**O9-2** **Liposomes in situ gelling system for mucosal vaccination.**

**Tiwari S.** [1#] **and Vyas S.P.** [1*]
[1] Department of Pharmaceutical Sciences, Dr. H. S. Gour University, India,
shailjadops@gmail.com

## INTRODUCTION AND OBJECTIVES

Novel carriers have been widely used as carriers of protein or peptide antigens. But their low encapsulation efficiency largely stalls their use for delivery of these expensive molecules (Shailja 2009a). In the present work with an effort to enhance encapsulation efficiency of novel carriers, we developed a novel drug delivery system, liposomes in situ gelling system (LIGS) of biodegradable polymers for nasal mucosal immunization against Hepatitis B for induction of cellular, humoral and mucosal immunity. This system is conceived from a combination of the polymer and lipid-based delivery systems and can thus integrate the advantages and avoid the drawbacks of the two systems. Gel core liposomes were prepared by extrusion method in which the dried lipid film was hydrated with aqueous solution of antigen containing 0.3%w/v polymer poly acrylic acid (PAA). Then the one part of prepared formulation (gel core liposomes suspended in PAA gel administration medium-liposomes in situ gelling system) will be use as such and the other one will be processed for separation of unentrapped polymer and antigen (gel core liposomes) (Shailja 2009b).

## MATERIALS AND METHODS

| ***In vitro* optimization and characterization:** | |
|---|---|
| Size and Zeta potential | Zetasizer (Malvern UK) (Table-II) |
| Shape and lamellarity | Transmission electron microscope (JEM-200, JEOLTokyo, Japan) (Fig-1) |
| Entrapment efficiency | BCA method for protein antigen (Table-II) |
| *In vitro* release | Dialysis method using dialysis membrane MWCO 25-30KDa (Pall Pvt,Ltd, India) (Fig-2) |
| In process stability | SDS-PAGE (Fig-3) |
| Storage stability | Evaluation vesicle size and residual antigen content of liposomal formulation stored at 4±1°C and 25±1°C. |
| ***Ex-vivo* study** | |
| FACS analysis | To evaluate uptake of liposomal formulation in dendritic cells and human bronchial epithelial cells (calu-3 cell line ). (Fig-6) |
| ***In vivo* study: performed with permission of institutional animal ethical study on Balb/c mice** | |
| Fluorescent uptake study | To evaluate uptake of formulation in nasal mucosal tissue. (Fig-5) |
| Gamma Scintigraphy study | The nasal clearance of various radiolabelled formulations was evaluated by using gamma camera (Seimens, E-Cam, Germany) at 0, 10, 20, 30, 60 and 120 min.(Fig-4) |
| Immune response | Determination of IgG and IgA antibody titer by ELISA .Following administration of different formulation in Balb/C mice (Fig-7) |
| Cytokine response | Splenocyte culture study followed by measurement of cytokines by ELISA assay.(Fig-8) |

## RESULTS AND DISCUSSION

Table-II: Particle size, zeta potential and loading capacity of various liposomal formulations (mean±s.d.:n=6)

| Formulation | Average vesicle size (μm) | Entrapment efficiency | | Zeta potential | PDI |
|---|---|---|---|---|---|
| | | Liposomal vesicle | Gel administration medium | | |
| CL | 1.12±0.64 | 45.32±3.4% | - | -10.4±0.21 | 0.247±0.019 |
| GC | 1.23±0.53 | 46.76±2.9% | - | -36.1±0.16 | 0.141±0.007 |
| LIGS | | 46.76±2.9% | ~54% | -36.1±0.16 | 0.141±0.007 |

