Plan of the work
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In process of pursuing the objectives, this work was aimed to develop a generalized and usable methodology for nanocapsulation of delicate drug molecules. Generalized protocol was attempted using hydro polymer material and a continuous process was preferred for its industrializability over batch processes.

Hydro-based biocompatible polymer materials calcium alginate and BSA were experimented with and Methotrexate (MTX) has served as a model drug for coating. The protocol was basically a sequence of micro emulsion and polymer cross-linking.

Methotrexate is a broad-spectrum antineoplastic drug that acts by specific inhibitions of enzyme dihydrofolate reductase. It has biological $t_{1/2}$ at 7.2 ± 2.1 hrs and on administration, 50% of the drug is bound to plasma proteins. Very low dosage (0.1 mg/kg) is absorbed from GIT and both metabolism and excretion of the drug in human is almost nil. The drug is accumulated in all tissues, slowly reducing immunity and damaging tissues like kidney. MTX associated tumor resistance occur largely due to defective transport of the drug. Wide application of MTX in arthritis, immunoprotection and as antineoplastics therefore associate serious side effects including risks of fatal cross infections. Reservoir type MTX nanocapsules were therefore perceived as it might provide slow and continues supply of the drug in desired tissue target site. Moreover successful development of nanocapsulation techniques with zwitterionic compound like MTX can provide a basis for nanocapsulation of peptide and vaccine drugs in later stages for wider applications.
Bovine serum albumin (BSA) and Calcium Alginate were chosen as representative coating polymer materials. They are of well known structure, naturally occurring, available in sufficient purity, aqueous soluble and can provide specific advantages in novel antineoplastic drug delivery devices\textsuperscript{4,5}.

The hydrophilic polysaccharide alginate can be crosslinked to exist as positively charged micro or nanoparticles. BSA nanoparticles on the other hand are also charged particles were the surface charge is largely dependent on surface available amino groups\textsuperscript{6,7}.

For preparation of nanocapsules two different preparative techniques were used; in method-I, the drug in organic phase was microemulsified in aqueous polymeric solution and cross linker solution was slowly added into it under gentle stirring conditions. The nanocapsules formed were subsequently washed and collected by ultracentrifugation. In method-II, drug in organic phase was microemulsified in aqueous polymeric solution and this microemulsion was passed through a pneumatic nozzle for the microemulsion mist to come in contact with a polymer precipitator or cross linker solution. The nanocapsules prepared were similarly harvested.
Preparation of nanocapsules (in-situ precipitation method)

Method -I

Core Material
drug
in organic solvent

Coating Material
in aqueous phase
containing surfactant

Sonication

Oil in water Emulsion

Co-surfactant

Mechanical stirrer

Microemulsion

Cross linker /
Polymer precipitator

Nanocapsulation in
Preparative media

Ultracentrifugation

Nanocapsules

Fig. 1: Steps in nanocapsulation technique method I
Preparation of nanocapsules (Nebulisation/spray method)

Method –II

Fig. 2: Steps in nanocapsulation - method II
Nanocapsules prepared were observed in transmission electron microscopy (TEM) and were later studied & characterized in photon correlation spectroscopy. The drug loading efficiency, of different formulations were studied by complete dissolution of nanocapsules and measurements of the total drug released in HPLC by area normalization approach. Residual solvents if any were studied in Gas Chromatography of the redissolved nanocapsules and the drug loading was further confirmed in FT-IR observations of prepared nanocapsules.

*In vitro* release kinetics were studied and release pattern was mathematically modeled in order to get some insight into drug release pattern.

*In vivo* tissue distribution for selected methotrexate nanocapsules were studied in animal models against parallel administration of standard drug.