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Formulation Development and In Vitro Evaluation of Dendrimer Stabilized Paclitaxel Nanocrystal

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ABSTRACT

Due to its rapid development, nanotechnology has significantly improved treatment methods by enabling the site-specific release of chemotherapeutic medicines depending on their physicochemical and biological characteristics. The preparation and assessment of a dendrimer stabilised paclitaxel nanocrystal formulation were the goals of the current investigation. First, pure paclitaxel nanocrystals were created by ultrasonically precipitating an antisolvent. It is discovered to be practical, affordable, and more effective to make paclitaxel nanoparticles utilising the anti-solvent method methodology using methanol, ethanol, ether diethyl sulphoxide, and tween 80 as hydrophilic colloidal carriers and stabilisers. These findings point to the potential value of paclitaxel and dendrimer nanocrystals as a promising drug delivery paradigm for anticancer therapy.

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INTRODUCTION

A naturally occurring diterpenoid known as paclitaxel is derived from the bark of the Taxus brevifolia, often known as the Pacific yew tree.[1] Paclitaxel showed anticancer action in clinical trials by binding to microtubules with high affinity, stabilising and promoting tubulin polymerization, and suppressing spindle microtubule dynamics.[2-4] These processes, which result in apoptotic cell death, include cell motility, mitosis, and intracellular transport. Unfortunately, paclitaxel's poor water solubility (0.3 µg/ml) has hindered the development of the drug's clinical use in its natural form.[5] Chemically altering the naturally occurring paclitaxel molecule to boost solubility is challenging because it lacks a functional group in its structure.[6] To enhance the clinical use of this anticancer drug, it is therefore especially important to choose the right delivery vehicles for paclitaxel. To increase the solubility and pharmacological characteristics of paclitaxel, a number of delivery methods have been studied. [7–10] These include micelles, liposomes, microparticles, nanoparticles, the use of cosolvents, and cyclodextrins.

With increasing dendrimer production, dendritic macromolecules often acquire a more globular form and linearly expand in diameter. In order to study the effects of polymer size, charge, and composition on biologically relevant properties like lipid bilayer interactions, cytotoxicity, internalisation, blood plasma retention time, biodistribution, and filtration, dendrimers have thus emerged as an ideal delivery vehicle candidate.[11] Beginning with a centre atom or cluster of atoms known as the core, dendrimer molecules have this structure. Through a number of chemical processes, 'dendrons'—branches of other atoms—grow from this central structure. The precise structure of dendrimers, namely whether it is fully stretched with the greatest density at the surface or whether the end-groups fold back into a closely packed interior, is still up for debate.[12-14] One benefit of this technology is the ease with which the dendrimer's surface can be modified with the proper functional groups during the last step of the synthesis process. Additionally, it is a fast synthesis that makes it possible to make enormous dendrimers. This process needs a lot of purification because the final product and the intermediate reactants have similar molecular weights, charges, and polarities. Additionally, branching defects are more likely to occur at higher generations because it is more difficult to couple new branches to existing ones when there are bulky branches present. Despite these difficulties, this process is still the most used way to make dendrimers today due to its benefits. Conversely to divergent synthesis, convergent synthesis allows for the production of dendrimers from the surface up. [15-17] The multiple branching sites are

integrated with new monomers to create the dendrimers as the molecule develops at the ends of the chain.

These branches are linked to a central core once the proper generation size has been reached. Contrary to divergent growth, this method enables simpler purification due to higher disparities between the final products and the initial reagents. Additional benefits include lower branch faults and more monodispersity for low generations. The main drawbacks include a lower yield and difficulty reaching higher generations due to steric obstacles encountered when the branches are connected to the core. It is obvious that novel paclitaxel administration methods that improve drug solubility while removing side effects are needed.[18, 19] A nanocrystal formulation is a potential substitute for the standard paclitaxel delivery mechanism. A nanocrystal is made up of crystalline particles that are nanosized and may or may not be stabilised by one or more suitable stabilisers.[20, 21] Hydrophilic polymers and/or surfactants are frequently added to nanocrystal formulations in order to stabilise them. When these polymers or surfactants are adsorbed onto the surface of the nanocrystals and/or change the liquid environment's dielectric constant, the nanocrystals are stabilised by repulsion caused by either steric or electrostatic hindrance.[22]

In the current study, formulations of paclitaxel nanocrystals with dendrimer acting as a stabiliser are developed and evaluated. Successful fractionation of dendrimer into various fractions allowed for analysis of the stabilising effect. The major components of the most effective fraction were then analyzed further as potential stabilizers for the paclitaxel nanocrystal formulation.

