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FORMULATION AND EVALUATION OF POLYMERIC MICELLES FOR A POORLY ABSORBED DRUG

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ABSTRACT

Many of the Drugs are having poor water solubility and usually associated with low bioavailability. Among different types of systems Micellear systems are having excellent property of Enhancing solubility and oral bioavailability. The main aim of the study is to formulate and evaluate the polymeric micelles of lipophilic drug paclitaxel using pluronic F-68 by sonication method. The Drug is having excellent antineoplastic activity but poor bioavailability and poor aqueous solubility. The prepared micelles were subjected to particle size, zeta potential, morphology, Encapsulation Efficiency, *in vitro* dissolution studies. The mean particle size was 100nm with PDI of 0.241 and zeta potential of +34mV. FTIR studies confirms that there is no interaction between Drug and Pluronic F-68. DSC studies showed that the drug was properly incorporated in to micelles. *In vitro* dissolution profiles shows 4 times more than that of pure paclitaxel. It was concluded that prepared polymeric micellar system acted as a better carrier for Paclitaxel and showed increased solubility.

KEYWORDS

Paclitaxel, Pluronic F-68, Zeta potential, FTIR, DSC and Micelles.

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INTRODUCTION

Paclitaxel (Taxol) is a diterpenoid derivative obtained from the bark of the Pacific Yew tree (Taxus brevifolia) which is an anticancerous agent for the treatment of various cancers including breast and ovarian cancers. Paclitaxel as a potent inhibitor of cancer cell replication is related to its ability to block cancer cells in the late G2-mitotic phase of the cell cycle by stimulating microtubule polymerization and suppressing their dynamics. Paclitaxel has very low water solubility and

clinically it is used as a solution in Cremophor EL/ethanol (1/1, w/w) To overcome these problems and increase paclitaxel bioavailability, many types of drug delivery systems, such as nanoparticles, liposomes, emulsions and various micelles, have been tried as pharmaceutical carriers for paclitaxel. Recently published data suggest that polymeric micelles may be of particular interest for delivery of sparingly soluble drugs including anticancer drugs. Micelles are spherical nanoparticles of a colloidal size, into which many amphiphilic molecules self-assemble. In water, hydrophilic parts of such molecules form the micelle corona, while hydrophobic fragments form the core of a micelle that may serve as a cargo space for poorly soluble pharmaceuticals. Because of their small size, micelles are able to spontaneously accumulate in pathological areas with the damaged ("leaky") vasculature, such as infarcts and tumors, via the enhanced permeability and retention (EPR) effect. Based on this background the objectives of present investigation were set in an attempt to develp a novel, safe, effective and stable micellar system comprised of Pluronic F68 (PF68) in corporating paclitaxel. The physicochemical properties of the formulations were evaluated and it *in vitro* drug release was also investigated.

MATERIAL AND METHODS

Pluronic F68 (PF68) and Paclitaxel were purchased from Sigma Aldrich, USA. All other chemicals and reagents were of analytical grade. Milli Q water (Millipore) was used through out the studies.

Methods

Preparation of polymeric micelles

The formulation of micelles was executed by sonication method. The sonication method consisted of the following steps; weighing of the PF68 (200.0mg) and paclitaxel (20mg) in to a screwtop glass vial, addition of 3.0mL distilled water and subsequent sonication using a sonica tor (Vibracell, USA) at for 10 minutes. Micelles formed were centrifuged at 13000rpm for min and filtered through 0.4µm filter. Empty micelles were reagents were of analytical grade. Milli Q water (Millipore) was used throughout the studies prepared according to the, same method in the absence of drug.

Solubilization efficiency of polymeric micelles

The solubilization efficiency of polymeric micelles was investigated for their solubilization enhancement capacity as compared to pure drug. The weighed amount of drug and drug loaded polymeric micelles were suspended in distilled water. The vials were placed on a mechanical shaker at ambient temperature. After equilibrium (24 h), the obtained suspension was centrifuged at 10,000rpm for 10min (Remi PR24 Centrifuge, India) and filtrate analyzed for paclitaxel concentration at 230nm using UV-visible spectrophotometer.

