing to find their stability and efficacy. Each formulation was divided into three portions of which was kept at 25ºC ± 2^0C/60 ± 5% RH, 30ºC ± 2^0C/65 ± 5% RH and 40ºC ± 2^0C/75 ± 5% RH respectively for two month. At 15 days intervals, samples were evaluated for physical testing, entrapment efficiency and % CDR study.

CONCLUSION

Based on the observations, it can be concluded that the nanoparticulate delivery system of methotrexate was formulated using widely accepted and physiologically safe polymer was capable of exhibiting sustained release properties for a period of 72hr. It was also concluded that the concentration of polymer and/or cross-linking agent was highly influence the formulation characteristics.

By administering such formulation, it may reduce the amount of drug to be administered along with frequency of dosing, thereby minimizing the occurrence of side effects, may improve bioavailability and the effectiveness of the drug. But it can be confirmed by further studies.

REFERENCES:

1. Wikipedia.(www.wikipedia.com).
2. http://www.medscape.com/viewarticle/585098. Accessed on 05/11/2011.
3. http://mohfw.nic.in/kk/95/i9/95i90e01.htm. Accessed on 05/11/2011.
4. Umadevi K. Current status of gynecological cancer care in India. *J Gynecol*
5. Oncol.2009; 20(2):77-80 Peppas LB and Blanchette JO. Nanoparticle and targeted

systems for cancer therapy. *Adv. Drug Delivery Rev*. 2004; 56:1649-59.

6. Bruce A, Chabner P, AmrinC. Possible treatment of cancer. The pharmacological basis of therapeutics. 11th Edition.2006;477-486.
7. Singh UV, Shenoy DB, Udupa N. Novel carrier in cancer chemotherapy.Advances in controlled and novel drug delivery system.2010:97-104 MagaliZeisser-Labouebe,Norbert Lange, Robert Gurny, Florence Delie. Hypericin-loaded nanoparticles for the photodynamic treatment of ovarian cancer. Int J of Pharmaceutics 2006; 326:174–181.
8. Evan CS,Zinav R, Daniel D Convection-enhanced delivery of methotrexate-loaded maghemite nanoparticles. Int J Nanomedicine,2011;77-80.
9. Tae HK, Jiang HH, Yu SY. Preparation and characterization of water-soluble albumin-bound curcumin nanoparticles with improved antitumor activity j 2009;77-80.
10. www.elsevier.com/locate/ijpharm 2007
11. Nair HB, Bokyung S, Yadav VR, Ramaswamy K Delivery of antiinflammatorynutraceuticals by nanoparticles for the prevention and treatment of cancer. j homepage: www.elsevier.com/locate/biochempharm 2010;901-908.
12. Das S, Banerjee R, and Bellare J. Aspirin loaded albumin nanoparticles by coacervation: Implications in drug delivery. Trends Biomater. 2005; 18(2):203-12.
13. www.elsevier.com/locate/carbpol, Development of chitosan nanoparticles as drug delivery systems for 5-fluorouracil and leucovorin blends. Carbohydrate Polymers 2011; 85:698–704.
14. M Sivabalan. formulation and evaluation of 5-fluorouracil loaded chitosan and eudragit®nanoparticles for cancer therapy. Int J of Comprehensive Pharm ; 2.
15. AzadeTaheri. Nanoparticles of Conjugated Methotrexate-Human Serum Albumin: Preparation and Cytotoxicity Evaluations. J of Nanomaterials 2011; 1:1-7.
16. WeiliQiao, Bochu Wang, Yazhou Wang, Lichun Yang, Yiqiong Zhang, and Pengyu Shao. Cancer Therapy Based on Nanomaterials and Nanocarrier Systems journal name 2010; .
17. VinodVenkatpurwar, Anjali Shiras, VarshaPokharkar. Porphyran capped gold nanoparticles as a novel carrier for delivery of anticancer drug: In vitro cytotoxicity study. Int J of Pharmaceutics 2011; 409:314–320.
18. AbhayAsthana, Abhay Singh Chauhan,PrakashVamanraoDiwan,and Narendra Kumar Jain. Poly (amidoamine) (PAMAM) Dendritic Nanostructures for Controlled Site-

specific Delivery of Acidic Anti-inflammatory Active Ingredient. AAPS Pharm Sci Tech 2005; 6:1-10.

19. http://www.webmd.com/vitamins-supplements/ingredientmono-625-chitosan.aspx?activeIngredient www.wikipedia.com/chitosanprofile.
20. www.wikipedia.com/sodiumtripolyphosphateprofile.
21. MuhammedRafeeq P E*, Junise V, Saraswathi R. krishnan P.N, Dilip.C,Development and characterization of chitosan nanoparticles loaded with isoniazid for the treatment of Tuberculosis;Research Journal of Pharmaceutical, Biological and Chemical Sciences RJPBCS 1(4)2010 October – December: 383-387

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