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Formulation and Evaluation of Lyophilized Nanocochleates of Paclitaxel – For Cancer Chemotherapy

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ABSTRACT

The nanocochleates are mainly prepared from the liposomes of active pharmaceutical ingredients (APIs) with the help of inactive ingredients like phosphatidylserine (PS) and calcium. The PS shows a considerable binding affinity towards calcium mainly due to the calcium's tendency to lose part of its hydration shell and to displace water upon complexation. Due to neutralization of the electrostatic charge, calcium causes aggregation & fusion of liposomes composed PS with each other. It has been reported that ca^{++} has an affinity to form a more tightly packed & highly ordered and less hydrated structure than mg^{++} with phosphatidylserine, even at a much lower concentration. It is well documented that ca^{++} plays a vital role in natural membrane fusion phenomena while other cations are ineffective in most such systems. The stability of Nano-cochleate can be increased by doing freeze-drying of finished product which in turn provides a longer shelf life to the product at, where solution form is most unstable. Based on various literature survey and laboratory work, 'The Ethanol Injection Method' was selected for the preparation of Nanoliposomes followed by Nanocochleates. The developed Nanocochleates exhibited higher encapsulation efficiency and controlled release of Paclitaxel. This technology may bring about a reduction in dose as well as cost and increase patient compliance. These Nanocochleates could therefore advantageously be employed to improve anticancer activity of Paclitaxel and an alternative to present intravenous administration. From the present study, it can be concluded that the developed Nanocochleates exhibited higher encapsulation efficiency and controlled release of Paclitaxel which may prove to be a suitable & potential candidate for healing a Cancer at a lower concentration.

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INTRODUCTION

Nanotechnology is a multifaceted area of science and has immense progress due to its applications in recent decades. Looking at the investment of multiple pharma companies in R&D, it is observed that the US, Japan and Germany dominate the current research and development efforts in nanotechnology. Nanotechnology is an exciting field of research and there is growing interest to formulators as it yields high drug loading, good drug stabilization, smaller sized carriers, increased solubility, better tissue targeting, lesser side effects and extended

drug release at lower doses (Nadaf and Killedar, 2015; Sankar and Reddy, 2010).

The polymer-based nanoparticles for insulin were developed to improve their oral bioavailability. The self-micro emulsifying drug delivery systems were developed to overcome the problems of poor solubility and bioavailability. The nanoemulsion was developed to improve transdermal delivery of celecoxib.

The nanostructured lipid carriers were developed to enhance oral bioavailability of raloxifene. The PEGylated liposomal nanoparticles were developed to enhance the anticancer activity of 5-fluorouracil against colorectal tumour growth (Egan and Lauri, 2002; Sudhamani *et al.*, 2013).

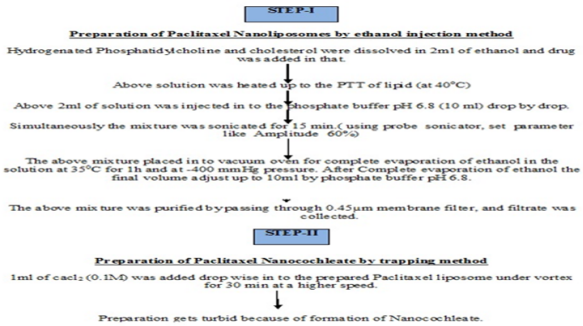


Figure 1: Schematicre presentation of preparation of Paclitaxel Nanocochelate

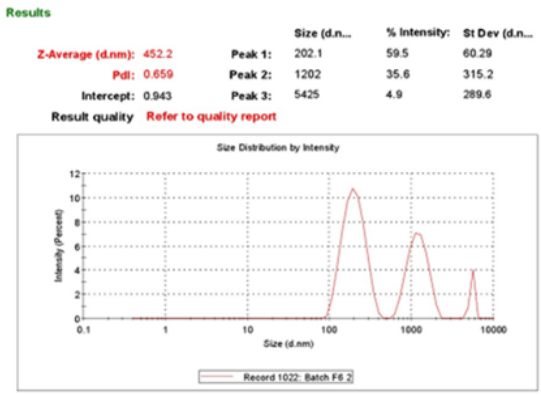


Figure 2: Result of particle Size (Batch F6 PTXNC)

Paclitaxel is an antineoplastic agent with a proven activity against a many types of tumors; it was patented in 1986 and approved for medical use in 1995. Paclitaxel is available as a generic medication in the market. It is on the world health organization list of essential medicines, the most effective and safe medicines needed in a health system.

However, its low bioavailability is a major problem in its development (Sadukhan *et al.*, 2014; Ochekpe *et al.*, 2009).

