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Research Article

POLYETHYLENE GLYCOL CONJUGATES OF PACLITAXEL AS PRODRUGS BY SIMPLE TECHNIQUE SUCH AS SOLVENT EVAPORATION

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ABSTRACT

Paclitaxel (PACL) is an anti-cancer drug used to treat breast cancer, lung cancer, ovarian cancer etc. It is available as IV infusion and not as oral formulation. It is practically insoluble in water and hence possesses problems for development as oral or per oral formulations. Hence in the present research work, polyethylene glycol conjugates of PACL were prepared by simple technique such as solvent evaporation technique using high molecular weight polyethylene glycols such as PEG15000 and PEG35000 without linking agents. Eight compositions in drug to PEG15000/PEG35000 ratio of (1:10), (1:20) (1:25) and (1:50) were prepared. The prepared conjugates were evaluated by solubility studies, drug content uniformity and analysis by FTIR and DSC. Solubility of paclitaxel was enhanced in case of all prepared PACL-PEG conjugates. PACL: PEG35000(1:50) exhibited 0.317±0.081 mg/ml and all other PACL: PEG35000 conjugates of ratios (1:10), (1:20) (1:25) and (1:50) also evidenced same values with slight variation.The low c.v. values in percent drug content values of all conjugates ensured the uniformity of drug in prepared conjugates. FTIR spectra of paclitaxel PACL:PEG35000 evidenced disappearance of –N-H vibrational stretch at 3311.35 cm^{-1}indicating conjugation of this end hydroxyl group with amine group of paclitaxel. The shift in the endothermic peak of the PEG conjugate (67.29^0 C) compared to the respective pure drug (221.57^0 C) confirmed the formation of drug-PEG conjugate. This work indicates PEG conjugates of paclitaxel will act as prodrugs and upon dissolution of polyethylene glycol moieties they parent drug in body fluids without effecting required therapeutic effect of paclitaxel.

Keywords: Paclitaxel, poor solubility, Solvent evaporation, High molecular weight PEGs, Prodrugs.

INTRODUCTION

Current status reveals that, the prevalence of cancer has enormously increased but the deaths due to cancer are reduced. This is due to appreciable advancements in the research of better formulations of chemotherapeutic drugs with site specific effects and less side effects. Cancer causing viral infections such as HBV/HCV and HPV are responsible for up to 20% of cancer deaths in low- and middle-income countries[1]

Paclitaxel (Taxol) and its derivatives are most widely used anti-cancer drugs to treat malignant glioma and brain metastases and active against various tumors. It was first isolated from the bark of the pacific Yewtree, Taxusbreviofolia. It is used to treat ovarian, breast and lung cancers[2].

Paclitaxel is highly lipophilic in nature and prescribed in the form of IV infusion for treatment of cancer other than brain tumors. Even for the development of IV formulations the water insoluble nature of paclitaxel causes trouble for development using aqueous solvents with painless administration. The research involving the conversion of paclitaxel into its prodrug form using conjugation with biopolymers or synthetic polymers wasexploited to achieve optimum therapeutic effect[3].

At this juncture, the technology of polyethylene glycol conjugation involving conjugation of paclitaxel to PEG through non-covalent interaction to enhance its hydrophilicity and aqueous solubility of paclitaxel can be exploited to prepare PEG conjugates of paclitaxel suitable for use in oral or IV formulations for treatment of various cancers including ovarian, breast ***etc.***

Advanced PEGylation especially refers to the use of high molecular weight polyethylene glycols for conjugation of biologically active molecules including anticancer drugs and other small molecules and Advanced PEGylation is the choice of technology for formulation development of this particular category drugs. [4, 5]. The PEG conjugates are of two types:

1. PEG conjugates with active substances that are loose and reversible[6].
2. PEG drug conjugates formed by covalent bonding in which the drug is covalently linked to macromolecular backbone through a physiologically labile bond[7].

Hence the present work is aimed at development of polyethylene glycol conjugation involving conjugation of paclitaxel by simple technique without sing chemicals oo spacers foor conjugation. The method expected to involve conjugation of end hydroxyl group of PEG with amine group of paclitaxel so that these conjugates will act as prodrugs and upon dissolution of polyethylene glycol moieties they release parent drug in body fluids without effecting required therapeutic effect of paclitaxel.

