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# Thermoreversible liposomal poloxamer gel for the delivery of paclitaxel: dose proportionality and hematological toxicity studies

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The currently existing treatment modalities of cancer suffer from a major drawback of systemic toxicity, which results from high systemic drug exposure. Delivery of chemotherapeutic agents by delivery systems that alleviate systemic side effects but at the same time provide therapeutic advantage by controlling tumor growth exists as a viable option. To achieve this objective, a thermo reversible poloxamer gel containing paclitaxel incorporated in liposomes was formulated at three dose loadings. These paclitaxel loaded formations were injected subcutaneously (s.c.) in Sprague Dawley rats. Blood samples collected at various time points were used in the determination of drug concentration as well as white blood cell and neutrophil counts for the estimation of systemic toxicity of the formulation. Absorption of paclitaxel after s.c. injection occurred slowly with prominence of absorption phase in plasma profile, suggesting presence of flip-flop pharmacokinetics. In spite of increase in dose of paclitaxel administered, no statistically significant increase in plasma levels and pharmacokinetic parameters occurred. Further, no significant increase in hematological toxicity was observed with increased drug exposure to animals. These results show that liposomal poloxamer gels reduce systemic toxicity of paclitaxel even at high doses; and thus, can serve as an effective delivery system for alleviating body burden of this toxic chemotherapeutic agent.

## 1. Introduction

The anticancer drug paclitaxel is a diterpenoid produced by an endophytic fungus that resides in Pacific yew, *Taxus bervifolia* (Wani et al. 1977). Interestingly, paclitaxel has a number of therapeutic attributes, it inhibits angiogenesis (Okaba et al. 1995), metastasis and promotes apoptosis (Yen et al. 1996). It also induces an increase in vessel diameter and thereby increases the vascular surface area for delivery of therapeutic agents (Griffon et al. 1996). However, it has a low therapeutic index, and the therapeutic response is always associated with side effects. The toxicity of conventional systemic chemotherapy and immunotherapy affords limited effectiveness and frequently compromises the quality of life for patients. Inadequate regional control of malignant disease, including recurrence of disease at the site of surgical resection, continues to be the major cause of morbidity and mortality (Hunter et al. 1997). In an attempt to reduce the body burden of chemotherapeutic agents, various nanodelivery systems like nanoparticles, micelles etc. have been explored (Cegnar et al. 2005). They release encapsulated drug in a slow and sustained manner, thereby decreasing the exposure of the body to high concentrations of drug.

Biodegradable *in situ* gel forming formulations offer an attractive alternative to implants, microparticles, nanoparticles and pastes for control of tumor and have gained lot of interest (Winternitz et al. 1996). These *in situ* gel forming formulations can be based on various phenomena like photpolymerisation, solvent diffusion from a water insoluble polymer matrix or temperature dependent sol-gel transformation (Amiji et al. 2002). Among these the thermo gelling approach seems to be free of major drawbacks and is most convenient for clinical use. A thermosensitive *in situ* gelling chitosan based hydrogel with gelling temperature of 37 °C was developed by Ruel-Gariepy et al. (2000) however, the formulation could not sustain the release of the drugs and release was complete in 24 h. With the objective to sustain the release of the drug from the chitosan based gel, liposomes were incorporated into the chitosan solution (Ruel-Gariepy et al. 2002). It was found that gels could control the release of drug for 2 weeks under *in vitro* conditions. A thermoreversible paste consisting of triblock copolymer of poly(D,L-lactide-co-caprolactone)-block-polyethyleneglycol-block-poly(D,L-lactide-co-caprolactone) was formulated by Jackson et al. (2004). The formulation could control the release of paclitaxel for 7 weeks under *in vitro* conditions; however, major disadvantage of the formulation was that it is in sol state at temperatures around 45 °C. This formulation needs to be heated prior to injection and thus, the approach seems to be less attractive.

Thermosensitive polymers include poloxamers, isopropyl acrylamide, poly(ethylene glycol) and poly(lactic acid) block copolymers, chitosan solutions neutralized with glycerol phosphate. Poloxamer has been widely explored as a

vehicle for parenteral drug delivery and in particular for sustained release parenteral formulations. It has remarkable ability to solublize water insoluble drugs that renders it an attractive vehicle for controlled release of water insoluble drugs. Due to its thermo reversible nature, the *in vivo* injection of this gel helps in formation of an implant at the site of administration. In addition, poloxamer has the ability to increase the time of permanence of drug at the injection site and to retard release of drug from the gel. This prolongs the plasma concentrations; as a result drug concentrations are within the therapeutic level for a longer time. Incorporation of drug in liposomes can additionally delay release from the gel (Paavola et al. 2000) and can act synergistically with poloxamer to decrease drug release from formulations. Poloxamer gel has been studied for sustained delivery of various molecules when administered subcutaneously (s.c.) and has shown to maintain concentrations in a plateau phase (Guzman et al. 1992). By virtue of these attributes, it has been used as vehicle for sustained delivery of various therapeutic agents. Further, poloxamer has been reported to increase therapeutic activity of antitumor agents and also has been proven to be effective for treatment of multi drug resistant tumors *in vitro* as well as *in vivo* (Batrakova et al. 2001).

