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## Biophysical Characterization of (DOX-NP<sup>TM</sup>): FTIR and DSC Studies

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### Abstract

Doxorubicin loaded into liposomes grafted with polyethylene glycol (PEG) has been demonstrated to have a longer circulation time and lower cardiotoxicity than doxorubicin (DOX). This study aims to investigate the biophysical characterization of a marketed formulation DOX-encapsulated liposome (DOX-NP<sup>TM</sup>). The interactions between doxorubicin and liposomal lipids can help in liposomal development. The liposome and DOX-NP<sup>TM</sup> were characterized in terms of differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR). The rheological properties of liposomal samples were also measured. Physical interactions may be occurred between the drug functional groups and liposomal lipids, probably by weak hydrogen bond formation or weak bond formation due to dipole-dipole interaction. There was no shift of existing peaks or appearance of new peaks was detected between the characteristic peaks of the liposomal lipids were present in the DOX-encapsulated liposome sample. This suggests that there were physical interactions that took place only between the drug and lipids and no chemical interaction between them. DSC information shows that the phase transition temperature shifts to lower temperature degrees after loading of DOX into the liposomes. The DSC curve has a small broadening. This may infer a little cooperativity decrease between acyl chains of liposomal membranes after DOX inclusion. The encapsulation of DOX into liposomes decreases the plastic viscosity of liposomes (from 1.64 to 1.48 cP), which shows that the membrane fluidity was increased.

**Keywords:** [Liposome, Doxorubicin, DOX-NP<sup>TM</sup>, Viscosity, FTIR, DSC.]

### 1. Introduction

A cytotoxic anthracycline derivative doxorubicin has some success when utilized as a chemotherapeutic in different human cancer forms. However, its treatment has a cardiotoxicity as side-effect. Doxorubicin loaded into PEGylated liposomes has been approved to decrease cardiotoxicity and increase tumor localization [1]. On the other hand, these liposomes moreover leads to increased accumulation within the skin and circulation times, limiting the drug release at the tumor site [2].

Liposomes may be defined simply as lipid (usually phospholipids) vesicles enclosing an aqueous space [3]. Liposomes are most frequently composed of phospholipids, particularly phosphatidylcholine, but may also include other lipids, such as egg phosphatidylethanolamine, when they are consistent with lipid bilayer structure [4,5]. A liposome design may employ surface ligands when attaching to unhealthy tissue [6].

Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC) are highly effective in the drugs and phospholipids interaction investigation [7,8]. The changes in the function and structure of the lipid assemblies can be investigated by using FTIR, by analyzing the changes in bandwidth or the different acyl chains vibration mode frequencies, the head group of lipid molecules, and the interfacial region [9].

The PC lipids have pre and main transitions in their DSC curves. The main transition indicates the gel phase to a liquid crystal phase change while the pre-transition is mainly related to the polar region of phospholipids [10]. The main transition process is closely related to the acyl chains of phospholipids bilayers, which can reflect the interaction between the acyl chains of phospholipids and exogenous substances [11].



The rheological behaviors is influenced by the drugs release from semi-solid carriers. The rheological measurements can be investigated the effect of storage time and temperature on the quality of liposomes as pharmaceutical products. So, the physical and pharmaceutical properties of DOX-encapsulated liposome can be predicted using the rheological analysis was employed as a sensitive tool.

DOX-NP™ is the market name for PEGylated liposomal doxorubicin. The circulatory half-life of the doxorubicin increases after its encapsulation into PEGylated liposomes resulting in its enhanced bioavailability at the tumor site. It has fewer side effects on healthy cells than doxorubicin. It is approved by the FDA, and is currently used to treat recurrent ovarian cancer and Kaposi's sarcoma [12,13]. The biophysical characterization of this marketed DOX-NP™ formulation was investigated in this work.

## 2. Materials and Methods

### 2.1 Materials

DOX-encapsulated liposome (DOX-NP™) is formulated and manufactured by Lipocure Ltd. Jerusalem Biotechnology Park, Jerusalem. DOX-encapsulated liposome for pre-clinical studies is produced by Avanti Polar Lipids, Inc. (Alabaster, USA) under license from Lipocure.