MATERIALS AND METHODS

Paclitaxel was obtained as a gift sample from Celon Laboratories Hyderabad, PEG 15000 and PEG 35000 were

purchased from S. D. fine chemicals ltd. All other chemicals used in the study were of analytical grade.

Preparation of drug-PEG conjugates by solvent evaporation technique

PEG conjugates of paclitaxel with PEG15000 and PEG35000 were prepared in the weight proportions of paclitaxel to PEG. Trial compositions 8 nos were prepared and are shown in Table 1. Drug and PEG were dissolved separately in possible minimum volume of acetone (≈50ml) and these solutions were mixed well using cyclone mixer for 30 min. Then the solvent was removed by evaporation in a water bath at 35^0 C under vacuum. Then the mass was dried at 35^0 C and scrapped and the powder fraction was sieved through # 100, collected and stored in 30 ml screw capped glass vials for further use. In each case 2 gm of dispersion was prepared.

Evaluation of drug-PEG conjugates

The PEG conjugates of paclitaxel were evaluated by solubility studies, drug content uniformity studies, FTIR and DSC studies.

Solubility analysis of paclitaxel-PEG conjugates

Solubility studies were carried out for pure paclitaxel and the prepared PACL-PEG conjugates. In each case excess sample was placed in 5 ml of pH 7.8 phosphate buffer in sealed glass tubes. The tubes were mixed occasionally in vortex mixer and kept aside for 24 hrs at room temperature for equilibrium to reach. Then the supernatant liquid was filtered through 0.45µm Millipore filter and aliquot samples were estimated for paclitaxel using UV-visible spectrophotometer, (Systronics) at λ_{max} of 232nm**.**

Uniformity of drug content

From each paclitaxel-PEG conjugate mixture of paclitaxel and PEG, four samples of 50 mg were analyzed for drug content. The sample was transferred into stoppered conical flask, and dissolved in minimum amount of in acetone. Then the volume was made up to 50 ml with pH 7.8 phosphate buffer. The contents were thoroughly mixed and kept aside for 24 hrs with occasional shaking to facilitate the extraction of drug from the solid mixture into solvent. The clear supernatant solution was collected by filtering through a 0.45 µm Millipore filter. The solutions were suitably diluted and assayed for their drug content by using UV-visible spectrophotometer (Systronics) at λ_{max} of 232 nm.

Differential Scanning Calorimetry (DSC) studies

Differential Scanning Calorimetry was carried out for pure paclitaxel, PEG 35000, and for promising conjugate PACL:PEG35000(1:50) using Metler toledo DSC thermal analyzer, Switzerland. Samples were placed in an aluminum pan and heated with an empty pan as reference. Heating was done at a heating rate of 10^0 C/min in the temperature range of 30^0 to 240^0 C.

FTIR analysis of drug-PEG conjugate

FTIR spectra of pure drugs and the promising dug-PEG conjugates were obtained on a FTIR Spectrophotometer, BrukerLabs, INDIA equipped with a DTSG detector. Samples were prepared by KBr pressed pellet technique. The scanning range was 4000-400 cm^{-1} and the resolution was 1 cm^{-1}. The spectra are shown in Figure 1 to 5.

Table 1: Weight proportion of PEG conjugates of paclitaxel

S.No	Weight proportion of PACL:PEG15000	Weight proportion of PACL:PEG35000
1	1:10	1:10
2	1:20	1:20
3	1:25	1:25
4	1:50	1:50

Table 2: Solubility of paclitaxel in water at 25ºC (n=3)

Type of mixture	**Solubility mg/ml**	**%Solubility**
Pure PACL	0.0012±0.03	0.12
PACL: PEG15000(1:10)	0.105±0.06	10.5
PACL: PEG15000(1:20)	0.174± 0.043	17.4
PACL: PEG15000(1:25)	0.185±0.02	18.5
PACL: PEG15000(1:50)	0.302 ±0.024	30.2
PACL: PEG35000(1:10)	0.301±0.04	30.1
PACL: PEG35000(1:20)	0.306±0.06	30.6
PACL: PEG 35000(1:25)	0.308±0.03	30.8
PACL: PEG 35000(1:50)	0.317±0.081	31.7