Particle size, polydispersity and zeta potential measurement by dynamic light scattering (DLS)

The average particle size distribution and charge of the resulting polymeric micelles was determined by dynamic light scattering (Zetasizer ZEN 3600, Malvern, UK). The experiment was performed using clear disposable zeta cell, water as a dispersant which has refractive index (RI) - 1.333 and viscosity (cP) - 0.88 and the temperature was kept constant at 25°C. The sample was analyzed for three times to minimize the error.

Encapsulation capacity of polymeric micelles

Weighed amount of drug loaded polymeric micelles were dissolved in methanol, sonicated for 5min to break the micelles, diluted suitably and then analysed by UV spectrophotometer at 287nm. The encapsulation capacity was determined by the following equation:

$$\text{Encapsulation capacity} = \frac{\text{Actual weight of Drug}}{\text{Theoretical Weight of Drug}} \times 100$$

Fourier transforms infrared spectroscopy (FT-IR)

The samples were subjected to FT-IR analysis by KBr pellet method using Fourier Transform Infrared spectrophotometer in the range of 4000cm-1 to 400cm-1.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry was performed on pure sample of drug and its formulation using DSC

60 apparatus (Shimadzu, Japan). Calorimetric measurements were made with empty cell (high purity alpha alumina discs) as the reference. The instrument was calibrated using high purity indium metal as standard. The Dynamic scans were taken in Nitrogen atmosphere at the heating rate of 10ºC.

***In vitro* release studies**

Release of Paclitaxel from the micelle formulation in vitro was monitored by a dialysis method. Dialysis was carried out at 37°C using Spectra/Pordialysis membranes (Spectrum Laboratories, Inc, Rancho Dominguez, CA, USA) with a molecular weight cutoff of 1 kDa and phosphate-buffered saline (pH 7.4) as the sink solution. The molecular weight cutoff of the dialysis membrane only allows for diffusion of the free drug. Briefly, 1mL of the drug loaded micellar dispersion was placed in a dialysis bag. The end sealed dialysis bag was immersed into100mL of PBS (pH 7.4) at 37ºC which was stirred at 100 rpm speed. At scheduled intervals, 3mL of the dialysis medium was collected and the same volume of fresh medium was added immediately.The samples from eachtime interval were analyzed spectrophotometrically at 230nm for Paclitaxel content.

Kinetic analysis of in vitro release data

In order to determine the release mechanism that provides the best description to the pattern of drug release, the *in vitro* release data were fitted to zero-order, first order, Higuchi model and korsmeyer peppas model using the software, PCP Disso v 2.08.

The model with the highest correlation coefficient values, analyzed using the Korsmeyer Peppas model and the release exponent (n) describing the mechanism of drug release from the matrices was calculated by regression analysis using the following equation:

$$Mt/M\infty = kt^n$$

Where Mt/M∞ is the fraction of drug released at tim e t and k is a constant in corporating the structural and geometric characteristics of the release device. When n = 0. 5, Case I or Fickian diffusio iis indicated, 0.5<n<1for anomalous (non Fickian) diffusion, n = 1 for Case II transport (Zero order release) and n>1 indicates Super case II transport.

Stability studies

Formulations were packed in a screw capped bottle and studies were carried out for 12 months by keeping at

25 °C ± 2°C and 60 ± 5%RH

30 °C ± 2°C and 65 ± 5%RH

And for 6 months for accelerated storage condition at

40 °C ± 2°C and 75 ± 5%RH

RESULTS AND DISCUSSION

Solubilization efficiency of polymeric micelles

It was carried out with a rationale of comparing the solubilization efficiency of pure drug and drug in polymeric micelles. Solubility of Paclitaxel increased 20 fold when formulated as polymeric micelles (Figure No.2). The total solubility effect exerted by micelles may be because of entrapment of drug in the hydrophobic core with exterior hydrophilic surrounding. This investigation showed that the primary purpose of synthesizing polymeric micelles i.e. enhancement in solubilisation efficiency of Paclitaxel is served.

The average particle size, polydispersity index and zeta potential for polymeric micelles were determined by dynamic light scattering.