In the present study poloxamer gels containing paclitaxel incorporated in liposomes were evaluated in rodents. The systemic concentrations of paclitaxel were determined at three doses to asses increase in body burden with increase in dose. An attempt was made to understand the influence of increased dose of paclitaxel on hematological toxicity, which was also used as a marker of body burden of paclitaxel.

## 2. Investigations and results

### *2.1. In vitro study*

The temperature, at which "sol" phase makes transition into "gel", is defined as gelation temperature. The poloxamer solution prepared at 30% w/w, was observed to solidify in the range of 10–13 °C, which is below the body temperature of 37 °C. A 30% w/w solution was previously reported to have gelation point in the range of $13.4 \pm 0.5$ °C.

Release of paclitaxel was studied to derive an idea about the duration of sustained effect of poloxamer gel. As shown in Fig. 1, release rate was not constant throughout and it was observed that 95% of the initial loading was released in 220 h. Further, when release rates were calculated, paclitaxel was found to be released at the rate of 1% per hour in the first 50 h, while it declined to 0.66% in the next 50 h. In the initial 2 h period of study only 0.1% of paclitaxel was released indicating a small lag time, though it is quite negligible.

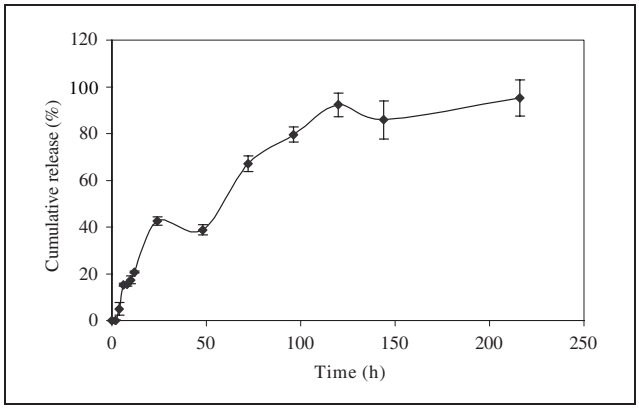


Fig. 1: Cumulative release of praclitaxel from thermoreversible poloxamer gel at 37 °C in phosphate buffer (0.1 M, pH 7.4). (Data is mean ± SD; n = 3)

### *2.2. In vivo study*

Plasma concentration profiles obtained following s.c. administration of gels are shown in Fig. 2. Absorption phase was considerably prolonged due to slow release of drug and $t_{max}$ for all three doses appeared at 25 h. Profiles were described by one compartment model and pharmacokinetic parameters calculated are listed in Table 1. The $t_{1/2}$ values show that elimination was occurring slowly. All the pharmacokinetic parameters ($C_{max}$, $AUC_{(0-\infty)}$, $k_{el}$, $Cl_T$) showed no significant difference with increase in dose when analyzed statistically by one way ANOVA ($p < 0.05$).

Interestingly, study of plasma concentration profiles revealed that release of paclitaxel from gel is occurring very slowly and this was further substantiated by the dominance of absorption phase in the plasma curve which existed until 25 h (Fig. 2). This indicated a possibility of a flip-flop pharmacokinetics and to support this observation, i.v. pharmacokinetics of paclitaxel was essential. Pharmacokinetics of paclitaxel vary considerably with species, and hence, i.v. administration of pure paclitaxel in ethanol as well as that of a Cremophor EL formulation (Intaxel®) was done in Sprague Dawley rats. Plasma concentration profile (Fig. 3) was bi-exponential and seen to follow a two compartment model. $AUC_{(0-\infty)}$ was calculated by the linear trapezoidal rule and $k_{el}$ was calculated by PCNONLIN as well as by linear regression analysis (Ritschel 1999). The elimination for pure paclitaxel was

**Table 1: Pharmacokinetic parameters of palitaxel after s.c. administration of thermoreversible poloxamer gel at three different doses**

| Dose (mg/kg) | Parameter | | | | | | |
|---|---|---|---|---|---|---|---|
| | $K_{el}$ ($h^{-1}$) | $AUC_{(0-\infty)}$ (g/L) h | $Cl_T$ (L/h) | $V_d$ (L) | $C_{max}$ (ng/mL) | $K_a$ ($h^{-1}$) | $T_{1/2}$ (h) |
| 15 | 0.0149 | 7446.32 | 2.01 | 134.66 | 86.71 | 0.1163 | 46.5 |
| 20 | 0.0174 | 8179.32 | 2.45 | 139.86 | 107.40 | 0.0935 | 39.8 |
| 25 | 0.0193 | 8248.29 | 3.03 | 157.03 | 118.05 | 0.1041 | 35.9 |

