Surface Modified Ultrathin Polyelectrolyte Nanoreservoir for Delivery of Proteins: Evaluation in Terms of Controlled Release and Biocompatibility

Girish K. Gupta, Vikas Jain, P.R. Mishra

Pharmaceutics Division, Central Drug Research Institute, Lucknow-226001 INDIA

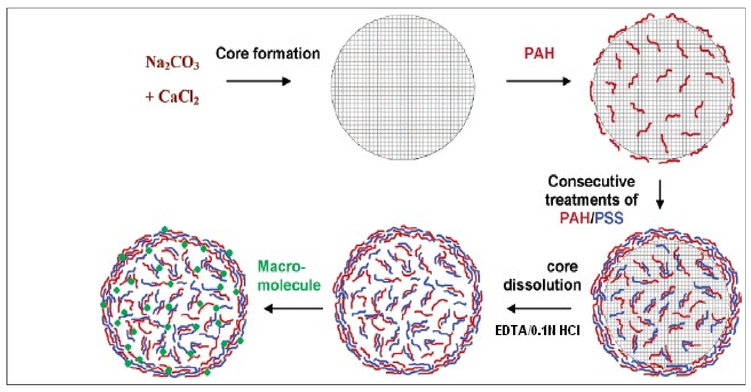
INTRODUCTION

Most of the colloidal polymeric systems based on synthetic and natural polyelectrolyte has been investigated by layer-by-layer self assembly technique for microencapsulation and controlled release of macromolecules using different templates with size ranging from nanometer to tens of microns, such as organic and colloidal particles, protein aggregates, biological cells and drug nano or microcrystals. System has been prepared by the sequential deposition of the oppositely charged polyelectrolyte using the phenomenon of electrostatic interaction between each other. Most of the colloidal templates can be decomposed at conditions where polymeric matrix is stable, which leads to the formation of hollow polyelectrolyte capsules with defined size, shape and shell thickness. Encapsulation of macromolecules, proteins, and other biotherapeutics into developed systems is of great interest for pharmaceutics and biotechnology due to its capability to use such systems as micro and nanocontainers for controlled drug delivery.

OBJECTIVE

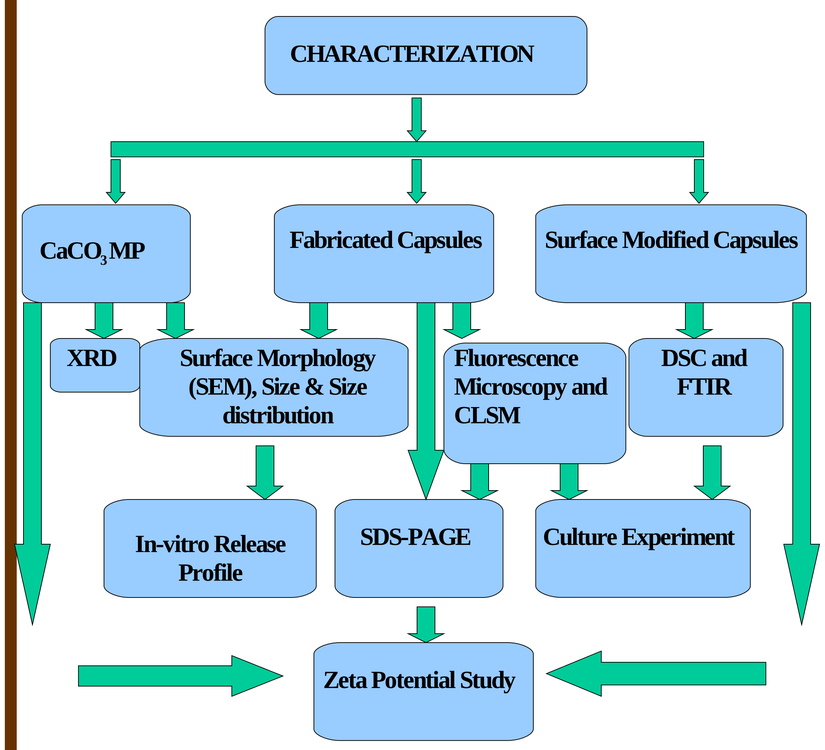
- To prepare ultrathin polyelectrolyte nanoreservoir (UPN, Capsules) using LBL technique with subsequent core removal at low pH (1.1) and subsequently surface modification by using pluronic (PF-68).
- To characterize the system in terms of encapsulation efficiency, surface morphology, LBL growth, in-vitro release profile, integrity of protein.
- To assess biocompatibility of the surface modified nanoreservoir.