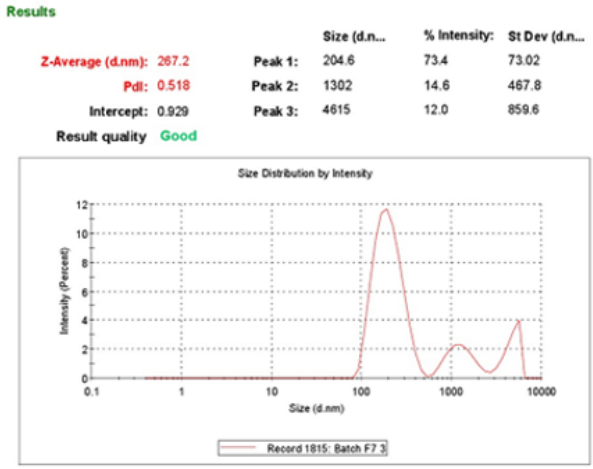


Figure 3: Result of particle size (Batch F7 PTXNC)

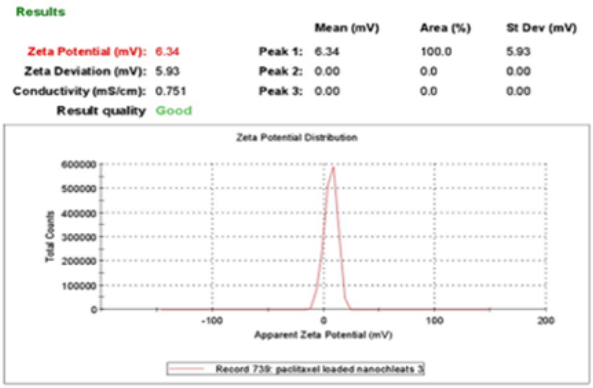


Figure 4: Result of zeta potential (Batch F6 PTXNC)

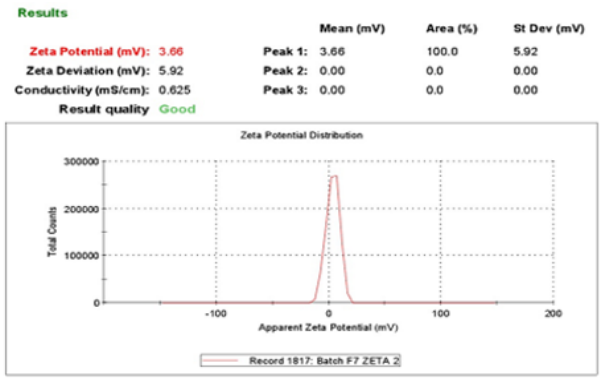


Figure 5: Result of zeta potential (Batch F7 PTXNC)

Table 1: Design of preliminary batches for selection of Phospholipidand Sterol Ratio for the formulation of liposomes

Batch Numbers	L1	L2	L3
Drug	5mg	5mg	5mg
Solvent (Ethanol)	2ml	2ml	2ml
HSPC: CH	1:1	2:1	3:1
Hydration medium	10 ml PB pH 6.8	10 ml PB pH 6.8	10 ml PB pH 6.8

Table 2: Design of Trial Batches for liposomes Formulation

Batch	F1	F2	F3	F4	F5	F6	F7
Drug	10mg	10mg	10mg	10mg	10mg	10mg	10mg
Solvent(Ethanol)	2ml	2ml	2ml	2ml	2ml	2ml	2ml
HSPC: CH	1:1	2:1	3:1	3.7:1	5:1	6.3:1	7.5:1
Hydration medium (PB pH6.8)	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml

Table 3: Preparation of Nanocochelates

Batch	F6 PTXNC	F7 PTXNC
Drug	10 mg	10 mg
Solvent (Ethanol)	2 ml	2 ml
HSPC: CH	6.3:1	7.5:1
Hydration medium PB pH 6.8	10 ml	10 ml
1 ml 0.1M Cacl2 solution	1 ml	1 ml

Table 4: Process Parameter For Lyophilization

Drying Parameter	Parameter Time	Temperature	Pressure
Freezing	Two h	-200C	-
Primary Drying	Six h	-760C	0.0010m Bar
Secondary Drying	Four h	-780C	0.0010m Bar

Table 5: Physical Stability of Preliminary Batches

Batch No.	Observation	Inference
L1	Thin white solution.	The clear white solution forms without any precipitate, which maybe indicates the formation of Nanoliposomes.
L2	Thin milky white solution	The somewhat thin milky white solution is formed because of the increasing concentration of lipid.
L3	Thin milky white solution	The somewhat thin milky white solution is formed because of the increasing concentration of lipid.