Note: Plasma profile were fit in one compartment model
All parameters were calculated by PCNONLIN and are mean values (n = 4)
$AUC_{(0-\infty)}$ was calculated by linear trapezoidal rule
$K_{el}$ by log linear regression
$K_a$ was calculated by back stripping of curve

**Table 2: Pharmacokinetic parameters of palitaxel after i.v. administration of paxlitaxel in ethanol and CrEL formulation**

| Formulation (Dose mg/kg) | Parameter | | | | |
|---|---|---|---|---|---|
| | $K_{el}$ ($h^{-1}$) | $AUC_{(0-\infty)}$ (μg/L) h | $Cl_T$ (L/h) | $V_d$ (L) | $T_{1/2}$ (h) |
| ETH (2.5 mg/kg) | 0.2295 | 1900.7 | 2.63 | 1.15 | 3.01 |
| CrEL (2.5 mg/kg) | 0.1642 | 6929.9 | 3.60 | 2.19 | 4.22 |

Note: Plasma profile were fit in two compartment model
All parameters were calculated by PCNONLIN and are mean values (n = 2)
$AUC_{(0-\infty)}$ was calculated by linear trapezoidal rule
$K_{el}$ by log linear regression

seen to occur faster with $K_{el}$ (PCL in ETOH) > $K_{el}$ (CrEL), and $t_{1/2}$ of paclitaxel alone was 3 h while that in Intaxel® it was calculated to be 4 h (Table 2). $K_a$ (s.c.) was found to be smaller than $K_{el}$ (i.v.), which indicates that absorption is occurring slowly and hence, flip-flop pharmacokinetics exits.

### *2.3. Hematological studies*

The hematological studies were performed in order to gain insight into the toxicity of paclitaxel after *in vivo* administration of gels. It is reported that severe leucopoenia and particularly neutropenia are dose-limiting toxicities of paclitaxel (Spencer et al. 1994). Frequent blood sampling can also produce bone marrow depression and can cause reduction in WBC count. Thus, WBC count of control rat i.e. rat to which no paclitaxel was administered was also done. WBC counts at various time points were determined for all rats after administration of formulation. A sinusoidal curve was observed both in case of control and treated

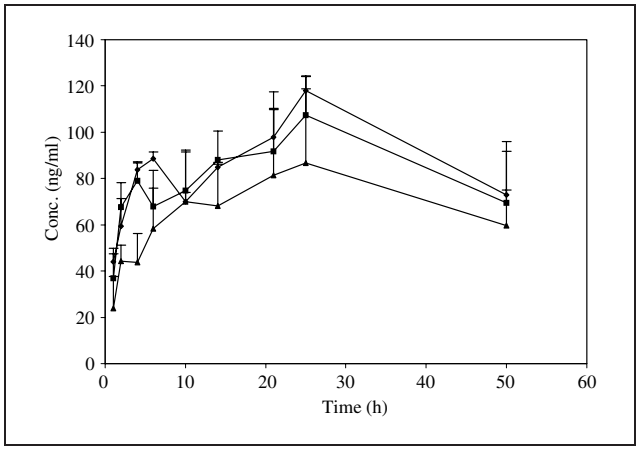


Fig. 2: Plasma concentration profile obtained after s.c. injection of paclitaxel loaded thermoreversible poloxamer gel at three different doses. Key: ● 25 mg, ■ 20 mg, ▲ 15 mg. (Data is mean n = 4 ± SD)

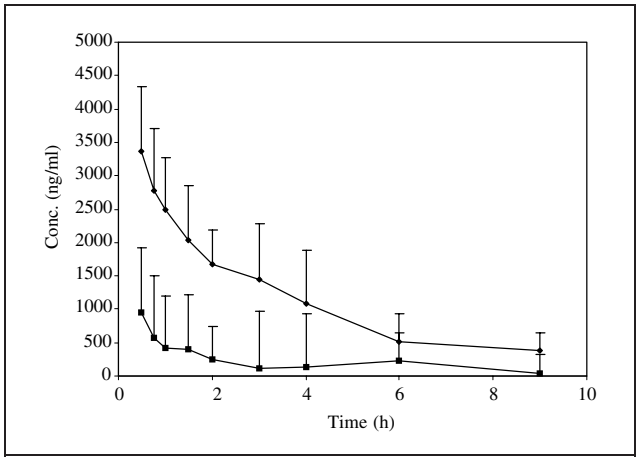


Fig. 3: Plasma concentration profile obtained after i.v. administration of paclitaxel in ethanol and CrEL formulation (Intaxel®). Key: ■ Paclitaxel in ethanol; ● Intaxel. (Data is mean n = 3 ± SD)