MATERIALSA AND METHODS

Material:

Paclitaxel was purchased from Yarrow Chem Pvt. Ltd, Mumbai. Methanol, Ethanol was purchased from S.D. Fine Chemicals. DMSO and Ether methanol were purchased from Sigma-Aldrich, USA. Acetone, dichloromethane, and acetonitrile were purchased from Merck, Germany.

Methods:

Fourier Transform Infrared Spectroscopy (FTIR):

Powders of paclitaxel, was examined by FTIR, using a spectrophotometer model ATR- FTIR Perkin-Elmer 100S and Samples were taken in a KBr pellet, and scanned in the IR range from 600 to 4000 cm^{-1}. [23]

NMR Characterization:

Paclitaxel drug "solution was freeze-dried and reconstituted in deuterated water (D_2O) or deuterated methanol (CD_3OD) at room temperature" to demonstrate the PTX properties of the for the PTX-loaded nanoparticles formulation. A Varian 400 MHz spectrometer was used to capture 1H-NMR spectra of free PTX in CD3OD, paclitaxel nanoconjugate in D_2O, and PTX in D_2O and CD_3OD (Varian, Palo Alto, CA, USA). [24]

Mass Spectra:

A Finnigan TM LCQ TM DECA instrument with an ion trap was used to collect mass spectra. For sample analysis, an ionisation device was utilised. The programme Xcalibur 2.0 SR2 was used (Thermo Electron Corporation 1998-2006). [25]

Ultraviolet Spectroscopy:

On a GBC Cintra-10 UV/Visible Spectrophotometer, spectral and absorbance measurements were taken at a "scan speed of 1400 nm/min with a data interval of 1.006 nm and a constant slit width of 2.0 nm". Because PTX is easily soluble in methanol but essentially insoluble in PBS 7.4, a mixture of Methanol and PBS was utilised (30:70). As a co-solvent, methanol was utilised. Dissolving 10 mg of medication in 30 ml of methanol and diluting to 100 ml with Methanol: PBS yielded a stock solution of PTX (100 µg/ml) (30:70). The Absorption Maxima (max) of the working standard solutions were determined by scanning them in the UV spectrum (between 200 and 400 nm). PTX's absorption maximum (max) was discovered to be 230 nm. At their respective absorbance maxima, PTX demonstrated strong linearity with absorbance in the concentration range of 2-20 g/ml, and the correlation value was determined to be <1.

Method for the Preparation of Standard Curve:

PTX (10 mg) was weighed precisely and dissolved in 30 mL methanol. To make a stock solution of 100 µg/ml, a volume of 30:70 methanol: PBS was prepared up to 100 ml. Volume was adjusted with 30:70 methanol: PBS to obtain final concentrations of 2.0-20.0 µg/l by transferring aliquots of 100 µg/ml solution into various 10 ml volumetric flasks. At 230 nm, the absorbance of 3 ml methanol made up to 10 ml with 30:70 methanol was measured: PBS as a starting point. [26]

FORMULATION DEVELOPMENT:

Precipitation of PNCs and Surface Functionalization:

PTX nanocrystal were prepared by anti-solvent method. 20 mg of PTX was dissolved in organic solvent .and then the solution was injected in to distilled (DI) water containing stabilizer under stirring for 5 min. Forming the suspension of PTX different solvent were evaluated including methonol, ether and diemethyl sulfoxide. (DMSO) and the ratio of solvent to antisolvent was also investigated. The stabilizer chose from dendrimer G4 tween 20 tween 80 the obtain suspension subjected to ultrasonication for 10 mnt. At 300 w using an ultrasonic probe after sonication the PNCs were centrifuged at 20000 g for 1h.

Preparation of PTX Nanocrystals:

Formulation-1 (With Dendrimer G4 PAMAM):

9.56 mg PTX dissolved in 2 ml methanol, then add 19.88 µl G4 PAMAM dendrimer. Now slowly-2 3 ml deionized water doing sonicated (Bath Sonicator) under high-speed stirring (4 °C Temperature) 10 min. The suspension then filtered with MILLE X. GV filter unit 0.22 µm and the retentate resuspended in deionized water by Homogenization.

Formulation-2 (Without Dendrimer):

3 gm PTX dissolved in 2ml ethanol and methanol mixture. Then introduced 3ml of deionized water in a flask the solution sonicated (Bath Sonicator) under high-speed stirring (4 °C temperature). The suspension then filtered MILLEX.GV filter unit 0.22 µm and the retentate resuspended in deionized water by homogenization.[27, 28]

Table No.1: Formulation Development of Paclitaxel G4 PAMAM Dendrimer

Sr. No.	Formulation	Dendrimer µml (G4)	PTX (µg)	Methanol (ml)	Deionized Water (ml)
1.	DPF1	70	2240	2	3
2.	DPF2	70	2240	2	3
3.	DPF3	70	2240	2	3
4.	DPF4	70	2240	2	3

EVALUATION OF FORMULATION

Surface Morphology:

Scanning electron microscopy (SEM) was used to examine the surface morphology of the generated formulation samples using a JOEL JSM-T330A Scanning Microscope. Double-sided conductive tape was used to secure samples in a metallic stub (58).