LAYER-BY-LAYER TECHNIQUE

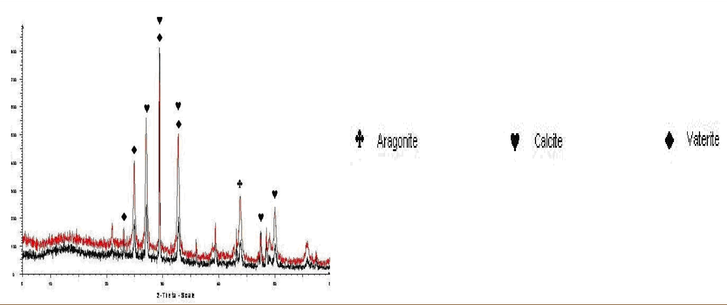


Volodkin et al., 2004

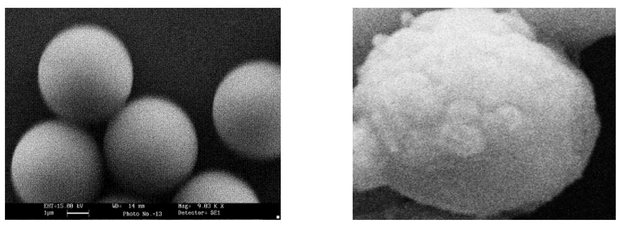
CHARACTERIZATION



POWDER XRD POROUS $CaCO_3$ MP



SEM OF POROUS $CaCO_3$ MP AND FABRICATED CAPSULES



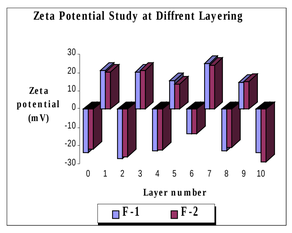
A B

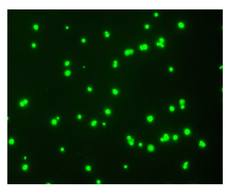
A: SEM of porous $CaCO_3$ MP

B: SEM of fabricated capsules

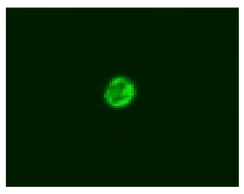
Scale bar 1 μ m

LAYER-BY-LAYER GROWTH BY ELECTROPHORETIC MOBILITY AND CLSM



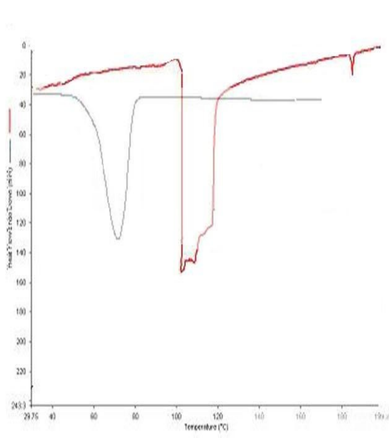


Fluorescence Microscopy

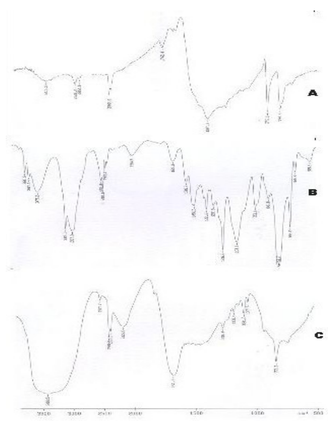


CLSM

PROOF OF SURFACE MODIFICATION BY DSC AND FTIR

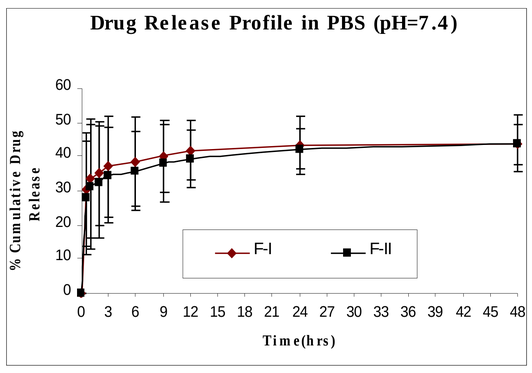


Gray and red line indicating Transition of PF-68 and Surface modified capsules respectively.



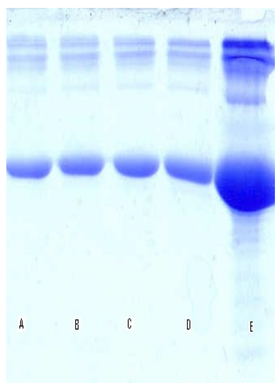
A, B & C: IR spectra of capsules, PF-68, surface modified capsules respectively.