### 2.2 Methods

#### 2.2.1 Formulation of DOX-encapsulated liposome:

Doxorubicin hydrochloride (HCL) loaded into PEGylated liposomes. The liposomes are composed of hydrogenated soy phosphatidylcholine (HSPC), 9.58 mg/ml; N-(carbonyl-ethoxypolyethylene glycol 2000) -1,2-distearoyl-sn-glycero- 3-phospho- ethanolamine sodium salt (DPEG-DSPE), 3.19 mg/ml; cholesterol, 3.19 mg/ml, ammonium sulfate, approximately 2 mg/ml; histidine as a buffer and sucrose to maintain isotonicity. Encapsulation efficiency is more than 90% as provided by Lipocure, Ltd.

#### 2.2.2 Size Measurements:

The size distribution of different samples is performed by Zetasizer Ver. 6.20 (Malvern instrument Ltd., UK). The technique is used for the measurement of the size of nanoparticles, colloids, and molecules.

**2.2.3 Fourier transformer infrared radiation (FTIR) spectroscopy:** Fourier transformer infrared radiation (FTIR) spectroscopy was conducted for structure detection (function groups) of the solution sample using a Thermo Scientific Nicolet 6700 in the range ( $4000-400\text{cm}^{-1}$ ), which caused stretching or bending vibrations of the molecules at peak. The wavenumber of peak indicated group function.

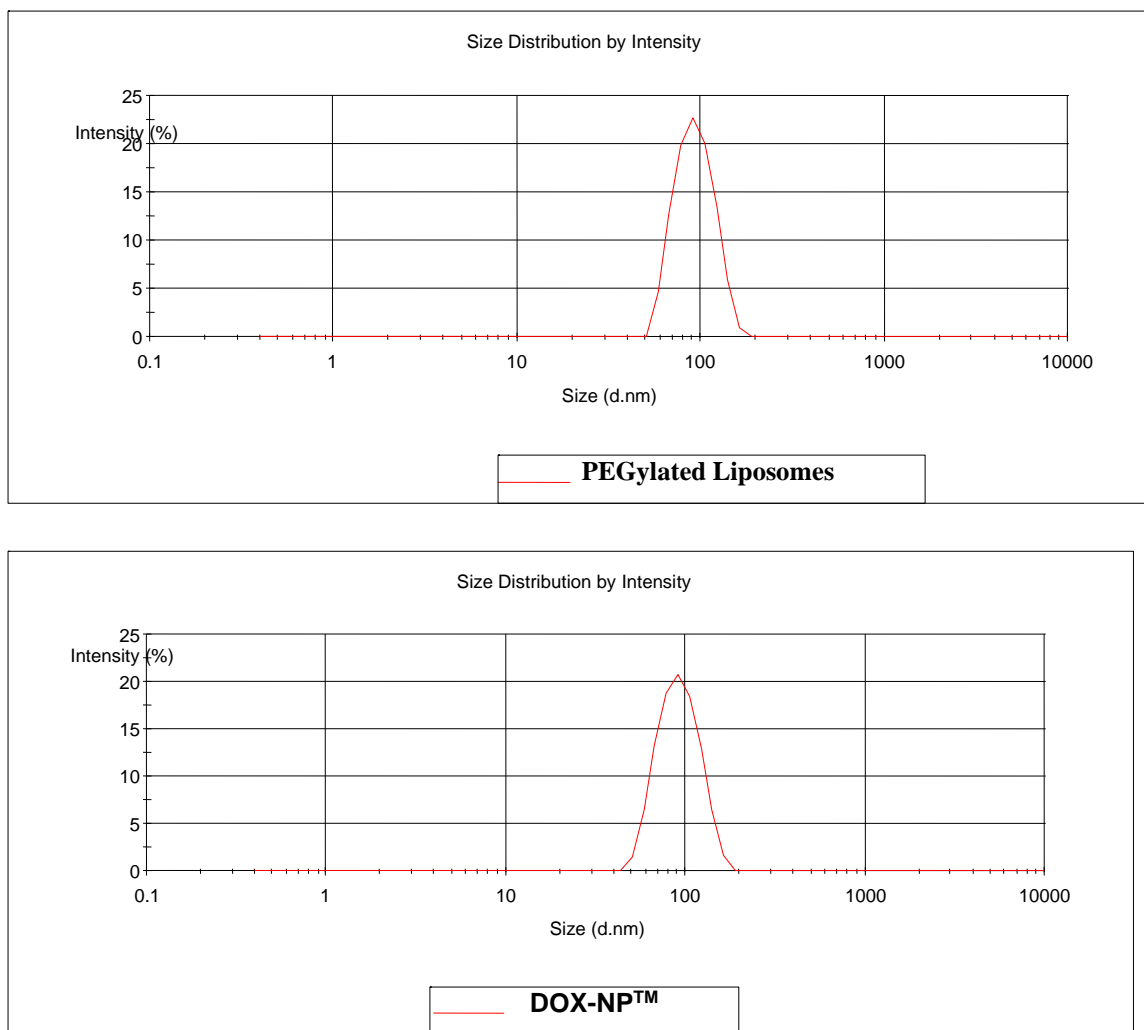
**2.2.4 Differential scanning calorimetry (DSC)** is a sensitive tool for the physicochemical characterization of liposomes. The thermal behavior of the liposomal samples was measured by a DSC Mettler Toledo AG, Analytical CH-8603. Here nitrogen atmosphere was maintained and  $2^\circ\text{C}/\text{min}$  was the heating rate.