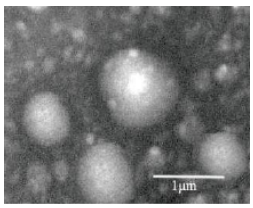


**Fig-1:TEM photomicrograph of gel core liposomes**

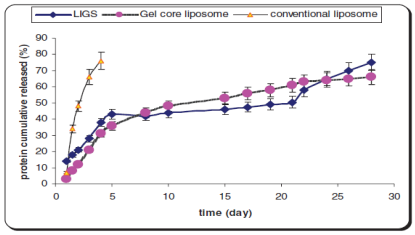


**Fig: 2-In vitro release profile of various liposomal formulations**

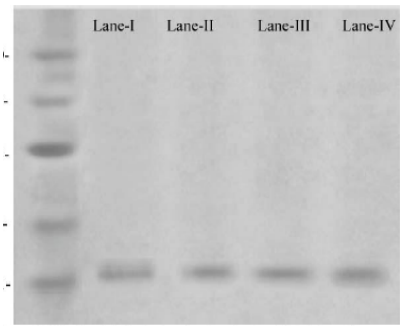


**Fig:3-SDS PAGE analysis showing integrity of protein antigen HBsAg in various liposomal formulations Lane-I=plain antigen, Lane-II, Lane-III, Lane-IV antigen extracted from plain liposomes, gel core liposomes, gel administration**

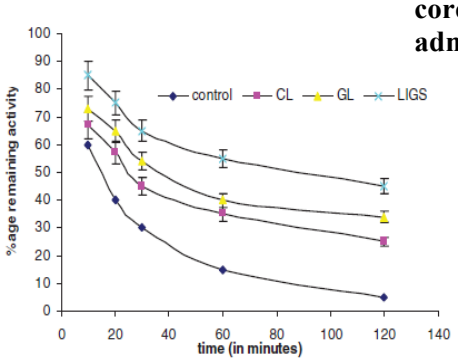


**Fig:4- Nasal clearance of various liposomal formulations as studied by gamma scintigraphy**

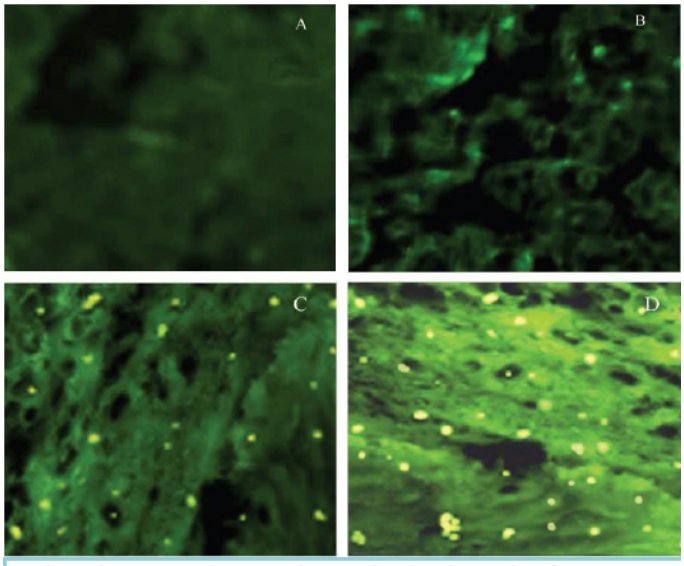


Fig:5-Fluorescent microscopy images showing the uptake of FITC-BSA loaded different liposomal formulations by nasal mucosal surface after intranasal administration. (A) soluble FITC-BSA (control); (B) CL (C) GC (D) LIGS.

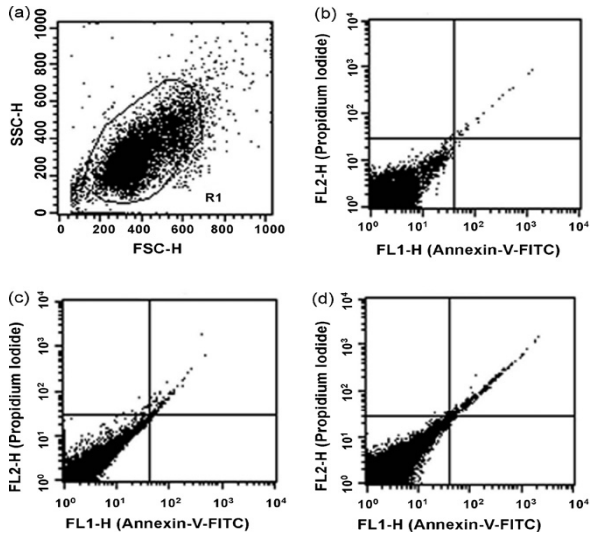


**Fig:6-Flow cytometric analysis for uptake in Calu-3 cell line following 6 h incubation (a) FSC/SSC plot showing the population of**