Table 6: Outcome of Trial Batches

Batch	Outcome	Conclusion
F1	It results in very low entrapment efficiency.	The batch is rejected because of low entrapment efficiency; this is due to the low amount of drug and HSPC: CH.
F2	It shows the low entrapment efficiency	The batch is rejected because of low entrapment efficiency; this is due to an insufficient amount of drug for the ratio of HSPC: CH.
F3	It shows the low entrapment efficiency	The batch is rejected because of low entrapment efficiency.
F4	It shows the low entrapment efficiency	The batch is rejected because of low entrapment efficiency.
F5	It shows the low entrapment efficiency	The batch is rejected because of low EE
F6	It results in good entrapment of drug & in-vitro drug release	The batch is accepted.
F7	It results in good entrapment of drug & in-vitro drug release	The batch is accepted.

Table 7: Optimization of preliminary batches of Liposomal Formulation

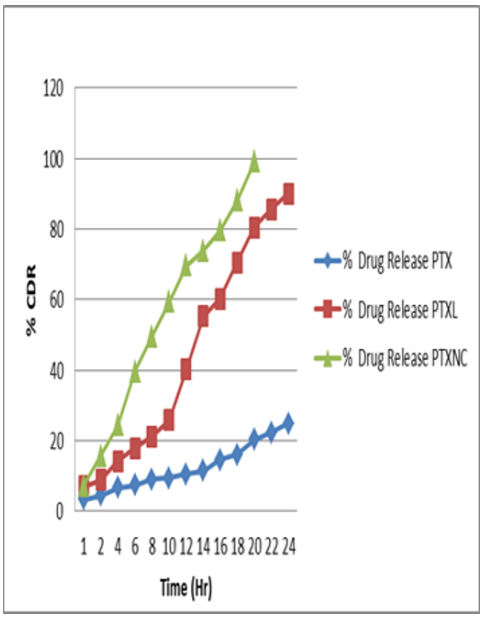
Batch	% Entrapment Efficiency
L1	20.5
L2	25.9
L3	30.2

Table 8: Optimization of liposomal Formulation

Batch	% Entrapment Efficiency
F1	35.2
F2	42.5
F3	46.7
F4	50.7
F5	55.2
F6	62.2
F7	72.8

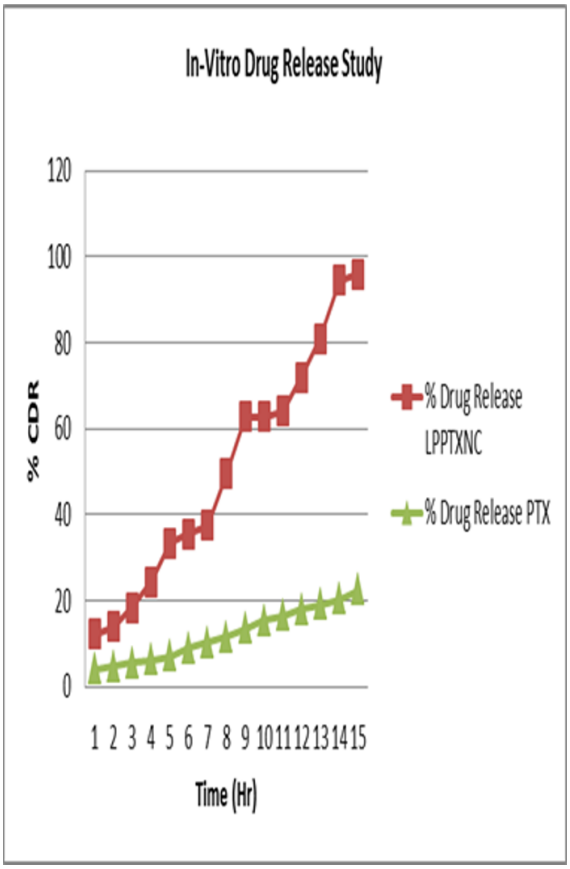
Table 9: Drug Release profile Data of Liposomes Batch F6, F7 and Plain Drug

Time (Hr)	% CDR		
	F6	F7	Plain Drug
1	6.85	7.10	3.37
2	8.53	8.82	4.50
4	13.78	14.19	6.65
6	17.37	17.85	7.50
8	20.67	21.20	9.10
10	25.23	25.79	9.5
12	39.13	40.15	10.5
14	50.1	55.2	11.5
16	60.1	60.3	14.6
18	65.4	70.5	16.1
20	80.5	85.6	20.4
24	90.5	96.0	25.0



Time (h)	% CDR	
	Batch F6 (PTXNC)	Batch F7 (PTXNC)
1	7.6	7.23
2	14.12	15.73
4	20.99	24.42
6	26.05	39.58
8	34.95	49.52
10	43.64	59.36
12	54.6	69.56
14	64.32	73.49
16	69.53	79.65
18	78.36	87.96
20	86.25	99.05
22	90.63	-
24	98.02	-