Zeta Potential: Using a zeta potential analyser, the prepared nanoparticle suspension was analysed in terms of zeta potential (Malvern Zeta sizer). A zeta potentiometer was used to evaluate electrophoretic mobility and zeta potential (Malvern Zeta sizer). Nanoparticles samples were diluted in KCl (0.1 mm) and put in an electrophoretic cell with a 15.2 V/cm electric field to determine the zeta potential (59-60) Each sample was examined three times.

***In vitro* Release of paclitaxel:** *In vitro* dissolution experiments for formulated formulations were conducted using a USP dissolution XXIII equipment, Type II, with 900ml phosphate buffer solution (pH 7.4±0.1), 37±0.1 °C, 75 rpm, and 900ml phosphate buffer solution (pH 7.40.1). Weighed nanoparticles containing 100 mg of paclitaxel were maintained for dissolution. Whatman filter paper No. l was used to filter 5 ml of the withdrawn material. In a 10 ml volumetric flask, 1 ml of the filtrate was made up to 10 ml with methanol. When necessary, more dilutions were prepared. The samples' absorbance was measured at 425 nm against a blank.[28, 29, 30]

RESULTS AND DISCUSSION

FTIR Spectrum of Drug:

The acquired sample's FTIR spectrum was obtained using the approach outlined in the materials and methods section, and the FTIR spectra of the sample medication showed identical distinctive peaks. The FTIR spectra of the sample drug were displayed in Figure No.1.

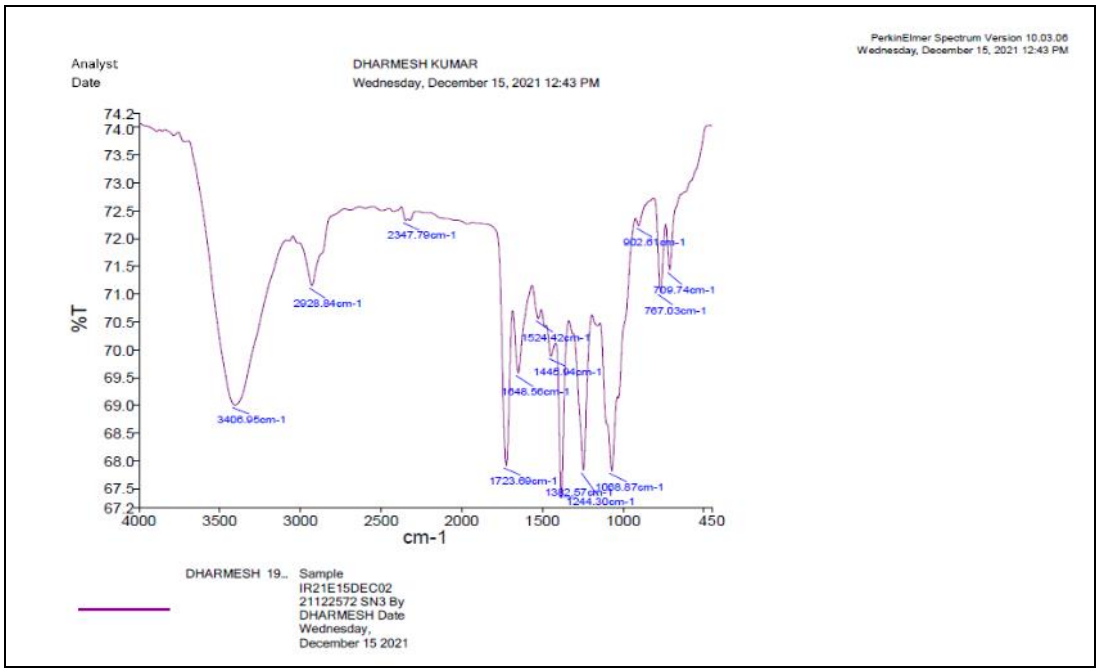
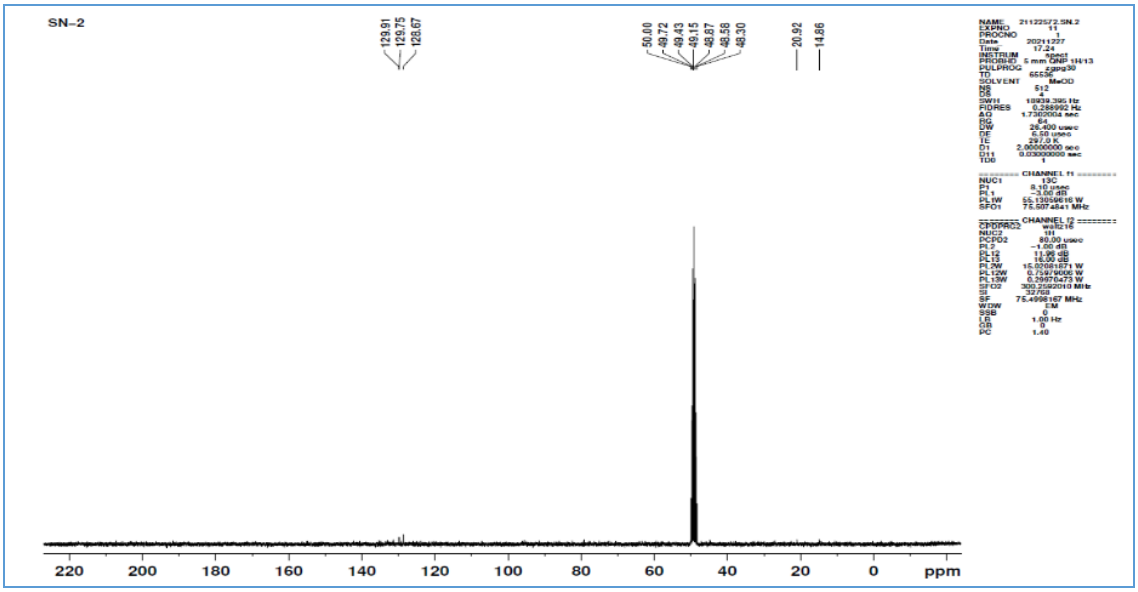


