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Self-assembled folates as potential nano-carriers for controlled delivery of cancer drugs

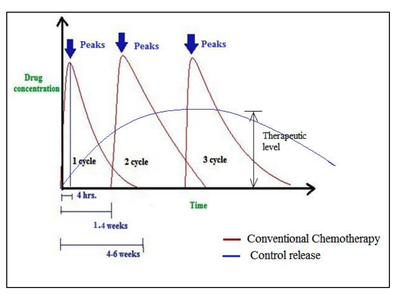
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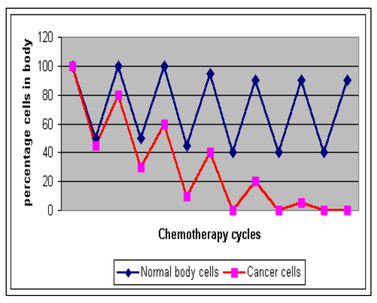
ABSTRACT

To make chemotherapy more effective, sustained release of the chemotherapeutic drug is desirable. By controlling the release rates, constant therapeutic levels can be achieved which can avoid re-administration of drug. This helps to combat tumors more effectively with minimal side effects. The present study reports the control release of methotrexate through liquid-crystalline folate nanoparticles. These nanoparticles are composed of highly ordered folate self-assembly which encapsulate drug molecules. These drug molecules can be released in a controlled manner by disrupting this assembly in the environment of monovalent cations. The ordered structure of folate nanoparticles offers low drug losses of about 4-5%, which is significant in itself. This study reports the size-control method of forming methotrexate encapsulated folate nanoparticles as well as the release of methotrexate through these nanoparticles. It has been demonstrated that methotrexate release rates can be controlled by controlling the size of the nanoparticles, cross-linking cation and cross-linking concentration. The effect of different factors like drug loading, release medium, and pH of the medium on methotrexate release rates was also studied.

1.Introduction

In conventional chemotherapy, drugs are administered intravenously into the bloodstream periodically (alternative days, weeks etc.), over "cycles". During this process, the drug concentration in the blood varies with time between a maximum concentration (which may represent a toxic level) and a minimum value (below which the drug is ineffective). In each cycle, the drug concentration inside the body reaches a toxic level (referred as "peak") and then decreases after about 4-12 hours. These toxic levels not only kill cancer cells but also affect healthy tissues of the body.

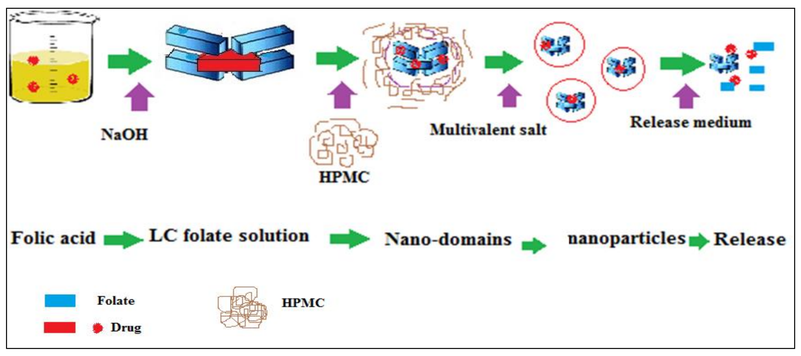




Conventional chemotherapy with drugs is normally effective in killing the tumors, but serious side effects of these drugs on liver, lungs, or kidneys has been reported. This is due to the uncontrolled levels of the drugs which reach in the body during the chemotherapy with direct drug.

Different nano-carriers have been developed for controlled release at target sites. Examples include polymeric nanoparticles, nano-micelles, dendrimers, liposomes, nanospheres. There remain some hurdles in controlled drug release with polymer and micellar based nanoparticles that have been extensively used in the past. Though most of these nano-carriers can effectively carry the loaded drugs to target site but they release the drugs in a "burst" as they are designed to carry drugs in "pockets" within the nanoparticle.