Figure 6: In- vitro release profiles of Paclitaxel, Paclitaxelliposomes and Paclitaxel nanocochleates



Time (h)	%CDR	
	Lyophilized powder	Plain drug
	12.3	4.05
1	13.9	4.72
2	18.4	5.62
3	24.5	6.3
4	33.5	7.2
5	35.7	9.0
6	37.5	10.35
7	49.5	11.7
8	62.7	13.5
9	62.77	15.4
10	64.00	16.6
11	72	18
12	81	19.12
13hr	96	22.5

Figure 7: In-vitro drugrelease profiles of Paclitaxel , Lyophilized powder of paclitaxel nanocochleates

Table 10: Optimization of Nanocochleate Formulation

Batch	Vesicle size (nm)	Entrapment efficiency (%)	Zeta potential (mV)	PDI	pH
F6 PTXNC	452.2	72.5	6.34	0.659	6.8
F7 PTXNC	267.2	87.7	3.66	0.518	6.8

Table 11: Solubility Study of lyophilized powder of paclitaxel nanocochleates

Formulation	Solubility
Plain drug	0.0055 μg/ml
Lyophilized powder	600 μg/ml

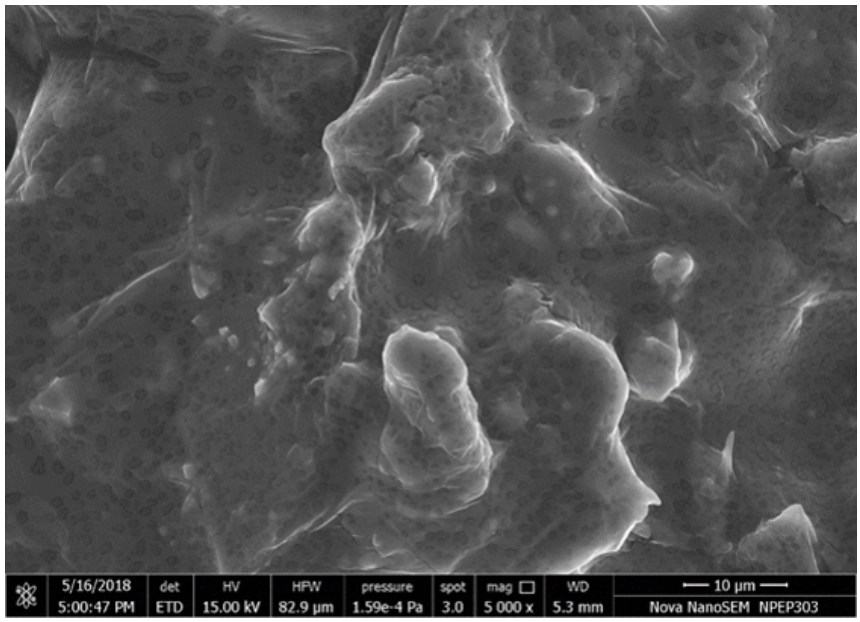


Figure 8: SEM image of Lyophilized of Nanocochleates. (Batch F7 PTXNC)

Several researchers formulated paclitaxel into various delivery systems such as mixed micelles, nanoparticles, nano-micelles, nano-capsules and self-emulsifying drug delivery system in order to improve its safety and efficacy. Considering the potential of Nanocochleates for improvement of molecular pharmaceutical properties of therapeutic agents, the present investigation involved the development of Paclitaxel-loaded Nanocochleates and its evaluation by *in-vitro* techniques. I had prepared Paclitaxel loaded Nanocochleate by the trapping method. Formulation of Nanocochleates involved two steps (Figure 1). The first step involved the preparation of paclitaxel liposome. I had prepared paclitaxel liposomes by Ethanol injection method. And the second step involved preparation of paclitaxel Nanocochleate. I had prepared paclitaxel loaded Nanocochleates by trapping method (Webster, 2007; Maroof and Zafar, 2015).

The batches were selected on the basis of trial and error. The different ratios of Hydrogenated soya phosphatidylcholine: cholesterol was used (6.3:1,7.5:1) for F6, F7. The entrapment efficiency for batch F6, F7, was found to be 62.2%, 72.8 %, respectively. On the basis of entrapment efficiency F6, F7, a batch was selected for *In-vitro* drug release study. These two batches were selected for the preparation of Nanocochleate on the basis of their entrapment efficiency and In-Vitro drug release. The batch F6 (PTXNC) showed the particle size of 452.2nm with entrapment efficiency of 72.5% study, whereas batch F7 (PTXNC) showed a particle size of 267.2nm with entrapment efficiency 87.7%. Both batches of Nanocochleate showed the value of zeta potential 6.34 mV and 3.66 mV, respectively. The batch F7(PTXNC) showed good particle size and entrapment efficiency as compared to batch F6(PTXNC). The batch F7 subjected to lyophilisation (Pawar *et al.*, 2015; Zarif, 2005).