The particle size analysis revealed that the average particle size is 285.8nm with low polydispersity index value of 0.243. As known, the polydispersity index is a parameter used to define the particle size distribution of nanoparticles.

It is a dimensionless number and it values ranges from 0.5 - 0.7 for mono dispersed particles, Values greater than 0.7 are characteristic of samples with a broad size distribution. Therefore, it can be stated that the particle size distribution is unimodel, having a narrow range and a homogeneous size distribution.

The zeta potentials of Paclitaxel polymeric micelles was sufficiently high (34.1mV) to prevent agglomeration of micelles.

Encapsulation capacity of polymeric micelles

The percentage entrapment efficiency of Paclitaxel loaded polymeric micelles was found to be

78.2±2.1% suggesting that Paclitaxel can be effectively loaded into polymeric micelles.

Differential scanning calorimetry (DSC)

The purity of the drug and the polymeric matrix influencing drug release can be determined by DSC.

***In vitro* release studies**

The release pattern of Paclitaxel was studied in PBS (pH 7.4) at 37±1°C. Figure No.6 shows the drug release behavior of the PF68 micelles and drug solution. Cellophane membrane with molecular weight cut off around1kDa was used for the experiments that retains polymeric micelles and only permits transfer of drug in solution form. The release of incorporated drug molecules form the micellar system is governed by transfer of drug from micelles to the surrounding aqueous medium followed by diffusion through the cellophane membrane into the receptor medium. In case of Paclitaxel solution drug was released at a slow rate due to its limited solubility whereas significantly more ($p<0.05$) amount of drug was released from micellar formulations.

Paclitaxel incorporated in the hydrophobic core is retained firmly by the micelles as observed by the controlled release with *in vitro* sink condition.

Kinetic analysis of *in vitro* release data Kinetic analysis of *in vitro* release data

The best fit model with the highest correlation coefficient value for the formulations was the Korsmeyer Peppas model. When the *in vitro* release data from the formulations were fitted to the Korsmeyer and Peppas equation, the values of n obtained were >1 in all the cases. Then value ranged from 1.53. Since the n value was >1, this indicated a super case II transport wherein multiple release mechanisms exists, predominant being swelling and relaxation. This is the ideal method of drug release in order to achieve a pharmacological prolonged, sustained and controlled action.

Stability studies

The observations of long-term storage conditions and accelerated conditions are shown in the Table No.1. Results indicate no significant changes in the parameter even when it was subjected to stress testing for a period of six months. When the polymeric micelle formulation was studies for long-term storage conditions and accelerated conditions, the drug content in the formulation with in the 95% confidence interval and hence the slight decrease in the drug content was statistically not significant.

Table No.1: Stability study data of the Paclitaxel loaded polymeric micelles

S.No	Stability condition	Sampling interval (months)	Physical appearance	% Drug content*
1	25◦ C ±2 C/60±5% RH	0	No change	98.30±0.10
		3	No change	98.12±0.14
		6	No change	97.98±0.08
		12	No change	97.85±0.12
2	30◦ C ±2 C/65±5% RH	0	No change	98.30±0.10
		3	No change	98.02±0.11
		6	No change	97.92±0.10
		12	No change	97.78±0.14
3	40◦ C ±2 C/75±5% RH	3	No change	98.30±0.10
		0	No change	98.40±0.32
		6	No change	96.80±0.39