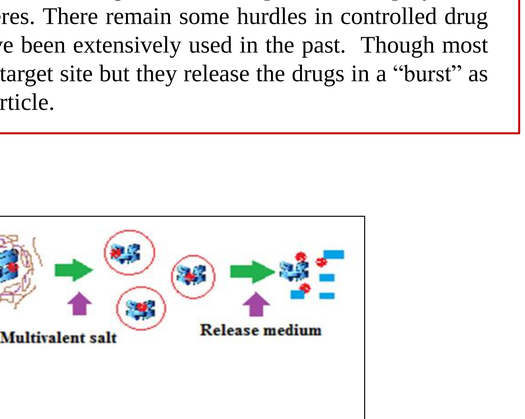
2. Methodology



2.1 Designing folate nano-carriers from liquid-crystalline folate solutions

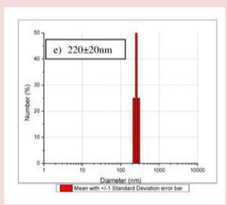
a. Folic acid is of interest as drug delivery carrier as it is a vitamin B, part of dietary supplements, biocompatible. Higher amount of folic acid does not cause any harm as it is excreted out through urine. Thus, there is lower risk of toxicity and side effects .

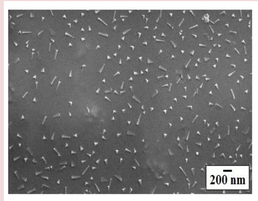
b. Folic acid being a chromonic molecule, show liquid-crystalline behavior and self assembles even at low concentrations of 0.1 wt. %.

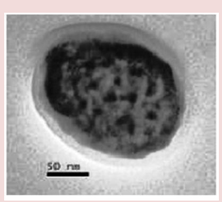


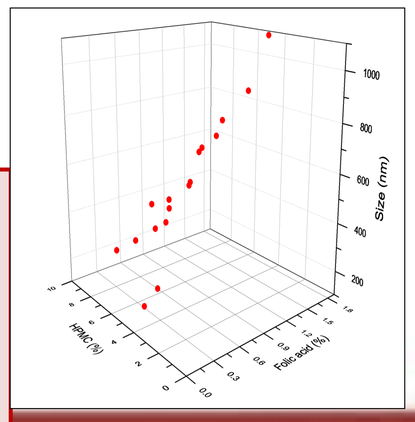
We have reported that drug encapsulated folate nanoparticles can be engineered from liquid crystalline folate solution by mixing it with HPMC (Hydroxy Propyl Methyl Cellulose) polymer and their size can be controlled by varying the relative concentrations of both components (folate & HPMC) in the solution.

Nanoparticles made of self-assembled folate



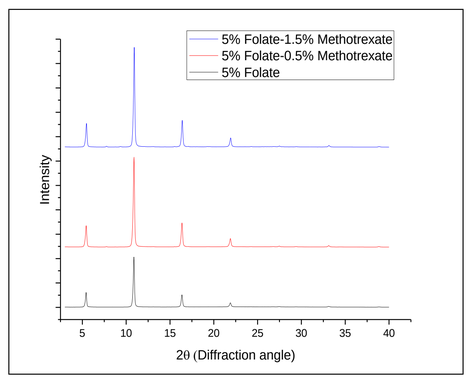






2.2 Drug loading and encapsulation efficiency

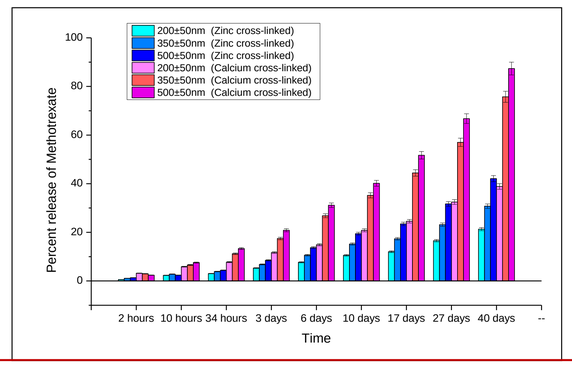
During encapsulation, drug molecules gets intercalated between/within the stacks present in folate assembly. Ordered structure of folates gives better control of release and high encapsulation. The total initial drug loading amount is kept at 10-30% of carrier content. Aqueous solution of drug is added to liquid-crystalline folate solution and continuously stirred for 30 minutes with the help of magnetic stirrer. **X-ray diffraction study of this folate-drug mixture shows that drug molecules participate in maintaining the ordered structure of folates.** Moreover, we have shown that **only 4-5% of drug (encapsulation efficiency 94-96%) was lost during the formulations**, which is significant since in most processes of formulation of nanoparticles, losses are in the range of 15-40%