MATERIALS AND METHODS

Materials

Paclitaxel was obtained as a gift sample from Glenmark Pharmaceuticals Ltd, Sinnar, and Nasik, India. The inactive ingredients like Hydrogenated Soya Phosphatidylcholine (HSPC) & Cholesterol (CH) were obtained from Glenmark Pharmaceuticals Ltd as a gift sample. Sodium Dihydrogenorthophsphate, Sodium Hydroxide & Calcium chloride were taken from Lobachemie. Solvent Ethanol was also taken from Lobachemie Pvt. Ltd. Mumbai as a gift sample.

Method

Paclitaxel Nanocochleate

Different trials were performed for making Paclitaxel Nanocochleate using different permutation combination & ratios of HSPC & CH (Harkare *et al.*, 2013; Anwekar *et al.*, 2011).

Selection of Phospholipid and Sterol Ratio

For the preparation on regular, uniform and stable Nanocochleate formulation it is necessary to select the proper ratio of the Phospholipid and Sterol, it was determined by taking ratio such as 1:1 and 1:2 and 1:3 likewise by taking further given below in table and selected on the basis of a literature survey to form stable and uniform vesicles, having good entrapment efficiency (EE) and zeta potential. This method is based on trial and error (Pai *et al.*, 2015; Immordino *et al.*, 2003).

Prototype formulation trials to optimise the ratio of Phospholipid and Sterol to achieve adequate liposomes required for preparation of Nanocochleate

With the objective of loading the maximum amount of drug into the lipid bilayers to achieve the optimum value of zeta potential, and to achieve the nano size of vesicles, the Nanoliposomes was prepared by taking a different concentration of lipid and cholesterol. Trials were designed based on a literature survey and preliminary trials at the laboratory (Table 1).

The phospholipids alone were used to form lipid bilayer vesicles as they are not soluble in aqueous media where they align themselves in planar bilayer sheet and minimize unfavourable interaction between the bulk aqueous phase and long fatty acid chain. The cholesterol was used in the preparation of Nanoliposomes for to maintain the integrity of the structure and also to stabilize the membrane in the presence of biological fluid such as plasma. The solvent (ethanol) is used to solubilise the drug and other components, and the phosphate buffer is used as hydration medium (Papahadjopoulos *et al.*, 1975; Yeole and Pimple, 2013).

Factors affecting the Nanoliposome Quality

1. The ratio of cholesterol to lecithin (w/w),
2. The concentration and physiological properties of drug,
3. Hydration temperature

Design of experiments of Liposomes formulation

With the objective of loading the maximum amount of PTX into the lipid bilayers, The Nanoliposomes were prepared bearing increasing amount of PTX (5, 10 mg) for different conc. of lipid as shown in Table 2 (Yousefi, 2009; Bhosale *et al.*, 2013).

Characterization of Liposomal formulation

Drug entrapment efficiency

The encapsulation efficiency of nanoliposomes was determined by separating non-encapsulated PTX from PTXNL suspension by centrifugation at 5000 rpm for 15 min at room temperature. The sediment nanoliposomes were disrupted with ethanol to release the entrapped drug; suitably diluted with phosphate buffer pH 6.8 and the absorbance measured at 230 nm to calculate the encapsulation efficiency using the calibration curve equation y = 0.04x + 0.0032. Here, 'y' is the measured absorbance and 'x' is the concentration of PXT in mg/mL. The percentage encapsulation efficiency was calculated using the following Equation (1).

Encapsulation efficiency %

$$= \frac{Total\ drug - free\ drug}{Total\ drug} \times 100 \qquad (1)$$

Optimization of liposomal formulation

Drug entrapment efficiency

To perform the drug entrapment efficiency study, the above-mentioned procedure was followed and calculation was done by the same equation (Yousefi, 2009; Immordino *et al.*, 2003).

In vitro drug release study

The *in-vitro* release of Paclitaxel from the liposomes was carried out in phosphate buffer saline (PBS, pH 6.8) using a dialysis bag diffusion technique. Formulation equivalent to 2 mg Paclitaxel solution was introduced into a dialysis bag hermetically sealed and immersed into 40 ml of release medium (Phosphate buffer pH 6.8). The entire system was kept at 37 $\pm$ 0.5^{o}C with continuous magnetic stirring at 1500 rpm/min.

At selected time intervals; the sample was removed and replaced with an equal volume of fresh medium in order to maintain the sink conditions.

The absorbance of Paclitaxel in the solution was determined using the double beam UV-Vis spectrophotometer (Yousefi, 2009; Lyseng-Williamson and Fenton, 2005).