**2.2.5 Viscometer:** A viscometer was used in the experimental setup to measure several rheological parameters (viscosity, shear stress, shear rate, consistency index, plastic viscosity, and activation energy) of the liposomal samples. The rheological parameters were. The Brookfield LVDV-III Programmable rheometer (cone-plate viscometer; Brookfield Engineering Laboratory, Incorporation, Middleboro, USA, supplied with a temperature bath controlled by a computer is used to measure these rheological parameters. a temperature sensor is used to monitor the temperature inside the sample chamber during the viscosity measurements. Rheological parameters were measured between temperatures of  $25^\circ\text{C}$  and  $55^\circ\text{C}$ .

## 3. Results and discussion:

### 3.1 Size Measurements

The size distribution of different samples is performed by Zetasizer. Figure (3.1) shows the size of liposomes and DOX-NP™ was 90.6 nm and 88.19 nm, respectively.

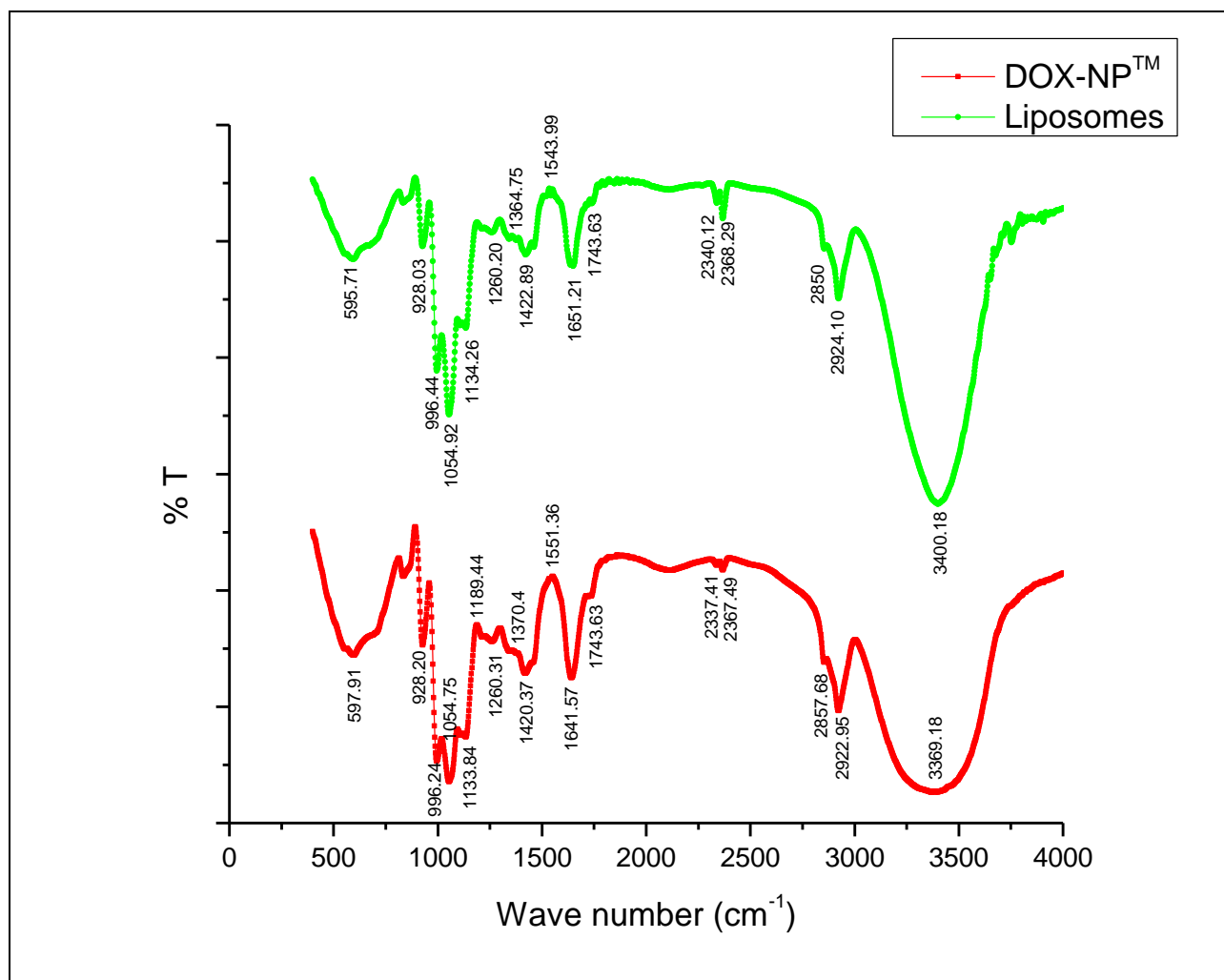


