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IN VITRO EVALUATION OF POLYMERIC NIOSOMAL FORMULATION FOR LOCALIZED DELIVERY OF PACLITAXEL

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ABSTRACT

The present study was focused on formulating and evaluating paclitaxel (PTX**) loaded** polymeric niosomal formulation for localize delivery and to provide prolonged and sustained release of drug. Niosomal formulations were prepared by using different ratio of surfactant and cholesterol by thin film hydration method and were evaluated for in vitro release study, Rheological evaluation. The entrapment efficiency (%EE) of optimize PTX niosomal formulation was found to be 82.31 ± 2.91 %. The percent entrapment efficiency (%EE) of noisome was depending on the ratio between surfactant and cholesterol. Formulation containing (2:1) molar ratio of span 80 and cholesterol shows maximum entrapment efficiency. % release of paclitaxel niosomes form the formulation at the end of 12 hours. The highest % of drug release (62.78 ± 2.80) was obtained with niosomal formulation having formulation code (N1). When the niosomes were gelified (formulation code N2, N3, and N4) by different concentration of carragenan (1.0%, 1.5% and 2.0%) the percent in vitro release were found to be range (66.12 ± 0.46 to 45.27 ± 3.08) which is lower than the pure niosomes. Percent paclitaxel release from polymeric niosomal formulation was slightly decrease in the concentration of polymer was increases up to 1.5%. Further increasing concentration up to 2.0% showed more decline in drug release that is 43.20 ± 3.08 %.The present study suggested that niosomal formulations provide sustained and prolonged delivery of drug with enhance bioavailability.

INTRODUCTION

Breast cancer is the most commonly diagnosed cancer among females worldwide, with an estimated 14.1 million reported cases. Cancer cells form a tumor when they have lost the ability to stop reproduction and enter the death phase at the proper time. Surgery, radiotherapy and chemotherapies are currently used therapies to treat breast cancer. Today, new technologies are used to increase the efficacy of chemotherapeutic agents reduce their side effects. Nanotechnology in medical field is one the new technologies used for both diagnosis and treatment [1]. The natural origin based anticancer agent like paclitaxel (PTX), isolated from the bark of *Taxus brevifolia* (northwest Pacific Yew tree), characterized it as a white crystalline powder, with the empirical formula ($C_{47}H_{51}NO_{14}$) and on the basis of characterization named it as Taxol. Later on, the Bristol-Myers Squibb Company developed this isolated compound commercially with the generic name Paclitaxel and then sold under the trademark Taxol. As

per as anticancer activity is concerned, PTX exclusively binds to the *β*-tubulin subunit through N-terminal side chain with 31 amino acid, in the microtubules causing depolymerization of *β*-tubulin subunit, with inhibition of mitosis and this combined effect can be directed to induction of cell apoptosis [2]. Based on previous reports, PTX shows anticancer activities towards breast cancer, ovarian cancer [3], lung cancer and pancreatic cancer [4]. Supportive to this, previously published reports by, shows that PTX gives antitumor activity by a formation of macrophage IL–12 through nitric oxide, which further caused to dysregulation of IL-12 p40 expression and finally reduces the tumour growth. Additionally, PTX also exhibits anticancer activity by removing phosphofructokinase from melanoma cells, accompanied by reducing the level of glucose and fructose 1, 6-phosphatase, with ATP. Niosomesthe concept of targeted drug delivery is designed for attempting to concentrate the drug in the tissues of interest while reducing the relative concentration of the medication in the remaining tissues. As a result, drug is localised on the targeted site.Different carriers have been used for targeting of drug, such as immunoglobulin, serum proteins, synthetic polymers, liposome, microspheres, erythrocytes and niosomes. The optimization of drug delivery through human skin is important in modern therapy. Clearly, the topical route of drug delivery for treating diseases offers an attractive alternative to the conventional drug-delivery methods of oral administration/injection, and it is becoming a most innovative research area in drug delivery. Niosomes are one of the promising drug carriers that have a bilayer structure and are formed by self-association of non-ionic surfactants and cholesterol in an aqueous phase. Niosomes are biodegradable, biocompatible, and non-immunogenic. They have long shelf life, exhibit high stability, and enable the delivery of drug at target site in a controlled and/or sustained manner. In recent years, the potential of niosomes as a drug carrier has been extensively studied. Various types of non-ionic surfactants have been reported to form niosomes and enable the entrapment of a large number of drugs with a wide range of solubility. The composition, size, number of lamellae, and surface charge of niosomes can be varied and optimized to enhance the performance of niosomes for drug delivery [5].Encapsulation of various anti-neoplastic agents in this carrier vesicle has minimised drug-induced toxic side effects while maintaining, or in some instances, increasing the anti-tumour efficacy. Doxorubicin, the anthracycline antibiotic with broad-spectrum anti-tumour activity, shows a dose-dependent irreversible cardio-toxic effect. Niosomal delivery of this drug to mice bearing S-180 tumour increased their life span and decreased the rate of proliferation of sarcoma. It has good control over the release rate of drug, particularly for treating brain malignant cancer [6] due to the presence of non-ionic surfactant and the lipid, there is a better targeting of drug(s) to tumour, liver and brain. Thus, they are useful in targeting of the drug for treating cancers, parasitic, viral and other microbial diseases more effectively [7].Niosomes have more advantages over the liposomes, such as entrapment of more substances, variable purity of phospholipids and high cost and needlessness of handling or storing in special conditions, and the availability as well as inexpensiveness of prepared materials. [8].in an aqueous system, liposomes have problems regarding degradation by hydrolysis of phospholipids molecules. The chemical stability as well as the relatively low cost of the materials used to prepare niosomes makes this vesicle more attractive than liposomes for industrial productions both in pharmaceutical and cosmetic applications [9].