Preparation of Nanocochleates

The optimized batches F6 and F7 are shows good entrapment efficiency and *in vitro* drug release study these two batches are selected for the preparation of Nanocochelates coded as F6PTXNC and F7PTXNC. The nanocochleates are prepared by trapping method.

Preparation of Paclitaxel Nanocochleates by the trapping method.

1 ml of $Cacl_2$ (0.1M) was added dropwise into the prepared Paclitaxel liposome under vortex for 30 min at a higher speed (Table 3).

Characterization of Nanocochleates Formulation

Determination of Encapsulation efficiency

To perform the drug entrapment efficiency study, the above-mentioned procedure was followed and calculation was done by the same equation (Yousefi, 2009; Immordino *et al.*, 2003).

***In vitro* drug release study**

The above-mentioned procedure was followed to perform the *in vitro* drug release study (Yousefi, 2009; Lyseng-Williamson and Fenton, 2005).

Optimization of Nanocochleate Formulation

Determination of particle size

Nanocochleate formulation (1 ml) was diluted with 10 ml deionized water in a beaker with constant stirring using a glass rod. The resultant solution was then subjected to particle size analysis.

The droplet size so formed determined by Dynamic light scattering (DLS) technique using a zeta sizer (Nano ZS, Malvern Instruments, UK) (Yousefi, 2009; Lyseng-Williamson and Fenton, 2005).

Measurement Condition

Temperature: 25.0 (°C)

Diluent Name: Water

Determination of encapsulation efficiency

To perform the drug entrapment efficiency study, the above-mentioned procedure was followed and calculation was done by the same equation (Yousefi, 2009; Immordino *et al.*, 2003).

Zeta Potential Determination

The Zeta potential of the selected formulation was determined by laser diffraction analysis using particle size analyzer (Malvern Zetasizer Nano Series ZS 90). The samples were diluted with a ratio of 1:100 (v/v) with distilled water and mixed for 1 min using a magnetic stirrer. All studies were repeated in triplicate.

Lyophilization of Optimized Batch of Nanocochleates

The optimized Nanocochleates Suspension was further lyophilized. The mannitol (5% w/v Solution) was added in Nanocochleates suspension as a cryoprotectant so avoid lysis of Nanocochleates (Table 4). The Sample was first subjected to deep freezing in a deep freezer for two-hand then it was freeze-dried in a freeze dryer (Model: Martin Christ, Model – Alpha 2-4 LD Plus).

Evaluation of Lyophilized Powder of Nanocochleates

Solubility of Nanocochleates of lyophilized powder

Excess amount of nanocochleates lyophilized powder was added to 10ml water, sonicated for three h. The suspension was filtered through a 0.45 μm filter and diluted with water, and analysed to determine concentration by UV-spectrophotometer at 230 nm.

***In-vitro* Drug Release Study of Lyophilized Nanocochleates**

The *in-vitro* release of paclitaxel from the nanocochleates was carried out in phosphate buffer pH 6.8 by dissolution study. Formulation Equivalent to 10mg paclitaxel lyophilized powder was added into 900ml release medium (phosphate buffer pH 6.8). The entire system was kept at $37 \pm 0.5\ ^0$C with continuous stirring at 50 rpm/min at selected time intervals; the sample was removed and replaced with an equal volume of fresh medium in order to maintain the sink condition. The absorbance of paclitaxel in the solution was determined using UV-Vis spectrometer (Musumeci *et al.*, 2006).

Determination of Surface Morphology

Scanning electron microscopy was employed to determine the shape and surface morphology of the produced nanocochleates (Kumar *et al.*, 2010).

RESULTS AND DISCUSSION

Formulation and Development

Physical stability of preliminary batches

Physical stability determined visually, the formulation was check visually for particles of drug any kind

of phase separation, drug precipitation, change in physical state, change in colour, change in thickness of solution (Table 5).

Milky white solution was formed by using an ethanol injection method. No any signs of change in appearance, phase separation, drug precipitation, change in physical state, change in colour, change in thickness of solution.

Result of preliminary batches for selection of method

On the basis of the literature survey, preliminary trials and their results it was concluded that, by taking the concentration of drug same (5mg) and changing the cholesterol and lipid ratio the batches were designed. The amount of cholesterol was taking the same in all batches because it is used to maintain the fluidity of the vesicle structure.

The results show that the increasing lipid concentration leads to increasing entrapment efficiency of drug the batch L3 shows good entrapment efficiency and as compare to L1 and L2. So, the further trials batches are designed to improve the entrapment efficiency.

Optimization of liposome formulation

Outcome of trial batches

On the basis of visual inspection and the study of Entrapment efficiency and *in-vitro* drug, the release was reported in Table 6 that explained the region of the batch rejection on the basis of percentage entrapment efficiency and the In-vitro drug release of liposomes batch.