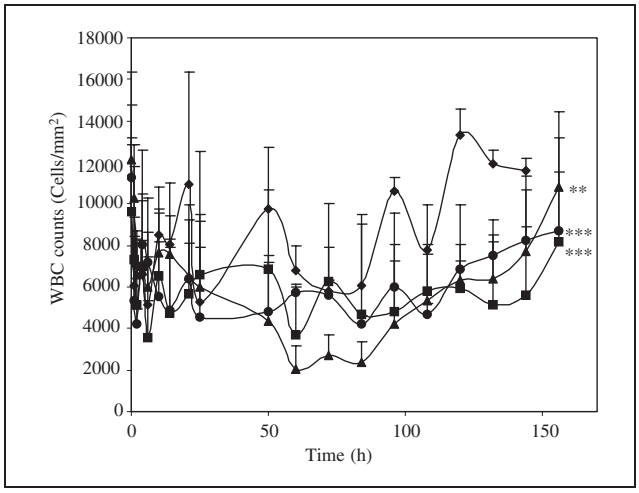


Fig. 4: WBC count determined in blood at different time intervals, both for control rats (no paclitaxel was administered) and rats that received poloxamer gel s.c. at three different doses. Data is mean (n = 4; ± SD). Key: ▲ 25 mg; ■ 20 mg; ● 15 mg; ◆ control
Note: ** Statistically significant difference between 25 mg and control (p = 0.01); *** statistically significant difference between 20 mg and control (p = 0.001); *** statistically significant difference between 15 mg and control (p = 0.002)

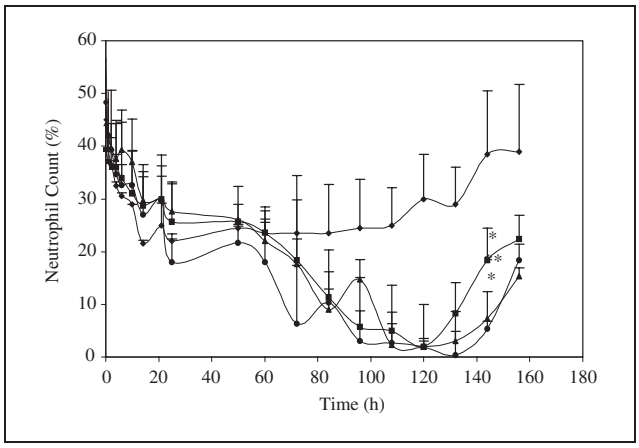


Fig. 5: Neutrophil count in blood as a function of time, both for control rats (no paclitaxel was administered) and rats that received poloxamer gel s.c. at three different doses. Haemacytometer was viewed at 40 X. Data is mean (n = 4; ± SD). Key: ▲ 25 mg; ■ 20 mg; ◆ 15 mg; control
Note: * Statistically significant difference between 25 mg and control (p = 0.036); * statistically significant difference between 20 mg and control (p = 0.015); ** statistically significant difference between 15 mg and control (p = 0.003)

rats. The reason for this could not be found. However, it was observed that leucopoenia was significant at about 72 h after gel administration and returned to normal after approximately 144 h (Fig. 4).
Differential leukocyte count of blood sampled at various time points was done in order to determine neutrophil present in blood. It was observed that neutropenia was significant at about 132 h after gel administration and neutrophil count returned to normal after approximately 144 h (Fig. 5).