MATERIAL AND METHOD

Preparation of niosomes (Thin film hydration method)

An accurately weighed surfactant and cholesterol were dissolved in chloroform and placed into a round bottom flask the required amount of drug were added in an optimized surfactant: cholesterol ratio as per batch size, then the organic solvent was removed by applying a vacuum. The temperature of the bath was set at 60°C and flask was rotated at 160 rpm until smooth film was formed. Film was removed from the round bottom flask using rotary evaporator equipment and put aside for 12 hrs. to remove traces of an organic

solvent. Then, hydration offilm was performed with an optimized Vol^m of 7% ethanol, at above the lipid-transition temp of surfactant. Niosomes were formed observe under an optic microscope.

Preparation of polymeric liposomal formulation

The prepared niosomal formulation was coated by Carrageenan in a final concentration of 1%, 1.5% and 2% w/w (formulation code N2, N3 and N4). Carrageenan was dissolved slowly in 100ml of demineralized water for 1 hour to avoid agglomeration. This gel was then directly added drop wise to the niosomal formulation by gentle stirring. The resulting translucent semisolid preparation was stored in suitable vessel.

Vesicular size determination

Motic Digital Microscope was used to characterize the vesicles size. Briefly, 2 ml of niosomal dispersion was placed over the clean slide and covered with a coverslip. The microscopic characterization of niosomes was examined at the magnification of (×40) using calibrated eyepiece micrometer. The images were recorded using Motic Image plus 2.0 ML software, accompanying with the instrument.

Percent entrapment efficiency (%EE)

Purification of paclitaxel was done by the ultracentrifugation method, to quantify the amount of entrapped paclitaxel, 2ml of vesicular dispersion was centrifuged at 10,000 rpm for 1 hr. at controlled temperature of 40^0C REMI cooling centrifuge, (REMI Elektrotechnik limited India) precipitate contain entrapped drug was withdrawn and measured UV spectroscopically at λ_{max} 230 nm against 30:70 ratio of methanol and phosphate buffer solution (pH 7.4)

A calibration graph was produced by diluting stock solution of PTX with against 30:70 ratio of methanol and phosphate buffer solution pH 7.4 [14] (Tatode et al., 2017). Percent entrapment efficiency (% EE) was calculated and expressed as a % of the available dissolved solute actually encapsulated the amount of drug entrapped in liposomes was determined by% entrapment efficiency (EE) = [total drug – diffused drug/total drug] ×100

***In vitro* release study**

In vitro release study of paclitaxel from prepared niosomes and polymeric niosomal formulation was carried out as per the procedure described by [10] with little modifications. In brief, the Franz diffusion cell apparatus was employed for this study. The apparatus consists of donor and receptor compartment, with an effective surface area for dissolution was (2.54 cm^2). Were employed and pre-treated as per the directions were given by the manufacturer. After proper pre-treatment, the membrane was cut into desired size and shape, then mounted between the effective surface areas of D & R compartment. The PTX dispersion (1ml) was placed over the membrane, accompanied by the addition of phosphate buffer (10ml, pH 7.4) as dissolution media in the R compartment. The contents of the R compartment were stirred at 100 rpm using a magnetic stirrer at 37°C. At specified time intervals, 1 ml aliquots were withdrawn from sampling port of apparatus, diluted suitably with fresh media and the abs of the resulting solution was read at 230 nm using UV- spectrophotometer [10].