Figure No.1: FTIR Spectra of Drug

NMR Spectra:

The concentration-dependent examination of a tidy paclitaxel sample in $CDCL_3$ at 298 K was shown in Figure 2. Except for the three OH protons, the proton signals of paclitaxel a chemical changes in solution 1H NMR spectra. When the sample was diluted from 100 mg/ml to 20.3 mg/ml, the three OH protons migrated upfieid. Since this was a typical NMR detection of the phenomena of intermolecular hydrogen bonding of the OH groups, the result was as predicted. These three OH protons generate intermolecular hydrogen bonding, according to the crystal structure of paclitaxel.


SN-2
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129.75
128.67
50.00
49.72
49.43
49.15
48.87
48.58
48.30
20.92
14.86
220
200
180
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140
120
100
80
60
40
20
0
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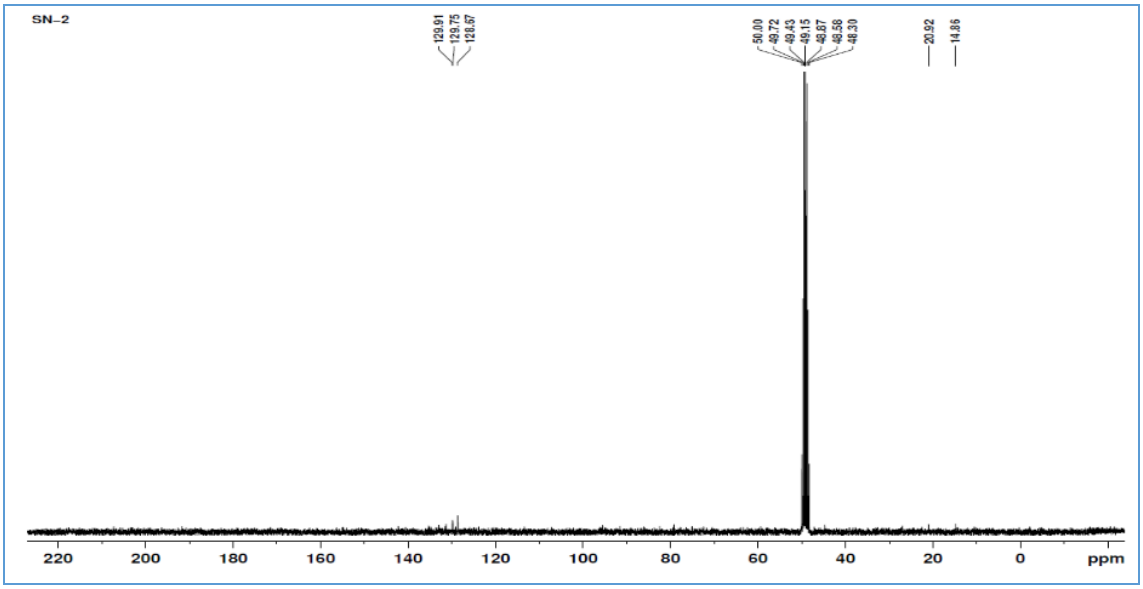
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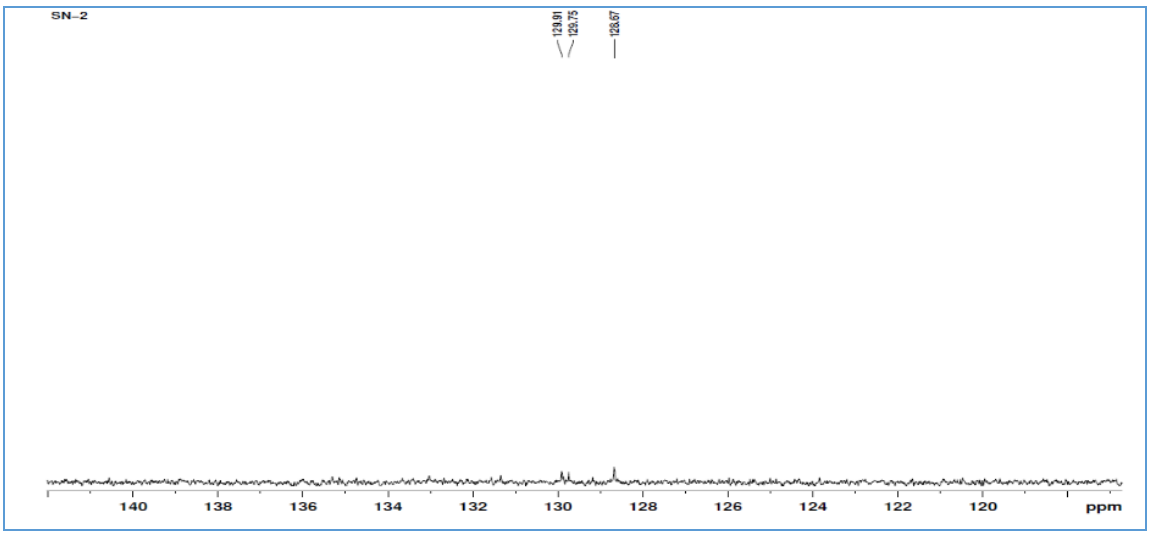
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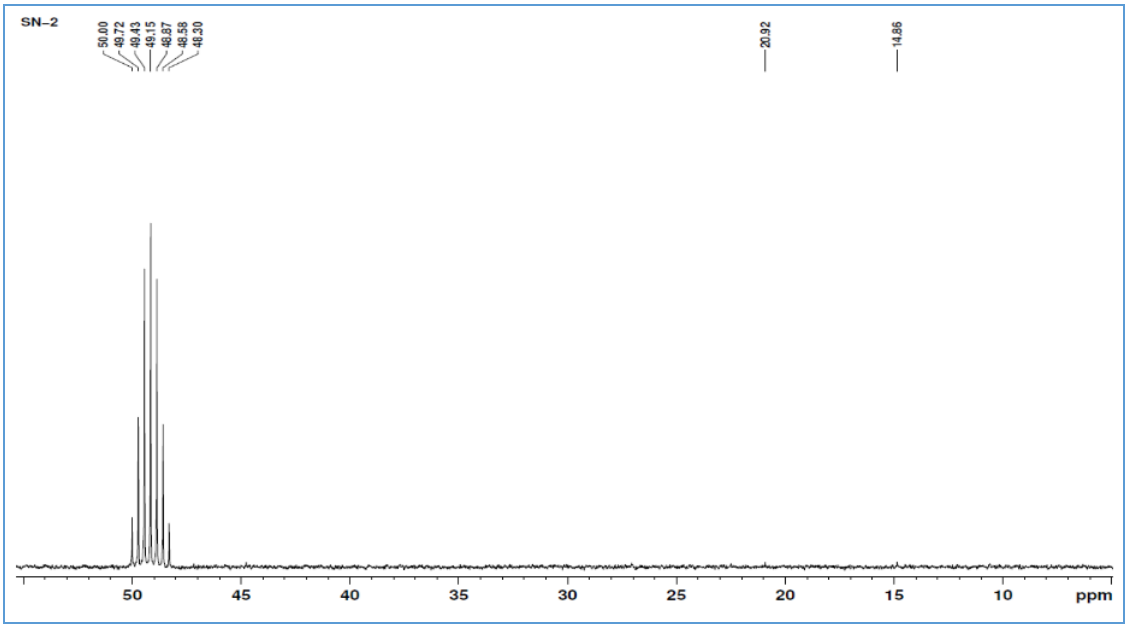
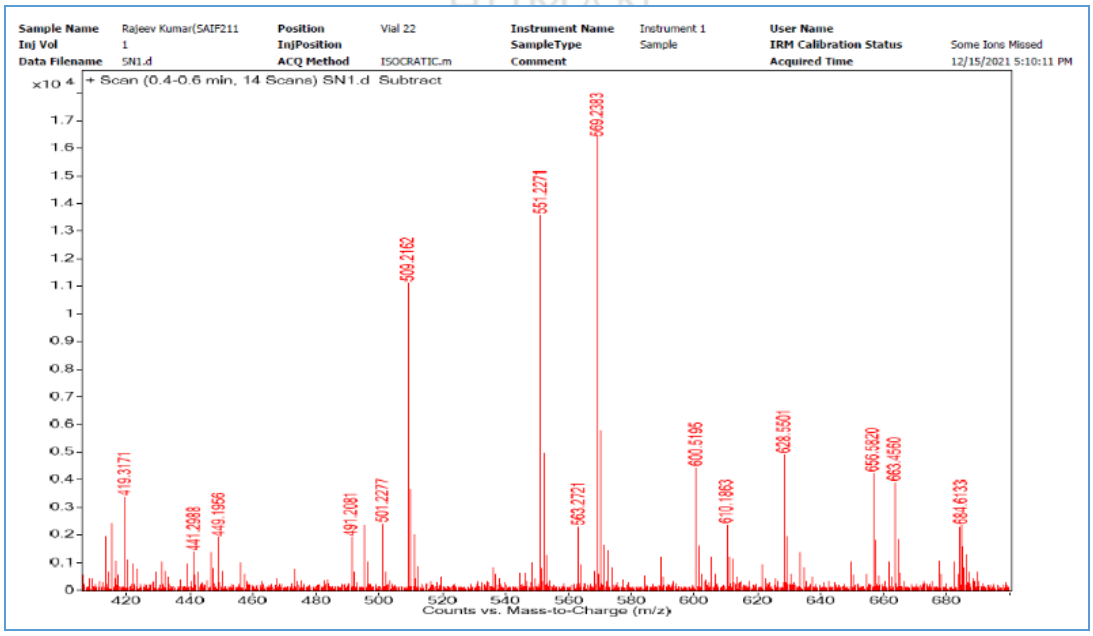