## 3. Discussion

Poloxamer is a biocompatible polymer which has been widely used in drug delivery by virtue of its thermal gelation property. It is reported that thermoreversible gels with high concentration of poloxamer retard the drug release, due to the rigidity of gels the rate of water diffusion gels is severely restricted and hence drug release is slowed (Moore et al. 2000). Incorporation of drug in liposomes can additionally delay the release of drug from gel and can act synergistically with poloxamer to decrease drug release from formulation. Bochot et al. (1998) prepared a thermosensitive liposomal gel at 27% poloxamer concentration and their study revealed the presence of oligolamelllar liposomes in the gel. They further reported that at higher polymer concentrations, mobility of poloxamer chains is greatly reduced and the bilayer chain interactions are minimal. However, at low concentrations, poloxamer is known to cause destabilization of liposomes due to perturbations caused by insertion of polymer chains into bilayers (Jamshaid et al. 1998). Based on the above understanding, paclitaxel liposomal gel was prepared with poloxamer at 30% w/v level containing drug : polymer in a ratio of 1 : 75 (w/w).
The transition temperature of the gel was not significantly altered by the inclusion of liposomes. It is anticipated that alteration in transition point of gel takes place due to interferences by liposomes with gelling process. This interference may arise from liposomes either due to hindrances in cross linking of micelles or increasing intermicellar distances. Moreover, role of intermicellar distances and solvation effects on gelation point of poloxamer gels, as influenced by concentration of poloxamer itself and ionic strength are also documented (Dhanikula et al. 2005). The nonexistence of these intermicellar phenomena serve to explain the absence of change in gelation point of liposomal gels.
The drug release from the gels under *in vitro* conditions was found to be sustained. A biphasic release pattern was observed with an initial faster release phase followed by a slower phase. Approximately, 95% of the drug was released in 220 h. When amount of paclitaxel released was plotted against square root of time linearity was observed. The release profile best complied with the Higuchi model indicating diffusion controlled release kinetics of paclitaxel from the liposomal gel. Thus, it is probable that release occurs by partitioning of paclitaxel into poloxamer micelles followed by diffusion of liberated paclitaxel through aqueous channels.
A previous study in our laboratory (Dhanikula et al. 2005a) has shown that blood levels obtained after administration of the formulation at the dose of 10 mg/kg showed lower plasma concentrations as compared to a Cremophor EL formulation (Intaxel®), liposomal and micellar formulation at the doses of 10 mg/kg. To obtain concentrations similar to Intaxel®, higher doses i.e. 15, 20 and 25 mg/kg were selected for liposomal poloxamer gels in the present study.
Peak plasma levels ($C_{max}$) and $AUC_{(0-\infty)}$ of paclitaxel have been reported to be dose related (Wiernik et al. 1987a), they also have patient variability (Wiernik et al. 1987b). In the present study, an increase in dose did not show dose dependent increase in $C_{max}$ and $AUC_{(0-\infty)}$ values. Further, the terminal elimination phase appeared to be independent of the dose administered, as has been reported earlier (Spencer et al. 1994). When pharmacokinetic parameters obtained at three doses were analyzed for significant difference by one way RM ANOVA, no statistically significant difference was observed for $C_{max}$ ($p = 0.878$), $AUC_{(0-\infty)}$ ($p = 0.894$), $k_{el}$ ($p = 0.848$), $Cl_T$ ($p = 0.521$) and $V_d$ ($p = 0.960$). This could be due to (a) slow release of drug from formulation, (b) the release of drug is independent of drug loading and approximately same amount of drug is getting released from all three gels, (c) fast elimination/distribution of drug from plasma. Since, studies in our laboratory have shown the release of the drug from gel matrix to be dependent on loading (Dhanikula et al. 2005a), reason (b) can be ruled out. Further, the elimination phase of paclitaxel is independent of the amount of dose administered, therefore, the extent and rate of elimination will be same at all doses. Hence, possibility of elimination and distribution of paclitaxel as factor can be ruled out. Thus, the possible reason could be (a) i.e. slow release of drug from the formulation.
The absorption phase in the plasma profiles of paclitaxel loaded poloxamer gels was considerably prolonged and $t_{max}$ at three doses appeared around 25 h. This is probably due to sustained and slow release of drug from poloxamer and liposome matrix, and/or due to very slow absorption of paclitaxel when gel was administered.
When the rate of absorption of the drug is the rate-limiting step in absorption, distribution and elimination of drug "flip-flop" exists. The literature shows that flip-flop pharmacokinetics is shown by slower absorption rate constant ($K_a < K_{el\ i.v.}$) (Spencer et al. 1994). Further, according to Boxenbaum (1998) existence of flip-flop pharmacokinetics can be determined by comparing KC with $\Delta C/\Delta t$. The Wagner-Nelson equation can also be written as follows;

$$\text{Rate of absorption} = V_z\{(K^*C) + (\Delta C/\Delta t)\} \quad (1)$$

where, $V_z$ is the terminal volume of distribution, K is the terminal disposition rate constant (determined from i.v. dosing), C is the plasma concentration at time t and $\Delta C$ is the change in plasma concentration over the time interval $\Delta t$.
When, $K^*C \gg (\Delta C/\Delta t)$,

$$\text{Rate of absorption} = (V_z)^*\,(K)^*\,(C)$$

$$(\text{Since, } (V_z)^*\,(K) \text{ is clearance (CL)} \quad (2)$$

$$\text{Rate of absorption} = (CL)^*\,(C),$$

$$\text{which is the rate of elimination} \quad (3)$$

Under these conditions, rate of absorption is approximately equal to rate of elimination and hence, flip-flop pharmacokinetic occurs.
To validate the existence of flip-flop pharmacokinetics, paclitaxel was administered intravenously. On comparison of plasma concentration profiles of paclitaxel obtained after s.c. administration with those obtained after i.v. administration, it was observed that detectable plasma concentrations were present for a prolonged period when gels were administered (Jhonston et al. 1992). The absorption rate