Rheological evaluation

pH Measurement:2.5 g of formulation was accurately weighed and dispersed in 25 ml of distilled water. The pH of the dispersion was measured by using a digital pH meter [11].

Viscosity Measurement: Viscosity was determined by Brookfield programmable DV III ultra-viscometer. In the present study, 100g of gel was taken in a beaker and spindle no. 64 was rotated with an optimum speed of 30 rpm to measure the viscosity of the preparation.

Spreadability Study: The Spreadability of polymeric niosomal formulations was determined by using Spreadability apparatus. 1.0 g of gel sample was placed on the lower slide and upper slide was placed on the top of the sample. The Spreadability was determined by the formula

$$S = \frac{m \; M \times l}{t}$$

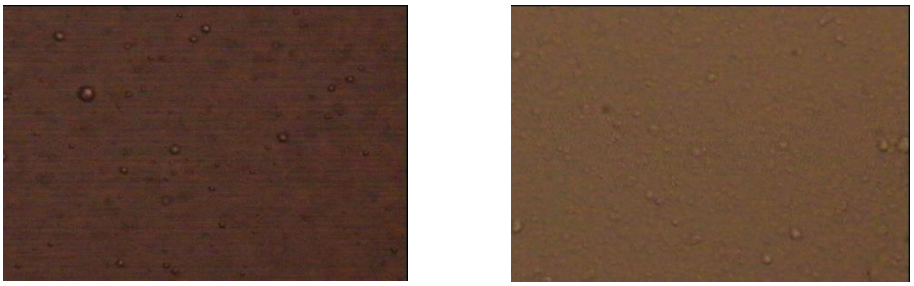
Where *S* is Spreadability, *m* is weight tied to upper slide, *l* is length travel by upper slide, and *t* is time [11].

Table no 1: Composition of Optimize liposomal formulation

Sr. No	Ingredients	Quantity (mg)
1	Paclitaxel	0.0085g
2	Span 80	0.0343g
3	Cholesterol	0.0156g

Fig 1: Franz diffusion cell

Figure 2: Motic Image of pure PTX niosomes (A) and PTX polymeric niosomes (B)



A and B

Table 2: Average vesicle size of pure PTX niosomes and polymeric niosomes

Formulation ratio	Size range (µm)	Area (sq.µm)	Perimeter (sq.µm)
N1	2.79 ± 1.07	17.44 ± 6.79	27.96 ± 20.56
N2	3.05 ± 0.96	19.13 ± 6.16	33.28 ± 21.29
N3	3.20 ± 0.96	20.07 ± 6.08	37.69 ± 20.76
N4	3.50 ± 1.09	18.42 ± 6.86	37.64 ± 21.30

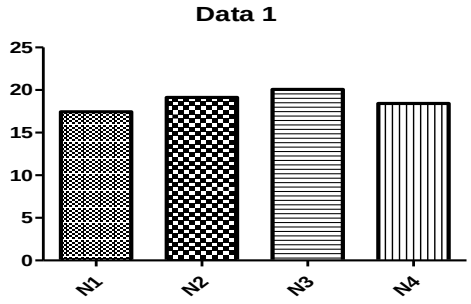


Figure 3: Average vesicle size of PTX niosomes and polymeric niosomes

Table 3: Calibration data for PTX at 230 nm

Sr. No.	Concentration (µg/ml)	Absorbance
1.	1	0.0211
2.	2	0.0606
3.	4	0.1565
4.	6	0.2691
5.	8	0.3565
6.	10	0.4967
7.	12	0.6090
8.	14	0.6893
9.	16	0.7968
10.	18	0.8878
11.	20	0.9728
REGRESSION EQUATION		Y = 0.052X + 0.034
CORRELATION COEFFICIENT		0.997