Figure No.2: ^{1}H-NMR Spectra of Drug

Mass Spectra:

The structural changes generated by cationization were investigated by looking at the IM distributions of PTX and its hydroxylated metabolites. The mass spectra of the PTX ions with different cations are displayed against the m/z values versus intensity in Figure No.3.



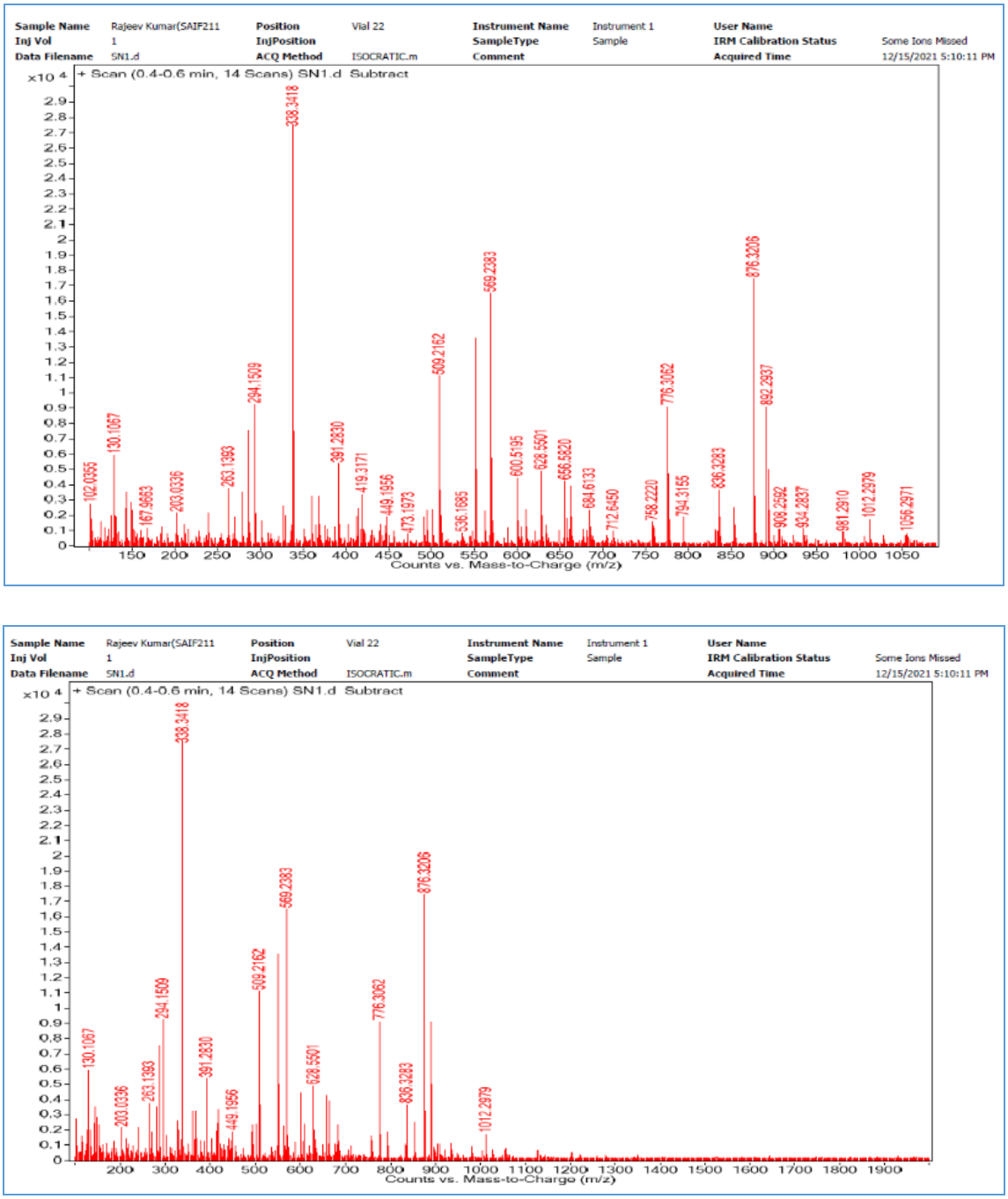


Figure No.3: Mass Spectra of Drug

UV Spectra of Drug:

As a result, more changes are necessary. The UV spectrum of paclitaxel in methanol: PBS was scanned, and typical