On the basis of the above trials, the batch F6 and F7 shows the good entrapment efficiency, and shows good *in-vitro* Drug Release. These two batches of liposomes are subjected to the preparation of nanocochelates (Tables 7 and 8).

On the basis of results of trial batches, it was concluded that the entrapment efficiency increases with increasing conc. of the drug for the particular ratio of HSPC: CH. On the basis of percentage entrapment efficiency, the F6 and F7 liposomes batch subjected to the *in-vitro* Drug Release study.

***In-Vitro* Drug Release of Liposomes Batch F6 and F7 and plain Drug**

From the above results I had concluded that the Liposomes batch F6 and F7 show the good value of entrapment efficiency and In-Vitro Drug Release So, these two batches were selected for the preparation of nanocochleates (Table 9).

Optimization of Nanocochleate Formulation

After the Preparation of nanocochleates, the percentage entrapment efficiency of liposomes increases from 62.2% to 72.5% and 72.5% to 87.7% for the batch F6 and F7, respectively. These two batches have the particle size and zeta potential value batch F6 (PTXNC) and F7 (PTXNC) is 452.2 nm and 267.2 nm, 6.34 mV and 3.66 Mv, respectively (Table 10). Hence, Batch F6 (PTXNC) and F7 (PTXNC) were further studied for *in-vitro* drug release.

Characterization of Nanocochleates

Particle size determination

The vesicle size of the optimized formulation is given in Table 8.14. The optimized batch F6 (PTXNC) and F7 (PTXNC) were showing vesicle size of 452 nm and 267 nm shown in Figures 2 and 3, respectively.

The nanoformulation shows the particle size of between 1-1000nm. Batch F6 Paclitaxel loaded Nanocochleates showed particle size of 452.2 nm, which is desirable.

The Nanoformulation shows the particle size of between 1-1000 nm. Batch F7 paclitaxel loaded nanocochleates showed particle size of 267.2 nm. The batch F7 (PTXNC) showed good particle size as compared to batch F6 (PTXNC) .

Entrapment efficiency

The encapsulation efficiency expressed as a percentage of drug get entrapped and calculated through the following relationship as Equation (2).

Encapsulation efficiency %

$$= \frac{Total\ drug\ -\ free\ drug}{Total\ drug} \times 100 \qquad (2)$$

The entrapment efficiency of formulations, i.e. Batch F6 PTXNC and Batch F7 PTXNC were found to be 72.5% and 87.7% respectively.

The Entrapment efficiency of formulation of batch F6 PTXNC and F7 PTXNC were found to be 72.5% and 87.7%. The batch F7 PTXNC had good entrapment efficiency as compared to batch F6 PTXNC.

Zeta potential measurement

Zeta potential of Batch F6 PTXNC and F7 PTXNC were shown in Figures 4 and 5, respectively. ZP governs the degree of repulsion between adjacent or similarly charged and dispersed droplet, it shows the practical application in the stability. ZP-values of F6 PTXNC and F7 PTXNC were found to be 6.34 mV and 3.66 mV.

The zeta potential value shows the stability of the formulation. It should be between in range 0 ± 25. The Zeta potential values of batch F6 PTXNC and F7

PTXNC were found 6.34 mV and 3.66mV, which is good and both batches have good stability.

In-vitro drug release

In-vitro release of PTX from PTXL and PTXNC were studied by dialysis bag diffusion and compared with PTX dispersion. Figure 6 **,** revealed that paclitaxel dispersion showed only 25% release after 24 h. However, paclitaxel release from PTXL and PTXNC showed controlled release up to 24h.

By comparing both F6 (PTXNC) and F7 (PTXNC), it was found that the F7 (PTXNC) batch is optimized than F6 (PTXNC). The formulation batch F7 (PTXNC) has a particle size of 267.2 nm zeta potential in a range of 3.33 mV; drug entrapment efficiency 87.7% and also the F7(PTXNC) formulation shows Controlled release of Drug up to 24 h. The Batch F7 (PTXNC) subjected to lyophilisation.

Evaluation of Lyophilized Paclitaxel Nanocochleates

In-vitro Drug Release Study of Lyophilized Powder of Nanocochleates

In-vitro drug release of PTX from and LPPTXNC was studied by Dissolution Study and compared with PTX dispersion. Figure 7 **,** revealed that paclitaxel dispersion showed only 22% release after 13 h. However, paclitaxel release from LPPTXNC showed controlled release up to 13h.

Solubility Study of Lyophilized Powder of Paclitaxel Nanocochleate

On the basis of solubility of lyophilized powder, it was concluded that the solubility of lyophilized powder is greater than plain drug (Table 11).

Surface Morphology Study

On the basis of SEM, it was concluded that the nanocochleates was formed because of its tubular shape structure (Figure 8).