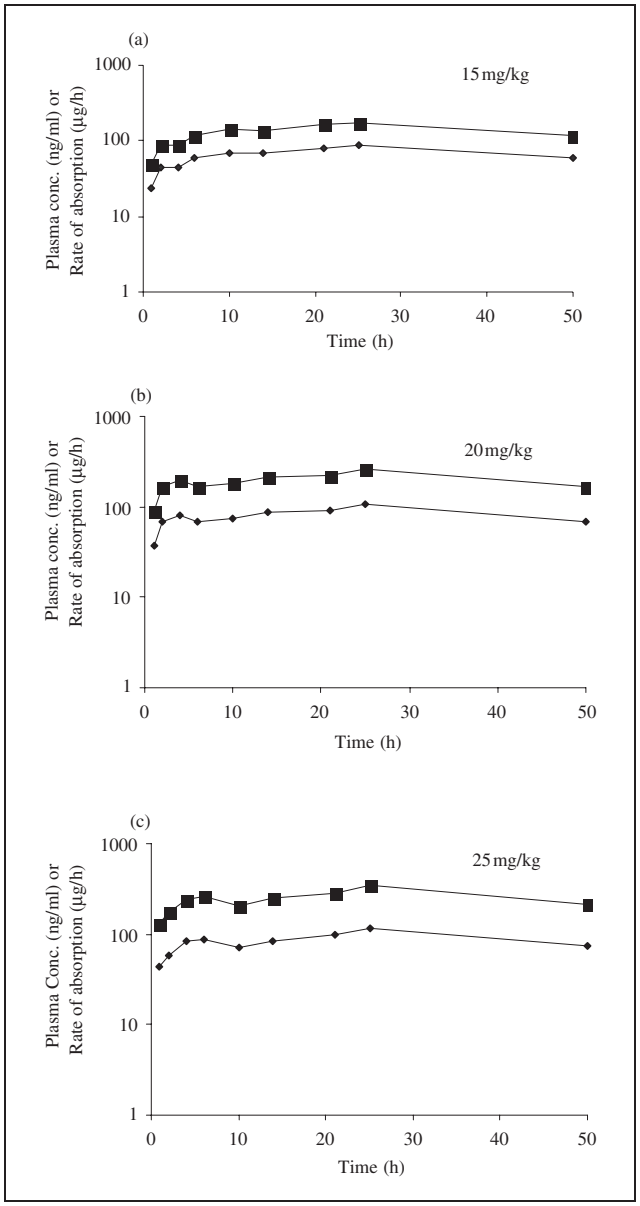


Fig. 6: The comparison of plasma concentration (ng/mL) of Paclitaxel obtained after s.c. injection of thermoreversible poloxamer gels at three different doses (a) 15 mg/kg (b) 20 mg/kg and (c) 25 mg/kg with rate of absorption (µ/h). Key: ■ rate of absorption; ◆ plasma concentration

constant calculated after s.c. administration of gel was smaller than the elimination rate constant obtained by i.v. administration. Further, KC was found to be greater than $\Delta c/\Delta t$, which indicates that the rate of absorption is approximately equal to the rate of elimination and this also substantiates that flip-flop pharmacokinetics occurs when the drug is administered as liposomal poloxamer gel. In the case of flip-flop the plasma concentration-time profile tends to closely parallel the rate of absorption. So, the shape of rate of absorption profile can be used as another effective way to identify "flip-flop" pharmacokinetics. The rate of absorption of paclitaxel from gels was calculated by a modified Loo-Riegelman equation (Wagner 1983). When rate of absorption was plotted as function of time (Fig. 6), the profile was found to be parallel to the plasma concentration profile at all three doses; this further confirms the existence of flip flop pharmacokinetics. It is also postulated that "flip-flop" condition exists throughout most of the profile, since the steepness of the plasma concentration-time in the terminal phase of profile from the gel formulation is comparatively less than i.v. administration.

Though absorption is slow, an initial higher concentration exists about 10 h, this could be probably burst release of drug from gel. This particular attribute of gels will make them amenable for control of tumor growth. Very slow release of drug in the initial period is not effective in control of tumor growth, and hence initial high release is the desired factor. This fact is supported by results where local delivery of paclitaxel to solid tumours by an *in situ* gelling poloxamer formulation was found to significantly control tumour growth and resulted in increasing survival of tumour bearing mice by 91% (Amiji et al. 2002).