peaks at 227 nm in the 200-400 nm spectral region were discovered. The UV spectrum report is shown below in Figure No.4.

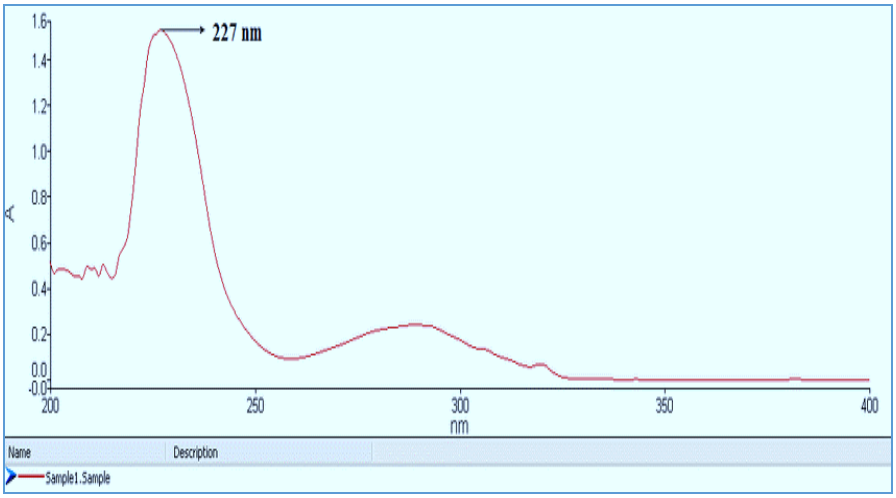


Figure No.4: UV Spectrum of Paclitaxel

Calibration curve of paclitaxel revealed that the graph obeyed Beers Lambert Law in the concentration range (0-20µg/ml). Regression equation was found to be: y 0978x + 0 0025 and high coefficient correlation of 0.9999 was also observed. Figure 5 showed the mean absorbance of different concentration of paclitaxel in Methanol: PBS (30:70).

