Annexure -A

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MAJOR RESEARCH PROJECT REPORT

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UGC approval Letter No. and Date	F.43-485/2014(SR), 15-10-2015
Title of the Research Project	**STATISTICAL DESIGNED NANOLIPOSOMAL DELIVERY OF ANTICANCER DRUG(S)**

INTRODUCTION:

Nanoliposomes comprise a bilayered membrane configuration and are superior and flexible pharmaceutical nanocarrier for low and high molecular weight active agents. Nanoliposomes are preferred as carriers because they are biologically compatible; cause slight or no pyrogenic, antigenic, toxic and hypersensitivity reactions. Nanoliposomes undergo biodegradation and shield the patient from the adverse effects of the encapsulated medicinal agent and simultaneously protect the entrapped active agent from the neutralizing action of the physiological medium. Nanoliposomes exposed the drug to the targeted cells for sufficient time period to augment the time of therapeutic action of exposed drug to the targeted cell. Gefitinib is a low-molecular-weight anilinoquinazoline derivative. It works by restraining the growth and multiplication of lung cancer cells. Imatinib mesylate is a small-molecule signal transduction inhibitor - protein tyrosine kinase inhibitor with antineoplastic activity, commonly used for targeted therapy.

Aims and Objectives of the Study:

The aim of the current work was to formulate imatinib/geftinib loaded nanoliposomes. The gefitinib loaded nanoliposomes were formulated using reverse-phase evaporation method and imatinib nanoliposomes by probe-sonication technique. The objective behind the study was to study the effect of critical variables on the formulation characteristics of nanoliposomes using experimental design.

Materials and Methods:

The gefitinib loaded nanoliposomes were formulated by using reverse-phase evaporation method These nanoliposomes were formulated according to Box–Behnken Design (BBD) to study the effect of sonication time (X_1), ratio of tween 80 and soya phosphatidylcholine (X_2) and ratio of cholesterol and soya phosphatidylcholine (X_3) (F1 to F17). Imatinib loaded Nanoliposomes were prepared by the modified solvent evaporation method. Thirteen batches of central composite designed imatinib loaded nanoliposomes (NLP-1 to NLP-13) were formulated to study the effect of Conc. of Cholesterol (X_1) and Conc. of Phosphatidyl Choline (X_2). For both of the formulations, the particle size, entrapment efficiency and percent Cumulative Drug Release (CDR) of drug were response variables. In nanoliposomes, cholesterol works to stabilize them and phosphatidylcholine acts as their structural backbone. Phosphatidylcholine is an amphiphilic in nature, which forms micelles with tail domain inside the aqueous media and with the head domain outside. The dimension of the micelle was abridged by sonication to nano range.

RESULTS AND DISCUSSION

The particle size and PDI analysis results for the geftinib loaded nanoliposomes ranged from 93.2 nm to 490 nm and 0.233 to 0.428, respectively. It was observed that the ratio of tween 80/soya phosphatidylcholine was the main factor which affects the particle dimension and the interaction between tween 80, cholesterol and sonication time plays a vital function in optimizing the particle size of nanoliposomes. The value of PDI demonstrated the narrow distribution of particles. The value of PDI was found to be less than 0.4 that was suitable for pharmaceutical applications. The highest encapsulation efficiency was found to be 87.56 % and loading capacity was 35.16 %. The sonication time and cholesterol/soya phosphatidylcholine ratio were the key factors affecting the encapsulation efficiency and the interaction between tween 80, cholesterol and sonication time plays a critical part in optimizing the encapsulation efficiency. The sonication time and cholesterol/soya phosphatidylcholine ratio were the chief factors affecting the % cumulative drug release and the interaction between tween 80, cholesterol and sonication time plays a decisive function in optimizing the % drug release. Design Expert 11.0 software was used to apply ANOVA on all the response variables so as to examine the relevance and the