Paclitaxel produces severe leucopenia and particularly neutropenia, both of which being dose-limiting toxicities (Spencer 1994). Further, it is also stated that hematological toxicity of paclitaxel is associated with some pharmacokinetic parameters. Severity of leucopenia and neutropenia is correlated with $AUC_{(0-\infty)}$ (Brown et al. 1991; Longnecker et al. 1987). In our study, paclitaxel administration produced up to 33–66% reduction in WBC counts when compared to control. It was seen that leucopoenia increased slightly with increase in dose; but when the data was analyzed statistically by Tukeys test, a significant difference was found for 25 mg ($p = 0.01$), 20 mg ($p = 0.001$) and 15 mg ($p = 0.002$) in comparison with control at all time points; which indicates that paclitaxel administration caused reduction in WBC count. However, no significant difference was observed in leucopoenia produced by three doses when analyzed statistically by one way RM ANOVA ($p = 0.773$). Since, in our study also $AUC_{(0-\infty)}$ values as well as leucopenia at three doses were not statistically different, the results are in accordance with those of Brown et al. (1991). It is also reported that decrease in neutrophil count is independent of $C_{max}$ and 24 h paclitaxel concentrations, but dependent on duration for which paclitaxel concentration is greater than 42.7 µg/L or 85.4 mg/L (Kreans et al. 1995). The results obtained in our study seem to be in agreement with those of the above studies since for all three doses, reduction in neutrophil and WBC count are not significantly different. In addition, terminal elimination phase of the plasma profile is independent of the dose administered. Thus, the duration for which plasma concentrations are above 42.7 µg/L will be almost the same for all three doses. Hence, it can be concluded that an increase in dose of paclitaxel administered *via* poloxamer gel did not produce significant increase in leucopoenia. Further, no significant difference in neutropenia at three doses was noticed.

It can be reasonably postulated from the study that with increase in dose there is no significant increase in body burden and toxicity. This is very advantageous because, it will allow higher doses of paclitaxel to be administered without influence on hematological toxicity.

Release of paclitaxel from liposomal poloxamer gel was found to be slow and sustained after *in vivo* administration. They served to increase the time of permanence of drug in the body as compared the commercial formulation. The rate of absorption of paclitaxel from these gels was very slow resulting in flip-flop pharmacokinetics. Administration of poloxamer gel at three doses produced no significant increase in AUC and other pharmacokinetic parameters. Further, the hematological toxicity did not show any significant increase with increase in dose of paclitaxel administered. Thus, it can be reasonably concluded that administra-

tion of paclitaxel via poloxamer gels did not increase body burden of drug in spite of increased loading. Hence, these liposomal-poloxamer gels can serve as a potential delivery system for sustained delivery of paclitaxel.

## 4. Experimental

### *4.1. Materials*

Paclitaxel and Poloxamer 407 were obtained as gratis sample from by Dabur India Ltd (India) and BASF (Germany), respectively. Radioactive paclitaxel ($^{14}$C) (specific activity 42.5 mCi/mmol) was purchased from Sigma (USA). Intaxel® 6 mg/mL (paclitaxel concentrate in Cremophor EL-alcohol solvent system) was purchased from Dabur India Ltd. Soyaphosphatidylcholine (Phospholipon 90), Soya phosphatidylglycerol sodium salt (Phospholipon G Na) were supplied from Nattermann Phospholipids, Germany. Diethyl ether was procured from Central Drug House Ltd. (India) and absolute ethanol from Merck KgaA (Germany). All other solvents were of analytical or reagent grade.

### *4.2. Methods*

#### *4.2.1. Preparation of thermo reversible poloxamer gel*

Thermo reversible poloxamer gel was prepared by cold method with poloxamer 407 at 30% w/v level containing drug : polymer at 1 : 75 (w/w) ratio. Paclitaxel was encapsulated in liposomes because of two reasons; first, to obtain a slow and sustained release of drug from the gel. Second, paclitaxel has poor aqueous solubility and it was observed that at higher loadings direct incorporation in gel lead to its precipitation (Dhanikula et al. 2005). Amiji et al. (2002) also reported that at low concentration paclitaxel completely dissolves in poloxamer gel; however, at higher concentration it forms a suspension. Thus, paclitaxel was encapsulated in liposomes which were subsequently incorporated in poloxamer gel. Liposomes were prepared by the film hydration method, which was previously optimized in our laboratory (Dhanikula et al. 2005).
Definite quantities of paclitaxel and radiolabelled compound (2.5 μCi/mL), required volumes of phosphatidylcholine and phosphatidylglycerol solutions (prepared in chloroform) were taken into round bottom flask and were subjected to vacuum at temperature of 60 °C to obtain a lipid film. The film obtained was redissolved in a cyclohexane : *tert*-butanol solvent system 1 : 2 (v/v) and was further dried, sufficient time was allowed to remove the traces of solvent left in the film. Thereafter, film was hydrated with normal saline pH 7 to obtain liposomes (encapsulation efficiency). Finally, a weighed quantity of poloxamer was added slowly and with constant stirring to the liposomal dispersion that has been cooled to 4–5 °C. Gel which was obtained after overnight cooling of the dispersion in refrigerator, showed thermo reversible property being sol in refrigerated conditions and gel at temperature above 25 °C (Miller et al. 1984).

#### *4.2.2. Drug content*

Gels were analyzed for content of drug by dispersing them in 1 : 1 solvent mixture of acetonitrile and ethanol (v/v). Drug content in pooled extract was determined by radioactive counting in liquid scintillation counter (Wallac 1409, Finland).