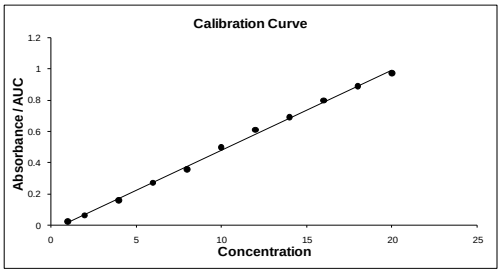


Figure 4: Calibration curve for PTX at 230 nm

Table 4: Percent *In vitro* drug release of PTX loaded niosomal formulation

Time (Hrs)	Formulation Code							
	N1		N2		N3		N4	
	% DR	± SD	% DR	± SD	% DR	± SD	% DR	± SD
1	14.15	2.60	12.42	1.55	12.12	3.75	8.55	1.80
2	25.50	3.07	24.85	2.33	24.51	3.89	15.56	2.66
3	35.40	3.12	32.40	3.01	29.99	1.79	19.47	2.90
4	38.80	2.15	38.58	2.15	36.79	3.56	25.25	3.88
5	45.12	3.11	46.15	2.04	41.90	3.50	25.56	3.90
6	48.10	2.69	42.38	2.80	43.45	2.50	30.21	2.54
7	51.45	1.20	50.26	2.50	47.84	1.98	34.45	2.98
8	52.03	3.40	52.47	1.90	47.21	1.90	36.90	2.70
9	57.14	2.25	55.70	0.46	50.81	1.88	35.47	2.20
10	55.27	1.40	56.45	3.78	53.48	5.04	41.20	2.40
11	61.13	1.70	58.22	2.02	55.97	3.40	42.27	2.08
12	62.78	1.90	66.12	1.14	57.64	3.30	45.27	3.08

Data are mean %DR ± SD (n=3)

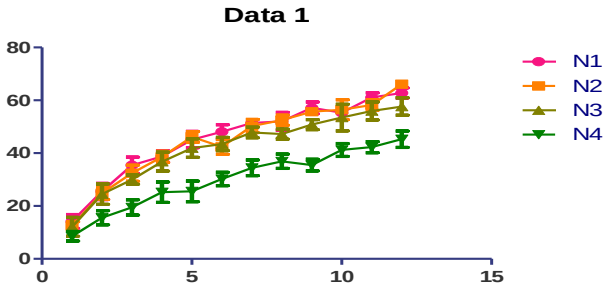


Figure 5: percent in vitro drug release of ptx niosomal formulation

Sr.No	Formulation	pH
1	N2	4.38 ± 0.36
2	N3	5.12 ± 0.16
3	N4	4.74 ± 0.96

Data are mean pH ± SD (n=3)

Table 6: Viscosity of polymeric niosomal formulation

Sr. No.	Formulation	RPM	poise	% VISCOSITY
1	N2	30	54.64	45.5%
2	N3	30	76.10	63.2%
3	N4	30	174.00	87.0%

Data are mean %Viscosity ± SD(n=3)

Table 7: Spreadability of polymeric niosomal formulation

Sr. No.	Formulation Code	Spreadability
1	N2	1.667 ± 1.34
2	N3	2.084 ± 1.06
3	N4	1.785 ± 1.09

RESULTS AND DISCUSSION

Vesicle Size

The size of 20 niosomes was measured using an optic microscope with calibrated eyepiece micrometer to determine diameter of each individual particle &expressed as average diameter. The coating of niosomes by carragenan polymer was confirmed by microscopic evaluation. The coating of niosomes by carragenan resulted in a marginal increase in the size of niosomes by a coating layer of polymer. The existence of carragenan surrounding the niosomes was visualized on the surface of carragenan coated niosomes (fig. 2 B).

Percent entrapment efficiency (%EE)

Entrapment efficiency is an important parameter for niosomes to provide their usage as drug carriers. The entrapment efficiency (%EE) of optimize PTX niosomal formulation was found to be 82.31 ± 2.91 %. The percent entrapment efficiency (%EE) of niosomes was depending on the ratio between surfactant and cholesterol. Formulation containing (2:1) molar ratio of span 80 and cholesterol shows maximum entrapment efficiency this might be due to the fact that span 80 has longest alkyl chain length. The thickness of the niosomes bilayers, the drug solubility in water and the compatibility between the drug and niosomes material was also responsible for percent entrapment efficiency.