**Figure (3.1)** Size measurement of different samples

### 3.2 Fourier transformer infrared radiation (FTIR)

FTIR spectroscopy investigated the drug-liposomes interaction at the level of functional groups. The FTIR spectra of the liposome and DOX-NP<sup>TM</sup> samples have been shown in Fig (3.2). The spectrum of the DOX-NP<sup>TM</sup> displayed the main characteristic bands: the antisymmetric stretching vibrations of the CH<sub>2</sub> in the acyl chain (2,924 cm<sup>-1</sup>), the CH<sub>2</sub> bending vibration CH<sub>2</sub> (1,422 cm<sup>-1</sup>), the OH stretching and bending vibrations (3,400 cm<sup>-1</sup>), the carbonyl stretching vibration C=O (1,651 cm<sup>-1</sup>) and the symmetric and antisymmetric PO<sub>2</sub> stretching vibrations (1,054 and 1,134 cm<sup>-1</sup>, respectively).

There were mild interactions observed in wave numbers between 3350 cm<sup>-1</sup> and 3450 cm<sup>-1</sup>. The stretching vibration of free and bonded hydroxyl (OH) and amine (NH<sub>2</sub>) groups is observed between wave numbers 3350 cm<sup>-1</sup> and 3450 cm<sup>-1</sup>. Peak variation may be due to weak hydrogen bonds formation [14].