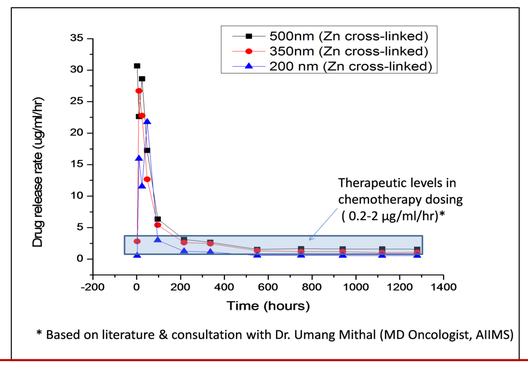


Nanoparticle specifications		Method of development			Initial drug amount and loss		Drug amount after encapsulation		
Nanoparticle size	Cross-linking	Folate %	HPMC %	MTX %	Amount of drug after cross-linking (gms)	Drug loss (µg)	Weight of dry Pellet (gms)	Drug encapsulated (µg)	Encapsulation percentage (%)
300 ± 20 nm	Zinc (10%)	2.5	1.5	0.25	0.005	**253.8**	0.145	4746	**95.32**
250 ± 20 nm	Zinc (10%)	2.5	4.5	0.25	0.005	**141.6**	0.162	4859	**97.18**
100 ± 20 nm	Zinc (10%)	2.5	10	0.25	0.005	**96.84**	0.185	4903	**98.48**

2.3 *In-vitro* controlled release of cancer drugs

0.04 grams of lyophilized nanoparticles were taken in a vial and suspended in 10 ml release medium. At different time intervals, release medium was removed and replenished with fresh medium. The concentration of released drug was in release medium was determined through HPLC. **We have reported that the release rates can be controlled by changing the size of the nanoparticles, cross-linking cation and cross-linking intensity. We are also able to target therapeutic levels through our system for the period of 40+ days successfully** .



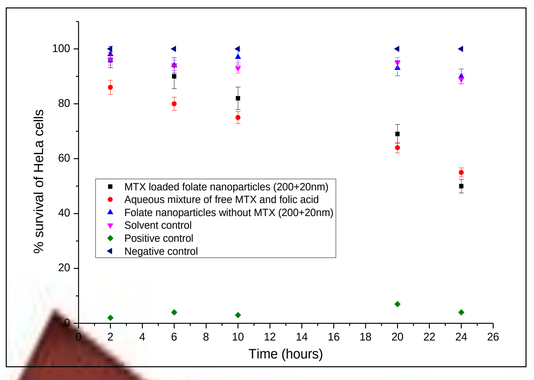


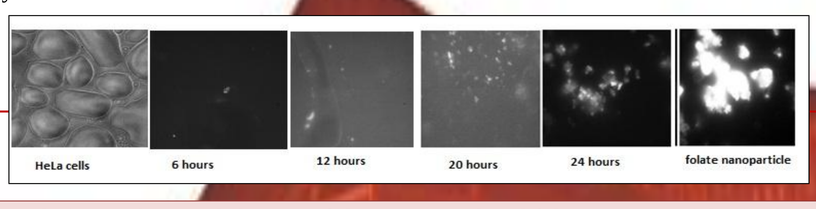
* Based on literature & consultation with Dr. Umang Mithal (MD Oncologist, AIIMS)

2.4 Cytotoxicity and targeting studies with HeLa cancer cells

HeLa cells were cultured and maintained in DMEM growth media in 96 well plate. Cultured HeLa cells were exposed to methotrexate encapsulated folate nanoparticles at pre-determined cytotoxic concentration (3µg/ml). The cytotxocicity was studied at different time points between 2-24 hours through MTT assay. It was observed that cytotoxic behavior of free MTX and bound MTX was closely the same.

Zinc ions in bound state fluoresce by themselves. Therefore, Zinc cross-linked folate nanoparticles when exposed to HeLa cells, increase in fluorescence was recorded over time. This clearly indicates the active targeting of folate nanoparticles.





3. Conclusion

The present study reports a method to design and control the size of drug encapsulated folate nanoparticles in the size range of 80-500nm. It has been shown that drug molecules can be encapsulated in these nanoparticles with significantly low drug loss of 4-5%. Drug molecules intercalate between or within the ordered folate stacks of the nanoparticles, which are cross-linked with a multivalent cation. Cross-linking cations play major role in keeping the drug encapsulated folate assembly intact. Due to exchange of ions present in release medium with the cross-linking cation, this assembly is disrupted. The rate of disruption of this assembly (measured by the rate of release of drug) is dependent on the size of the nanoparticles, choice of cross-linking cation and its concentration. These are three parameters that can be independently controlled to control the release rate from the nanoparticle.