IN-VITRO RELEASE PROFILE



F-1: $(PAH/PSS)_5$; F-II: $(PAH/SA)_5$

SDS-PAGE



A and B: Entrapped and free protein for F-2 [$(PAH/SA)_5$];

C and D: Entrapped and free protein for F-1 [$(PAH/PSS)_5$];

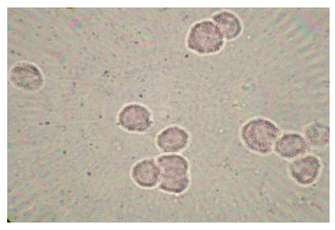
E: Pure protein solution.

PAH: Poly(allylamine) hydrochloride;

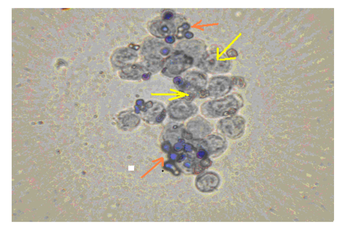
PSS: Polystyrene sulfonate sodium;

SA: Sodium alginate

PHAGOCYTIC UPTAKE AND BIOCOMPATIBILITY STUDIES

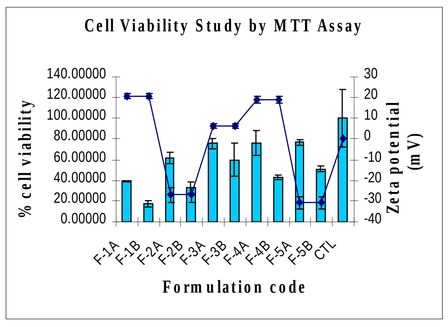


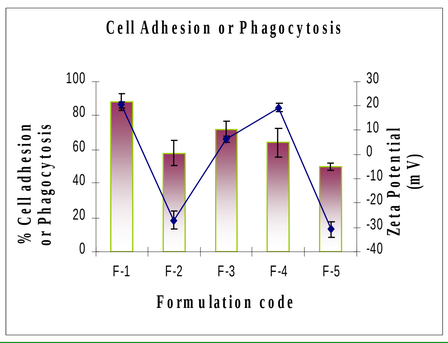
Plane macrophages cells



Adhered capsules to cells

Yellow arrows indicate phagocytosis of capsules where as **orange** indicates for cell adhesion.





F-1= $(PAH/PSS)_5$–PAH;
F-2= $(PAH/PSS)_5$;
F-3= $(PAH/PSS)_5$–PAH-PF68
F-4= $(PAH/SA)_5$- PAH;
F-5= $(PAH/SA)_5$;
CTL= J 774 macrophages.
A=10 µg/ml; B= 100 µg/ml.

DISCUSSION

- Inorganic decomposable core (charge substrate) has been selected for LBL assembly of polyelectrolyte using electrostatic interaction due to its great importance in geo-, bio-, and material sciences, as well as due to its wide industrial, technological and drug delivery applications.
- Shape, surface morphology and narrow size distribution ranging from 4-6 µm of the $CaCO_3$ microparticles and fabricated capsules were presented by the SEM micrographs. The nanoreservoir are promising carrier for proteins since structural integrity of protein was not significantly affected by the entrapment procedure or any harsh conditions or the type of polymers used in the study.
- Zeta potential study and CLSM reveals layer-by-layer growth of the systems.
- Both the systems exhibited biphasic release profile with initial burst followed by controlled release.
- Further, surface modification renders the capsule more biocompatible compared to plane capsules. It would help combat undesirable biological processes, such as phagocytosis of the colloidal carrier.

CONCLUSIONS

The fabrication of nanoreservoirs using combination of synthetic and natural polyelectrolyte seems to be promising carrier for therapeutic proteins without affecting its integrity. The surface modification of nanocapsules has successfully demonstrated biocompatibility and also reduced adhesion/phagocytosis to biological cells. Studies are still under way to obtain suitable combination by using combination of natural polyelectrolyte.

ACKNOWLEDGEMENTS

ICMR and CSIR for providing Senior Research Fellowship to G. K. Gupta and V. Jain and DST for providing fund under Fast Track Scheme. IITR , Lucknow for providing facilities for Zetasizer NanoZS.

BIBLIOGRAPHY

- Volodkin, D.V, A.I., Petrov, M., Prevot and G.B. Langmuir, 2004, 20(8), 3398.
- Decher G., Hong J.D., Schimtt, J. Thin Solid Films, 1992, 210(1-2), 831.
- Sukhorukov, G., Donath, E., Moya, S., Susha, A., Voigt, A., Hartmann, J., Mohwald, H. J. Microencapsulation. 2000, 17, 177-185.

Nature Precedings : doi:10.1038/npre.2008.2548.1 : Posted 21 Nov 2008