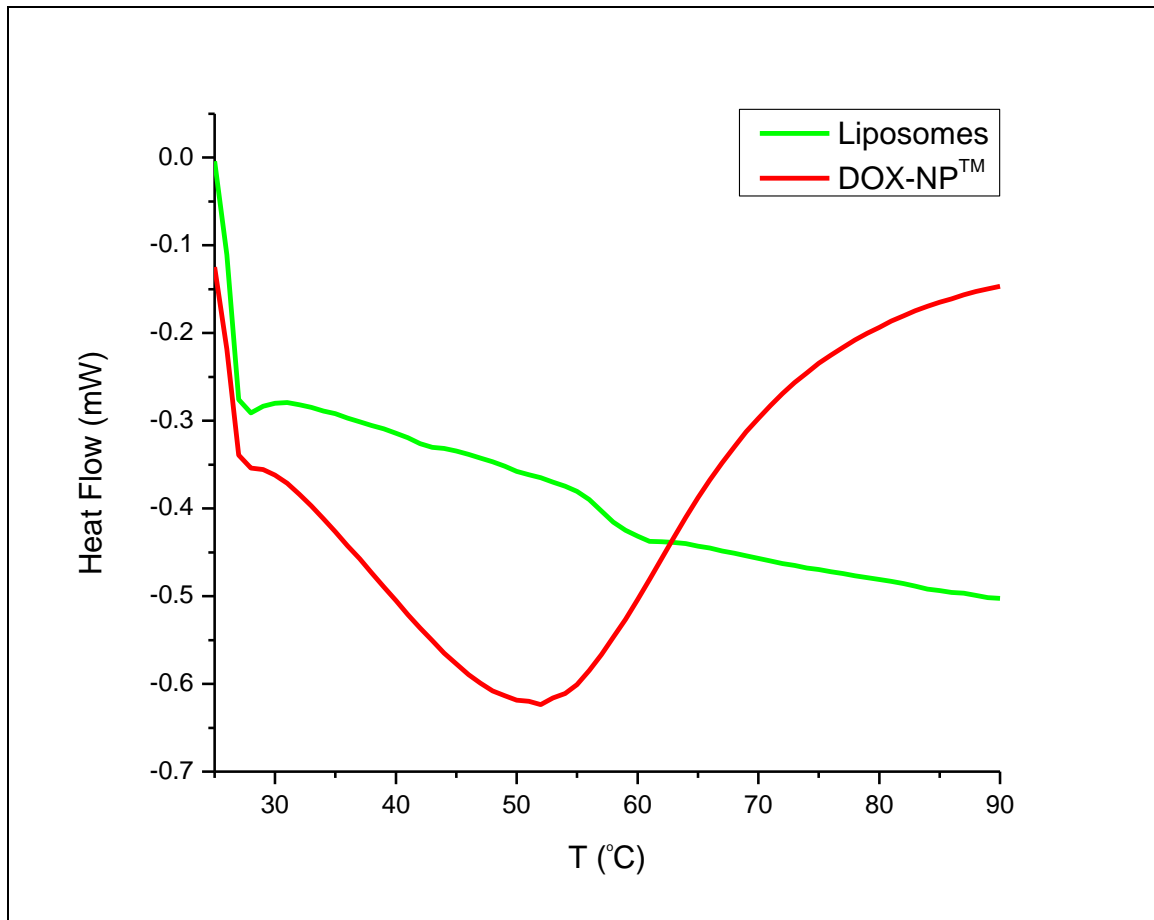
**Figure (3.2)** FTIR spectra for different samples.

In the DOX-encapsulated liposome FTIR spectrum, the DOX has not any significant effect on the order of the membrane and does not change the number of gauche conformers since there was no significant change in the  $\text{CH}_2$  stretching bands frequency, as observed in Fig (3.2). There was no hydrogen bond between the  $\text{C}=\text{O}$  group in the phospholipid and DOX as revealed from the  $\text{C}=\text{O}$  band investigation. The  $\text{PO}_2$  antisymmetric and symmetric stretching bands, which were located at 1,134 and 1,054  $\text{cm}^{-1}$ , respectively, reflected the interaction between the head group of liposomes and DOX. The wavenumber was shifted to other values (1,133  $\text{cm}^{-1}$ ) after the encapsulation of DOX into liposomes indicating the presence of hydrogen bonding between the head group of liposomal lipids and DOX. The  $\text{PO}_2$  antisymmetric double-bond stretching band (at 1,543  $\text{cm}^{-1}$ ) is shifted to higher wavenumber (1,551  $\text{cm}^{-1}$ ) for in the DOX-encapsulated liposome sample. DOX may be located in the membrane interfacial region. There is no formation of new peaks or predominant shifting of existing peaks was detected in the DOX-encapsulated liposome sample. This suggests that there is no chemical interaction between functional groups of the lipids and drug. There may be physical interactions between them, probably by weak bond formation due to dipole–dipole interaction or formation of weak hydrogen bond or van der Waals force of attraction.

### 3.3 Differential scanning calorimetry (DSC)

It was used to study the thermal behavior of the liposome and DOX-encapsulated liposome samples. DSC strategy is utilized to determine the phase transition temperatures of liposomes [5]. The endothermic phase transition temperature of the liposomes and DOX-NP<sup>TM</sup> was found to be 55-61°C and 52°C, respectively Figure (3.3). There is a strong hydrophobic interaction between DOX and the phospholipid forming the liposome due to the negative shift in the transition temperature [15]. The DSC curve has a small broadening

and shows that the phase transition temperature of DOX-NP<sup>TM</sup> has a lower temperature degree. This may infer a little cooperativity decrease between acyl chains of liposomal membranes after DOX inclusion [16].