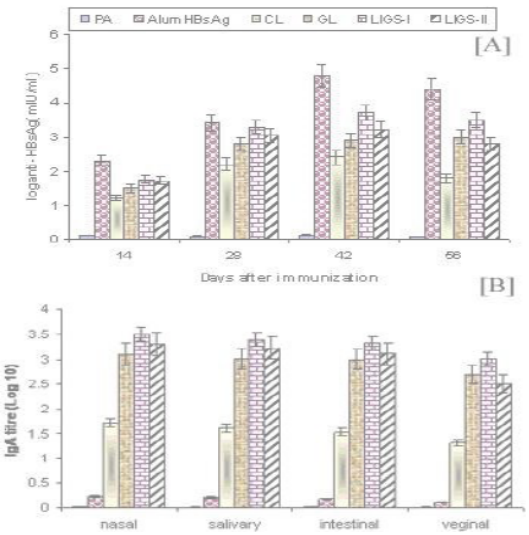


**Fig:7-Immunonological studies: [A]. Serum antibody (IgG) profile of mice immunized with different formulations. [B]. sIgA level in the salivary, intestinal, vaginal and nasal secretion after immunization with various formulations**

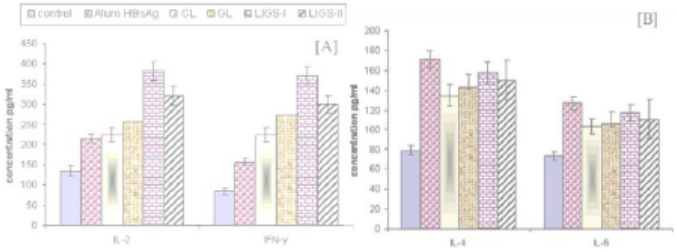


**Fig:8-HBsAg specific recall responses after stimulation of splenocytes derived from BALB/c mice immunized subcutaneously with various liposomal formulation. Cytokine concentration in culture supernatants was determined in splenocyte culture stimulated with 5 mg/ml of soluble HBsAg: (A) IL-2 & IFN-γ (B) IL-4 & IL-6 (n =4, mean ±s.d.)**

The purpose of this study was to compare the systemic and mucosal immune responses induced after intranasal vaccination of mice with LIGS, gel core liposomes, and plain liposomes associated to HBsAg antigen by encapsulation methods. These antigens were successfully encapsulated within various liposomal formulations without causing any damage in the protein structure. Even so, both LIGS and gel core liposomes induced significantly higher specific immune responses to HBsAg antigens, which were confirmed by the in vivo immunization study.

## CONCLUSION

It can be concluded from these results that the prepared systems can be utilized as a potent adjuvant for noninvasive mucosal immunization through nasal routes. The prepared system is capable to elicit humoral, cellular as well as mucosal immune responses and can be a potential carrier system for a number of antigens along with HBsAg. Though the humoral response was only comparative to alum based standard but the developed formulation gave these results after single dose compare to the booster dose given in case of standard. Moreover, developed liposomal carrier systems also elicited significant mucosal and cell mediated immune responses, which were not elicited by conventional alum-HBsAg. In addition LIGS offer advantage of 100% utilization of the protein antigen and serve the purpose to improve entrapment efficiency which is the main drawback associated with conventional novel carriers.

## REFERENCES


- Shailja Tiwari et al., *Gel core liposomes: An advanced carrier for improved vaccine delivery.* Journal of Microencapsulation, 26(1), (2009a) 75–82.
- Shailja Tiwari, *Development and characterization of novel carrier gel core liposomes based transmission blocking malaria vaccine.* Journal of Controlled Release, 140 (2009b) 157–165.


## ACKNOWLEDGEMENT

The author Shailja Tiwari wants to acknowledge Council of Scientific and Industrial Research, New Delhi (CSIR) for providing SRF.