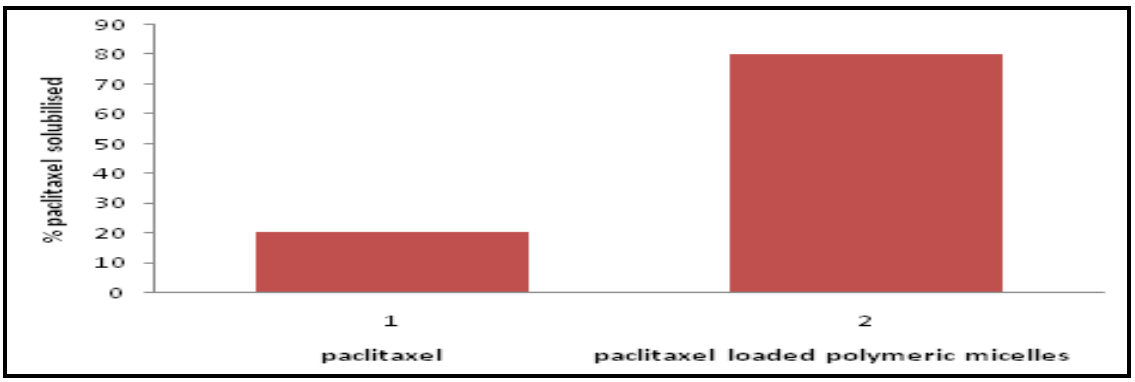

Figure No.1: Solubilization efficiency of Paclitaxel and drug loaded polymeric micelles in distilled water, Particle size, polydispersity and zeta potential measurement by dynamic light scattering (DLS)

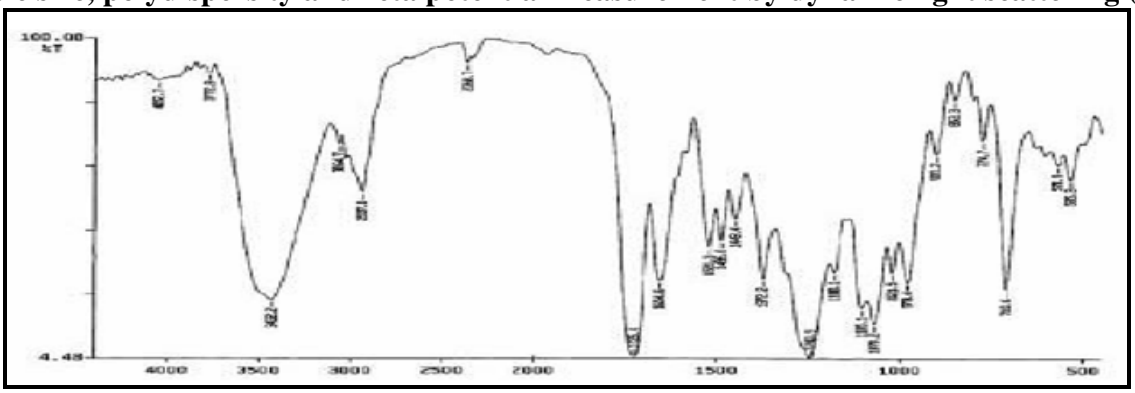

Figure No.2: Fourier transform infrared spectroscopy (FT-IR)

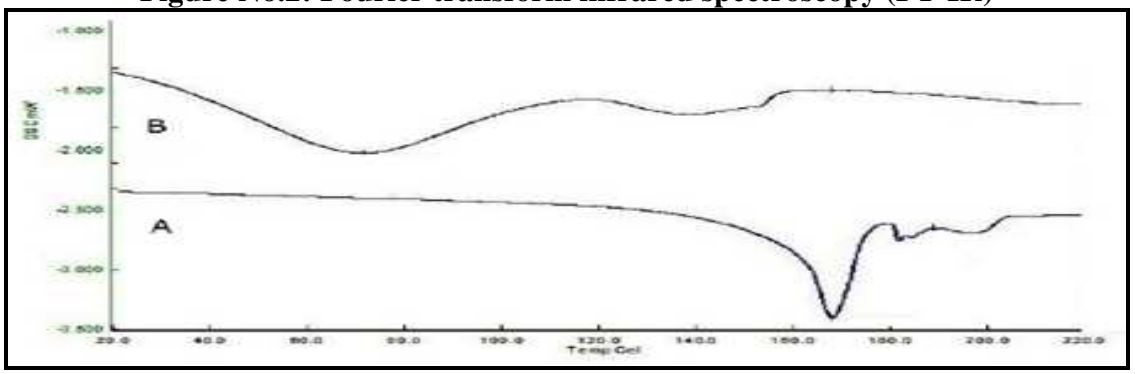

Figure No.3: DSC spectra of (A) pure paclitaxel and (B) paclitaxel loaded polymeric micelles