implications of model under scanner. The data from multiple regressions had shown that the square terms should be maintained in the mathematical model to elucidate the response curvature. The DSC analysis of geftinib had shown a pointed endothermic peak at 197.51°C with percentage purity 99.42 mol %. The disappearance of endothermic peak of the drug in nanoliposomal formulation showed the drug may either disperse or dissolve in the polymer medium or may appear as amorphous form. Neither a new peaks nor considerable shift of functional peaks nor overlaps of attribute peaks were observed in the formulation spectrum upon comparison with reference spectrum. The results suggested that throughout their encapsulation course drug remains stable. The % cumulative drug release was found to be 82.16±0.65% in simulated gastrointestinal fluid containing 0.1N HCl, 58.25±0.39% in acetate buffer pH 4.0 and 48.72±0.55% in simulated intestinal fluid pH 6.8, after 24 h. Gefitinib was found to be more soluble at low pH, it releases more rapidly in acidic media than in slightly basic media. The release rate of gefitinib loaded nanoliposomes exhibited sustained release behaviour in all three media and the release rate of gefitinib from prepared nanoliposomes was found to be highest in simulated gastrointestinal fluid. This is because gefitinib being a free base, more prone to release in acidic medium than in slightly basic medium. The sustained release manner of nanoliposomes in simulated gastrointestinal fluid may prolong the duration of drug absorption that might be helpful in reducing the side effects and improving the therapeutic index of drug. The release of gefitinib from gefitinib nanoliposomes was found to be of first-order. The 0.377 value of n characterized that the drug had shown the Fickian type diffusion ($0.45 < n$). It means that gefitinib liberated from the nanoliposomes by solvent penetration effect, swelling, polymer chain disentanglement and relaxation procedures. The IC_{50} values for free gefitinib were 1.92±0.86 and 1.06±0.7 μM respectively in A549 and H1299 cells, respectively after incubation, while the IC_{50} values for gefitinib loaded nanoliopsomes were 7.31±0.85 and 7.03±0.98 μM, respectively. Free gefitinib had shown more cytotoxicity than gefitinib loaded nanoliposomes, representing stable confiscation of gefitinib by nanoliposomal encapsulation. The less cytotoxicity of gefitinib loaded nanoliposomes might be due to the slow release rate of gefitinib from the nanoliposomes that might be beneficial as it provide protection to the cells from the altogether exposure of large amount of drug and improve the therapeutic index of drug. The results obtained had shown that the formulated vesicles were physically and chemically stable at 4°C for 6 months. During the

time period of stability study, the samples stored at 4°C did not show so much modification in the particle size and % encapsulation efficiency.

The particle size, PDI and zeta potential analysis results for the central composite designed imatinib loaded nanoliposomes ranged from 167.4 nm to 562.4 nm, 0.201 to 0.381 and -29.3 mV to -7.3 mV, respectively. The results showed that the least particle size was obtained at a high value of the concentration of cholesterol, high value of the concentration of phosphatidyl choline and highest zeta potential achieved at the mid value of concentration of cholesterol, and high value of the concentration of phosphatidyl choline. The high encapsulation efficiency was obtained at a high value of the concentration of phosphatidyl choline and mid-value of cholesterol. High loading capacity was obtained with a low value of the concentration of cholesterol and high value of the concentration of phosphatidyl choline. The DSC thermogram of imatinib showed an endothermic peak at 229.54°C with percentage purity of 96.24%. The dissipation of endothermic peak of the drug in the formulation proved that the drug may have been dispersed or dissolved throughout in the nanoliposomal matrix during the formation of nanoliposomes. The total incorporation of the imatinib into the nanoliposomes indicated a molecular dispersion of drug inside the nanoliposomal system. The nano-encapsulation process offered a considerable reduction in crystallinity of the drug and allows a nearly amorphous state. FTIR studies showed no major shifting of functional peaks and no overlapping of characteristic peaks. Additionally, no new peaks appeared upon the comparison of obtained spectra with reference spectra. The results suggested that the drug was stable during the encapsulation process. The FTIR data suggested that no molecular interactions occurred and no chemical interaction between the functional groups of imatinib and other components exist. The TEM image of the optimized batch of central composite designed imatinib loaded nanoliposomes was found to be nearly spherical in shape. Mathematical models developed for the estimation for encapsulation efficiency, loading capacity, zeta potential, % cumulative drug release in 0.1N HCl and % cumulative drug release in phosphate buffer pH 7.4. ANOVA was applied using Design Expert Software (Version 11.0.4.0, Stat-Ease Inc., Minneapolis, MN)) to study the fitting and significance of models. F-test was performed to compare the regression mean square with residual mean square. The ratio F shows the models are significant. These mathematical models can be retrospectively utilized to model the desired characteristics. Cumulative drug release for