Table 3: Percent paclitaxel in PACL -PEG conjugates

Type of mixture	Percent PACL content [n=4 (c.v)]
PACL: PEG15000(1:10)	99.19(1.08)
PACL: PEG15000(1:20)	99.64(0.97)
PACL: PEG15000(1:25)	99.21 (0.81)
PACL: PEG15000(1:50)	99.87 (1.05)
PACL: PEG35000(1:10)	99.57()0.94
PACL: PEG35000(1:20)	98.08(0.93)
PACL: PEG 35000(1:25)	99.78 (0.72)
PACL: PEG 35000(1:50)	99.87 (0.82)

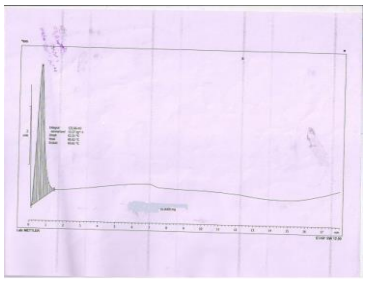

Figure 1: DSCthermogram of PEG35000

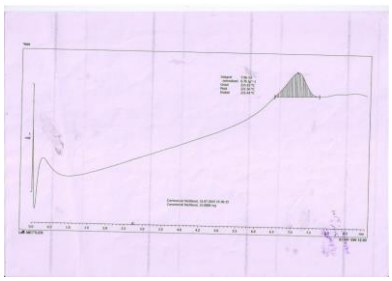

Figure 2: DSC thermogram of pure paclitaxel (Peak=221.93^{0}C)

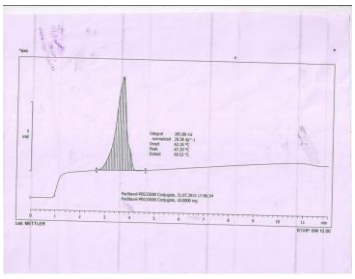

Figure 3: DSC thermogram of PACL:PEG 35000(1:50) (Peak=67.29^{0}C)

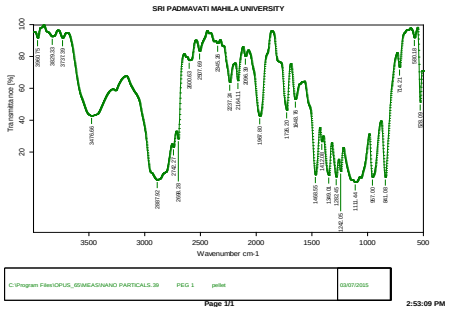

Figure 4: FTIR spectrum of PEG 15000

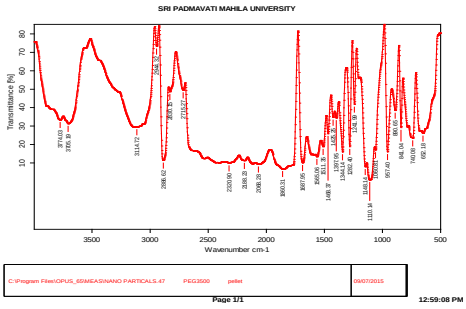

Figure 5: FTIR spectrum of PEG 35000

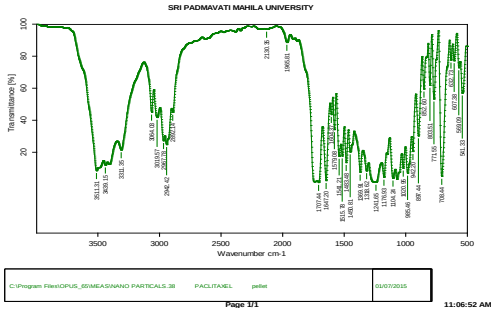

Figure 6: FTIR spectrum of pure Paclitaxel

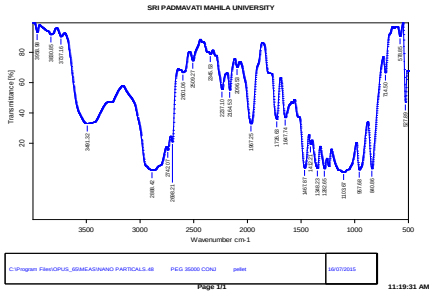

Figure 7: FTIR spectrum of pure Paclitaxel-PEG35000 conjugate

RESULTS AND DISCUSSION

All the conjugates, PACL: PEG15000 and PACL: PEG35000 prepared in weight proportions of (1:10), (1:20), (1:25) and (1:50) were free flowing and the results of various evaluation parameter such as are discussed as followsdrug content uniformity, solubility analysis, FTIR analysis and DSC analysis.