#### *4.2.3. In vitro study*

4.2.3.1. Sol-gel transition point

Inversion technique was adopted in this investigation to study "sol to gel" transformation of the gel (Valnere et al. 1984). For the purpose, 3 mL of gel at 4 °C was transferred into a 10 mL conical flask and was placed in a water bath at 6 °C (accuracy is ± 0.2 °C; Heto, Denmark). Then temperature of the bath was slowly raised to 25 °C with increments of 1 °C and at each point 15 min time of equilibration was allowed. The temperature at which flow of "sol" ceased was defined as gelling point.

4.2.3.2. Release rate studies

Paclitaxel release rate studies from gel were conducted using unjacketed Franz diffusion cells with an area of 0.79 cm$^2$ at 37 °C placed on an aluminum heating and stirring module (Permgear, USA). The poloxamer sol 500 μL was withdrawn with positive displacement pipette and placed into the donor compartment, which was separated from receptor compartment by a 0.45 μm hydrophilic membrane filter. During the study, the receptor compartment was filled with phosphate buffer (0.1 M, pH 7.4) which was previously degassed and equilibrated at the same temperature and 5% poloxamer was used to maintain sink conditions. At appropriate time points 200 μL of receptor medium was removed with replacement and analysis was performed by liquid scintillation counting.

#### *4.2.4. In vivo study*

4.2.4.1. Subcutaneous administration of gels

Poloxamer gels prepared at three doses i.e. 15, 20 and 25 mg/kg, were evaluated *in vivo* for dose proportionality. All the *in vivo* experiments were performed as per the guidelines of Institutional Animal Ethics Committee. In bred, Sprague Dawley rats (200–250 g) used in the experiment were obtained from the Central Animal Facility of National Institute of Pharmaceutical Education and Research. They were randomly distributed in three groups, each group having four rats. The same dose was administered to all four animals in a group. Rats were anaesthetized by ether inhalation following which gel was injected s.c. in the neck region. Rats were allowed water and chow *ad libitum* during the entire study. Blood samples were collected by retro orbital plexus under slight anesthesia at requisite intervals (0, 1, 2, 4, 6, 10, 14, 21 and 50 h). The collected heparinized blood was centrifuged at 5000 rpm for 15 min to separate plasma, which was used for determination of paclitaxel concentration, neutrophils and total white blood cell count.

4.2.4.2. Intravenous administration

Pure paclitaxel in ethanol as well as Cremophor EL formulation (Intaxel®) were intravenously administered to rats. A dose of 2.5 mg/kg was selected because it was well tolerated by rats (higher doses resulted in mortality). For this purpose, depending on the weight of the rat, the volume of formulation to be administered was calculated. Intaxel® (6 mg/mL) was reconstituted in normal saline and administered within 30 min after reconstitution. Further, the required volume was diluted two times to avoid animal mortality, which was observed when undiluted formulation was given. All the rats were given the premedication with ranitidine, dexamethasone and chlorpheniramine maleate 12 h prior to i.v. administration. Rats were anesthetized with urethane (1 mg/mL) and slight ether whenever required. Jugular vein cannulation was done for the administration of formulation.

#### *4.2.5. Plasma sample analysis*

Plasma sample analysis was done by a radiochemical method, which was validated prior to experimentation. For analysis, plasma collected at each time point was mixed with 3 mL of scintillation cocktail by vortexing for 1 min. Subsequently, radioactivity was determined by counting in a β-liquid scintillation counter (Wallac 1409, Finland). Plasma levels of radioactivity were converted into equivalents of paclitaxel from the known ratio of paclitaxel to radiolabelled paclitaxel in each dose administered.

#### *4.2.6. Hematological studies*

4.2.6.1. Determination of WBC

Blood collected from rats at various time points in heparinized vials was used for the estimation of WBC in blood. Blood 20 μL was diluted 20 times with Turk's diluting fluid in a micro centrifuge tube and this was shaken slightly so as to mix blood and diluting fluid appropriately. Subsequently, after an interval of 5 min 10 μL of diluted blood was placed on Neubeaur's chamber and was allowed to stand for approximately 2 min. Thereafter, WBCs were counted at 40 X using the microscope (Vision 2000, India).

4.2.6.2. Determination of neutrophils

For determination of neutrophils, a small drop of blood was placed in central line of slide about 1–2 cm from one end and was spread quickly using a spreader. Slide so prepared was allowed to air dry and was fixed in methanol after 15–20 min. Subsequently, staining was done using Leishman's stain. The stain was poured on smear to cover it fully and was allowed to act for 2 min. Following which twice the quantity of phosphate buffer (pH 7.4) was put on the smear and mixed with stain by micropipette. This diluted stain was allowed to act for 10 min. Finally, smear was washed, dried and observed under microscope at 40 X.

#### *4.2.7. Statistical analysis*

All the pharmacokinetic parameters obtained at three doses were statistically analyzed by one way RM ANOVA. While the data of hematological study was analyzed by Tukeys test for comparison with control and one way RM ANOVA for difference between three doses.

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