all the central composite designed nanoliposomes in phosphate buffer pH 7.4 ranged from 42.9% to 64.4% after 24 h. Imatinib is a free base and is more susceptible to dissolve in the acidic solution, so that is the reason the release rate of imatinib from nanoliposomes exhibited a sustained release profile in the 0.1N HCl and phosphate buffer pH 7.4 and release rate of imatinib was highest in 0.1N HCl after 24h. The sustained release manner of the central composite designed imatinib loaded nanoliposomes may extend the drug absorption time in the gastrointestinal tract, which might be helpful to enhance the therapeutic activity of the drug and diminish the side effects. The Higuchi model had shown the highest R^2 value for *in vitro* release data and the value of n in Korsmeyer-Peppas model for *in-vitro* release data in 0.1N HCl and phosphate buffer pH 7.4 was found to be 0.745 and 0.777, respectively; which suggested that the release mechanism followed by the imatinib is non-Fickian anomalous diffusion. Cytotoxic activity of the nanoliposomes showed the maximum inhibition of the lung cancer (A549) cells. It has been reported that the drug uptake in cancer cells is highly dependent on the particle size and higher value of zeta potential facilitate cytotoxicity in cancer cells due to the stronger interaction with tumor cell membrane. The optimized formulation was found to be stable after six-month study. From the results, it can be concluded that for better stability in long-term storage, the formulation should be stored at 4°C. The optimized batch was also compared to marketed formulation and it was found that drug release slowly in comparison to marketed tablet formulation which may help in improving the therapeutic efficacy of the drug and reducing the side effects of the conventional tablet dosage form.

CONCLUSION

The gefitinib-loaded/imatinib loaded nanoliposomes have been successfully formulated by applying experimental design. The formulated nanoliposomes have shown particle size in nano range along with good encapsulation and loading efficiency and less cytotoxicity against A549 cell lines. It is eminent and acknowledged that the biodistribution of nanoliposomes was extensively exaggerated by their surface properties, size, and stability. Therefore, the prospect to target the entrapped drug to particular tissues will be highly inclined and these nanoliposomes with different quantities can regulate the cancer cells.

PUBLICATIONS

Seema Rohilla, Harish Dureja. Recent Patents, Formulation and Characterization of Nanoliposomes. *Recent patents in drug delivery and formulations.* 9(3): 213-224, 2015.

Seema Rohilla, Harish Dureja. Effect of Chitosan Coating on the Physiochemical Characteristics of Gefitinib Loaded Nanoliposomes. *International Journal of Pharmaceutical Sciences and Research.* 9(12): 1000-1013, 2018.

Nidhi Saiwal, Mandeep Dahiya and Harish Dureja. Recent Patents and Formulation of Nanopharmaceuticals using Ultrasonication Technique. *Recent Patents on Nanotechnolgy,* 12 (2), 86-100, 2018.

Mandeep Dahiya and Harish Dureja, Box-Behnken Designed Nanoliposomes of Imatinib for Drug Delivery (Communicated).

Mandeep Dahiya and Harish Dureja, Nanoliposomes for the Delivery of Imatinib by Probe type Ultrasonication (Communicated).

Mandeep Dahiya and Harish Dureja. Recent Developments in the Formulation of Nanoliposomal Delivery Systems (Communicated).

Seema Rohilla, Harish Dureja. Formulation and optimization of gefitinib loaded nanoliposomes for anticancer potential using box-behnken design (Communicated).

Seema Rohilla, Harish Dureja. Soya Phosphatidylcholine Nanoliposomes Loaded with Gefitinib: Preparation, Characterisation and Optimisation using the Central Composite Design (Communicated).

Seema Rohilla, Vinay Chawla, Harish Dureja. Cytoprotective Agents to avoid Chemotherapy Induced Sideeffects on Normal Cells: A Review (Communicated).

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