Solubility analysis of paclitaxel-PEG conjugates

The results solubility studies are shown in Table 2. As indicated pure paclitaxel possessing percentage solubility of 0.12% indicated that it is a practically insoluble drug[8]. There is reasonable enhancement in case of PACL-PEG conjugates. PACL: PEG15000(1:50) exhibited 0.302 ±0.024 mg/ml and all other PACL: PEG35000 conjugates of ratios (1:10), (1:20) (1:25) and (1:50) also evidenced same values with slight variation. These results indicated free solubility of paclitaxel when it is converted as conjugates with high molecular weight polyethylene glycols, PEG35000 and PEG35000. PACL: PEG35000 possessed high value of 0.317±0.081mg/ml.

Drug content uniformity

The percent drug content values of all the prepared conjugates are given in Table 3. Percent drug content values were obtained in the range of 98.08% to 99.87 %. There was no significant loss in the drug content was observed in all the methods used for the preparation of PEG conjugates. However, 2-3% loss was observed in conjugates which may be due to loss of the drug during the process. The low c.v. values in percent drug content values ensured the uniformity of drug in each batch. All the values were in agreement with the theoretical values.

DSC

DSC thermogram of PEG 35000, pure paclitaxel and PACL:PEG 35000 that exhibited highest aqueous solubility are

shown in Figure 1, 2 and 3 respectively. The DSC thermograms of PEG 35000 shown in Figure 1 exhibited sharp melting endotherms at 66.62^{0}C. correlating with reported value[9].

The DSC thermogram of paclitaxel, and the promising conjugates are presented in Figure 2 and 3 respectively. Endothermic peaks were obtained for paclitaxel at 221.57^0 C. In Figure 3, PACL:PEG 35000 (1:50) showed indicated its endothermic peak at low temperature of 67.29^0 C. There is a shift in the endothermic peak of the PEG conjugate compared to the respective pure drug. This indicates formation conjugate formation of paclitaxel with PEG s.

Fourier transform infrared spectroscopy of PEG 15000 and PEG 35000

FTIR spectra of PEG 15000 and PEG 35000 presented in Figure 4 and 5 revealed characteristic absorption peaks at 3418 cm^{-1} due to aliphatic –OH group, 1110.11 cm^{-1} due to primary alcohol. The presence of these end hydroxyl groups indicated their suitability for drug conjugation.IR spectrum of pure paclitaxel shown in Figure 6 presented the peaks of its functional groups i.e., primary amine –N-H vibrational stretch at 3311.35 cm^{-1} and–strtche vibrations due to hydroxyl groups between 3511.31 cm^{-1} to 3311.35 cm^{-1} . The C=O stretch was also observed at 1707.44 cm^{-1} .Absorption stretch of C-N- of primary amine present at 1241.93 cm^{-1}. No. of -C-H in plane deformations are present in between942.2 to 803.51 But in case PACL:PEG 35000 conjugate in Figure 7 peaks of all functional groups of paclitaxel are exept –N-H vibrational stretch at 3311.35 cm^{-1}. This may due to conjugation of end hydroxyl group of PEG with amine group of paclitaxel. At this juncture it is expected that PEG conjugates will act as prodrugs and dissolution of polyethylene glycol moieties leads to release of parent drug paclitaxel in body fluids without effecting required therapeutic effect.

CONCLUSION

Polyethylene glycol conjugates of paclitaxel can be prepared successfully by solvent evaporation technique using high molecular weight PEGs such as PEG15000 and PEG15000 to enhance solubility of paclitaxel so that these conjugates can be used successfully in oral or IV infusions to exhibit enhanced solubility, dissolution and bioavailability.

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