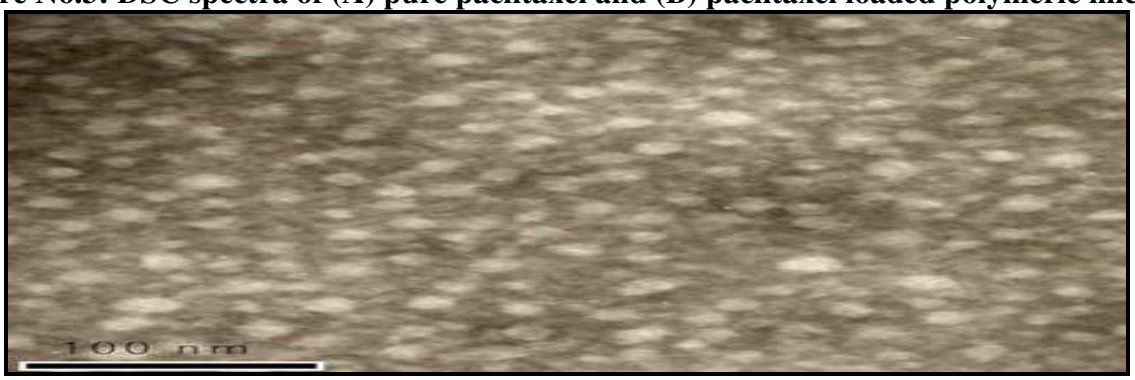

Figure No.4: SEM analysis of polymeric micelles

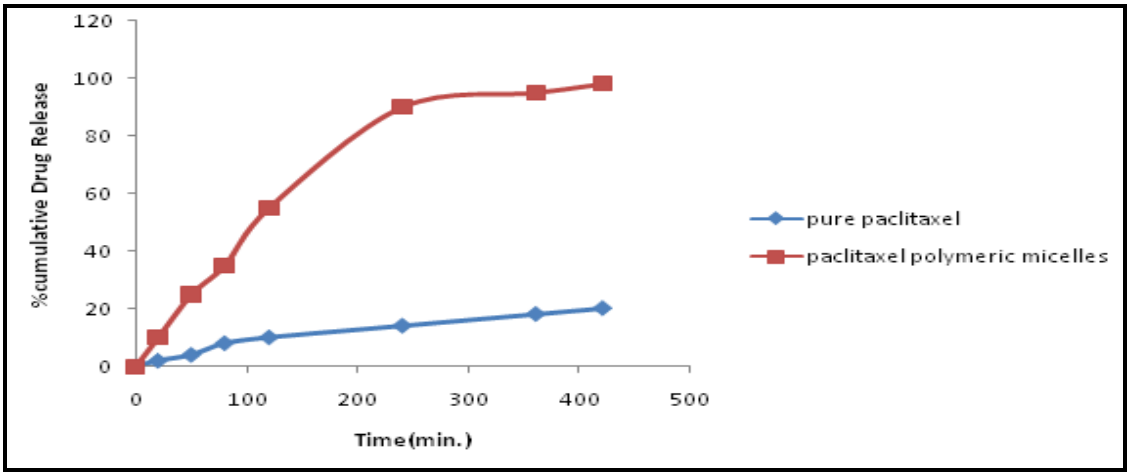

Figure No.5: *In vitro* release study of pure Paclitaxel and polymeric micelles

CONCLUSION

In the present study, Paclitaxel loaded polymeric micelles were prepared by sonication method and characterized for the delivery of Paclitaxel. Polymeric micelles showed high solubility than pure Paclitaxel. Paclitaxel was encapsulated in polymeric micelles and characterized by different techniques, which proved its encapsulation within the micelles. The polymeric micelles showed high drug encapsulation efficiency, enhanced dissolution and sustained drug release. Due to more solubilization, the bioavailability of Paclitaxel can be expected to be more as compared to plain drug. The micelles were stable under the conditions of storage. All of these properties are desirable features in effective therapeutic formulations and these results strongly suggest that the prepared PF68 polymeric micelles could be used as a functional drug carrier for Paclitaxel.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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