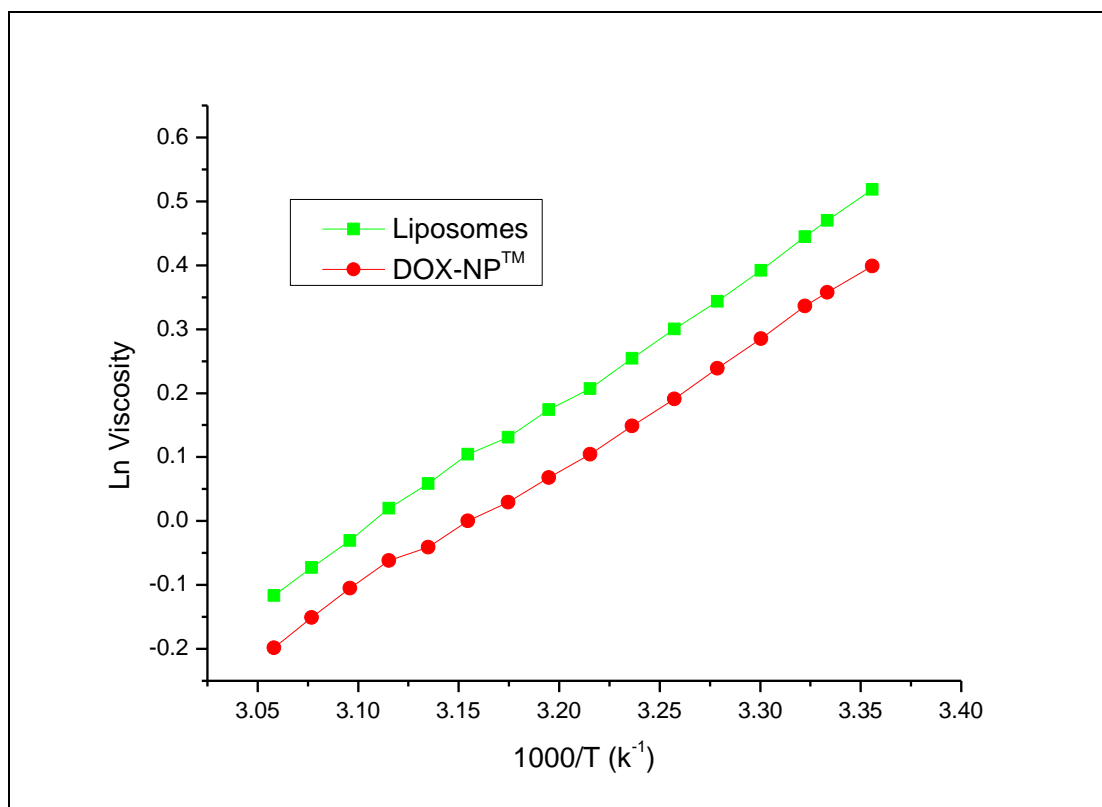


**Figure (3.3)** DSC thermogram of different samples.

### 3.4 Viscosity Measurements

The rheological properties of liposomes Figure (3.4) shows the linear relationship between the  $1000/T$  ( $K^{-1}$ ) and  $\ln$  viscosity of different samples. Table (3.1) shows the rheological parameters of the liposomal samples. The  $n$  and  $k$  values ranged from 1.005 to 0.999 and from 1.5 to 1.4, for liposome and DOX-encapsulated liposome samples, respectively. The values of the flow behavior index ( $n$ ) indicated a pseudoplastic behavior of liposome suspension. The viscous nature of liposomes is indicated by the consistency index ( $k$ ) [17, 18] that describes the plastic viscosity variation after the incorporation of DOX into the liposomes.

The plastic viscosity of liposomes decreases (from 1.64 to 1.48 cP) for the DOX-NP<sup>TM</sup> which shows that the increment of membrane fluidity [18].



**Figure (3.4)** The relationship between the  $1000/T$  (K<sup>-1</sup>) and Ln viscosity of different samples.

**Table 3.1** Rheological parameters of different liposomal samples

Sample	Plastic Viscosity (cP)	Consistency Index (cP)	Flow index	Activation Energy (kJ/mol)
Liposomes	1.64	1.5	1.005	17.34
DOX-NP <sup>TM</sup>	1.48	1.4	0.999	16.32

#### 4. Conclusion

The DOX lipid interaction was examined by noninvasive techniques, including DSC, FTIR, and viscosity measurements. The results showed that DOX affected the lipid bilayers thermo tropic phase behavior, fluidity and FTIR spectra of the membrane. The development of liposomal drug delivery systems can be based on the interactions between doxorubicin and lipid bilayers. The improved efficacy and safety of a novel class of anticancer therapeutics may depend on a better understanding of liposomal-drug interaction.

#### Conflict of interest

Authors have no conflict of interest.

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