CONCLUSIONS

From the present study, we can conclude that the lyophilized Nanocochleate of paclitaxel can be considered as a potential drug product for use in cancer therapy based on further evaluation *of in-vivo* studies. The result of the various experiment showed enhances solubility & efficient release of drug product without disturbing its inherent nature during lyophilization.

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Conflict of interest

None.

REFERENCES

Anwekar, H., Patel, S., Singhai, A. K. 2011. Liposome-as drug carriers. International journal of pharmacy & life sciences. 2:945–951.

Bhosale, R., Ghodake, P., Mane, A., Ghadge, A. 2013. Nano-cochleates: A novel carrier for drug transfer. *Journal of Scientific and Innovative Research*, 2(5):964–969.

Egan, W. J., Lauri, G. 2002. Prediction of intestinal permeability. *Advanced Drug Delivery Reviews*, 54(3):273–289.

Harkare, B., Kulkarni, A., Aloorkar, N., Suryawanshi, J., Wazarkar, A., Osmani, R. 2013. Nanocochleate: a new cornucopia in oral drug delivery . *International Journal of Innovations in Pharmaceutical Sciences*, 2(4):1–9.

Immordino, M. L., Brusa, P., Arpicco, S., Stella, B., Dosio, F., Cattel, L. 2003. Preparation, characterization, cytotoxicity and pharmacokinetics of liposomes containing docetaxel. *Journal of Controlled Release*, 91(3):417–429.

Kumar, K. N., Raju, N. A., Raju, S. S., Ramarao, B., Mukkanti, K. 2010. Estimation of Docetaxel in Parenterals by RP-HPLC. *Asian journal of chemistry*, 22(10):7513–7531.

Lyseng-Williamson, K. A., Fenton, C. 2005. Docetaxel. *Drugs*, 65(17):2513–2531.

Maroof, K., Zafar, F. 2015. Scope of Nanotechnology in Drug Delivery. *Journal of Bioequivalence & Bioavailability*, 08(1):1–5.

Musumeci, T., Ventura, C. A., Giannone, I., Ruozi, B., Montenegro, L., Pignatello, R., Puglisi, G. 2006. PLA/PLGA nanoparticles for sustained release of docetaxel. *International Journal of Pharmaceutics*, 325(1-2):172–179.

Nadaf, S., Killedar, S. 2015. Novel Liposome Derived Nanoparticulate Drug Delivery System: fabrication and Prospects. *Creative Journal of Pharmaceutical Research*, 1(3):117–128.

Ochekpe, N., Olorunfemi, P., Ngwuluka, N. 2009. Nanotechnology and Drug Delivery Part 1: Background and Applications. *Tropical Journal of Pharmaceutical Research*, 8(3):265–724.

Pai, P., Shabaraya, A., Kamath, K. 2015. Preparation, Characterization and In-Vitro Release Study of Liposomes Loaded with Artemether. *Am. J. Pharma Tech Res*, 5(4):497–506.

Papahadjopoulos, D., Vail, W. J., Jacobson, K., Poste, G. 1975. Cochleate lipid cylinders: formation by fusion of unilamellar lipid vesicles. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 394(3):483–491.

Pawar, A., Bothiraja, C., Shaikh, K., Mali, A. 2015. An insight into cochleates, a potential drug delivery system. *RSC Advances*, 5:81188–81202.

Sadukhan, S., Bakshi, P., Maiti, S. 2014. Bio-Polymeric Nanomicell Carriers: A Promising Approach for the Delivery of poorly Water Soluble Drugs. *Journal of Advanced Pharmacy Education and Reaserch*, 4(1):41–53.

Sankar, V. R., Reddy, Y. D. 2010. Nanocochleate-a new approach in lipid drug delivery. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2(4):220–223.

Sudhamani, G., Aboobakkarsidhiq, K., Kumar, D. J., Ganesan, V. 2013. Nanocrystallization: A novel solubility enhancement technology for poorly water soluble drugs. *Int. J. Univers. Pharm. Bio. Sci*, 2(5):539–556.

Webster, T. J. 2007. IJN's second year is now a part of nanomedicine history! *International Journal of Nanomedicine*, 2(1):1–2.

Yeole, S. E., Pimple, S. S. 2013. A Reviews on Nanocochleate. A Novel Lipid Based Drug Delivery System. *Journal of Biomedical and Pharmaceutical Research*, 2(1):539–555.

Yousefi, A. 2009. Preparation and In Vitro Evaluation of A Pegylated Nano-Liposomal Formulation Containing Docetaxel. *Scientia Pharmaceutica*, 77(2):453–464.

Zarif, L. 2005. Drug Delivery by Lipid Cochleates. *In Methods in Enzymology*, 319:314–329.