In vitro release study

Calibration curve- UV scanning based on the spectrophotometric scanning of paclitaxel (stock solution 100 ppm) the maxima was obtained at 230 nm in methanol: phosphate

buffer pH 7.4 (30:70 ratio). Standard calibration of paclitaxel was prepared for 1-20 µg/ml concentration in methanol: phosphate buffer pH 7.4 at 230nm (λ_{max}) value. The figure of absorbance v/s concentration was plotted and data was subjected to linear regression analysis

Percent *In vitro* drug release (%DR)

Particle size ranges less than 5 µm are required for passive delivery of drug through skin. The release profile of paclitaxel niosomes are shown in Figure 5 and % release of paclitaxel niosomes form the formulation at the end of 12 hours were given in the Table 4.The highest % of drug release (62.78 ± 2.80) was obtained with niosomal formulation having formulation code (N1). When the niosomes were gelified (formulation code N2, N3, and N4) by different concentration of carragenan (1.0%, 1.5% and 2.0%) the percent in vitro release were found to be range(66.12 ± 0.46 to 45.27 ± 3.08) which is lower than the pure niosomes. Percent paclitaxel release from polymeric niosomal formulation was slightly decrease in the concentration of polymer was increases up to 1.5%. Further increasing concentration of the concentration up to 2.0% showed more decline in drug release that is 43.20 ± 3.08 %. Our results are in agreement with those of previous investigation into the effect of surface coating with polymers to preserve liposome stability [12].

Rheological characterization

pH: The pH value of the polymeric niosomal formulation was found to be in the range of 4.38 ± 0.36 to 4.74 ± 0.96. pH value was slightly increased as the conc. of carragenan increases.

Viscosity (spindle no. 64): The viscosity of the gel was found to increase with the increase in concentration of polymer

Spreadability of polymeric niosomal formulation Better Spreadability and retention capabilities of polymeric niosomes (N3) as in compare to formulation N2 and N4. Applicability of niosomes was also improved by coating of niosomes

SUMMARY AND CONCLUSION

The majority of anticancer drugs has poor aqueous solubility, produce adverse effects in healthy tissue, and thus impose major limitations on both clinical efficacy and therapeutic safety of cancer chemotherapy. To help circumvent problems associated with solubility, most cancer drugs are now formulated with co-solubilizes. *In vivo* experiments demonstrated an interesting correlation between the better permeation capabilities of niosomes in comparison to other conventional dosage forms in terms of a better therapeutic efficacy at the affected site at lower doses of drugs present in the niosomal gel formulation These polymer-based prodrugs are macromolecular carriers, designed to increase the aqueous solubility of antitumor drugs, can enhance bioavailability. Additionally, polymer-based prodrugs approach exploits unique features of tumor physiology to passively facilitate intratumoral accumulation, and so improve chemodrug pharmacokinetics and pharmacological properties.Niosomal gels increased the therapeutic index of a drug leading to a reduction in dose.Due to maximum retention at the skin, the niosomal gel can maintain the at the target site for prolonged periods of time due to a niosomal “depot mechanism” Based on the above data, it can be concluded that the Nano vesicle Coated with polymer (i.e., niosomes)-based dosage forms developed here would have a better therapeutic efficacy at a lower dose in comparison to conventional dosage forms. **Conclusion is** Poor solubility and selectivity of anti-cancer drugs limits efficacy of the current cancer treatments. Conjugating drugs to niosomes coated polymer improves drug solubility, pharmacokinetic and pharmacodynamics, as well as increasing their tumor specificity. Thus, polymers provide a remarkable tool through which to increase the therapeutic efficacy of drugs. Despite decades of significant progress in polymer-based drug delivery, the number of FDA approved polymer-based drugs remains very small. Major objectives in designing new polymer based prodrugs include, Improving solubility of the drugs in aqueous solution or lipids; Conjugating the drug to the polymer without decreasing its potency; Binding the drug tightly to a polymer in circulation, but releasing it inside the tumor cells; andEnsuring that the polymer is non-immunogenic, biodegradable and can be cost-effectively produced in sufficient quantities. Successfully addressing

these challenges will yield novel and efficient polymer-based prodrugs and establish their significant role in current cancer therapy.The coupling of antitumor drugs with polymeric drug carriers, whether synthetic, natural or genetically engineered, can help overcome agent insolubility, rendering cancer treatment more effective. These polymers can potentially provide vehicles through which to improve application and target delivery of small, poorly water-soluble chemo drugs so as to improve therapeutic effectiveness and reduce the toll of treatment for cancer patients.

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