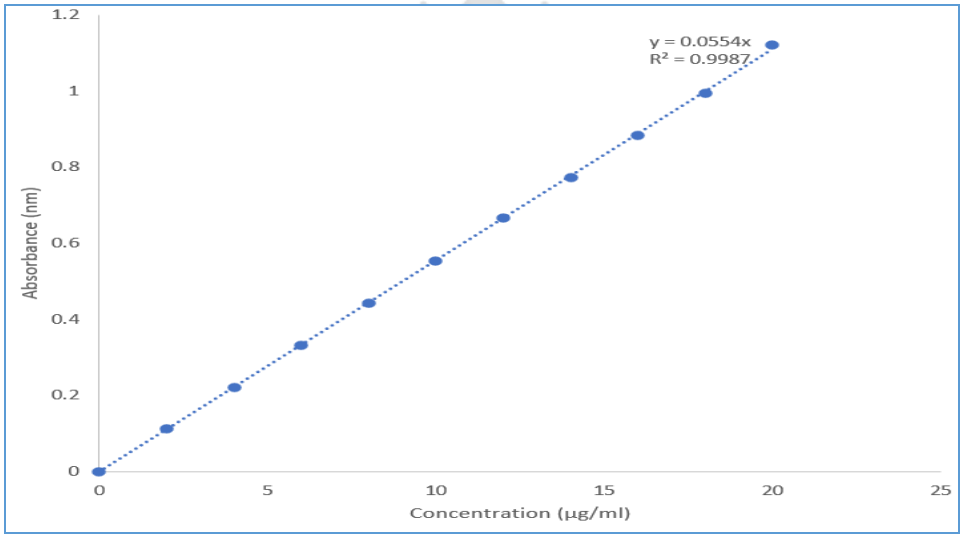


Figure No.5: Calibration Curve of Paclitaxel

EVALUATION OF DENDRIMERIC NANOCRYSTALS

Visual Examination:

The optical microscope examination confirmed that vesicle was found at 45 °C. The visual examination of dendrimer nanocrystal was confirmed by using optical microscope and the results were given below in Figure No.6.

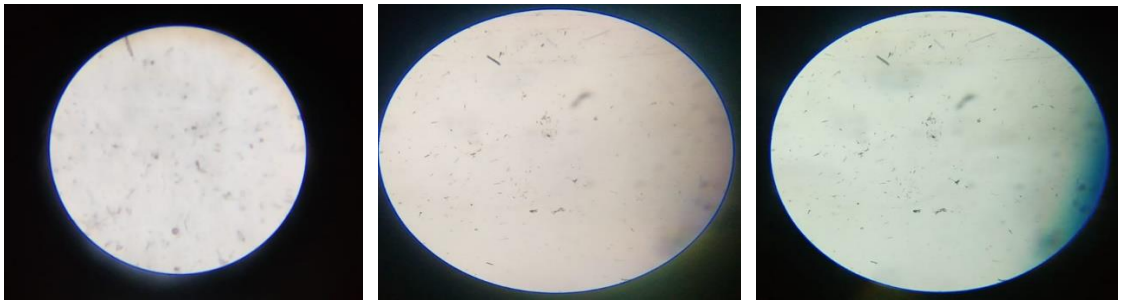


Figure No.6: Nanocrystal of Paclitaxel

Zeta Potential of Formulation: The surface charge of the nanoparticles was determined by using a zeta metre with a 3M resolution to measure the zeta potential of the nanoparticles. Table No.2 shows the results, and the zeta potential of all formed nanoparticles was in the range of 11.9 to 16.6 mV, as shown in Table 2, indicating that they are relatively stable according to the thumb rule.

Table No.2: Zeta Potential of Dendrimer Nanoparticles

Sr. No.	Formulation Code	Zeta Potential
1.	DPF1	11.9
2.	DPF2	16.6

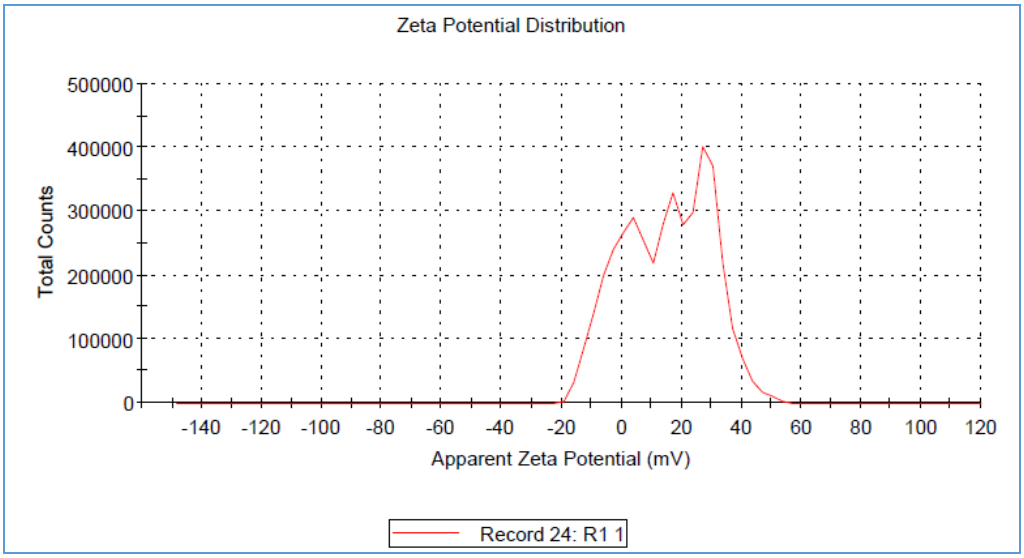


Figure No.7: Zeta Potential of Formulation DPF1

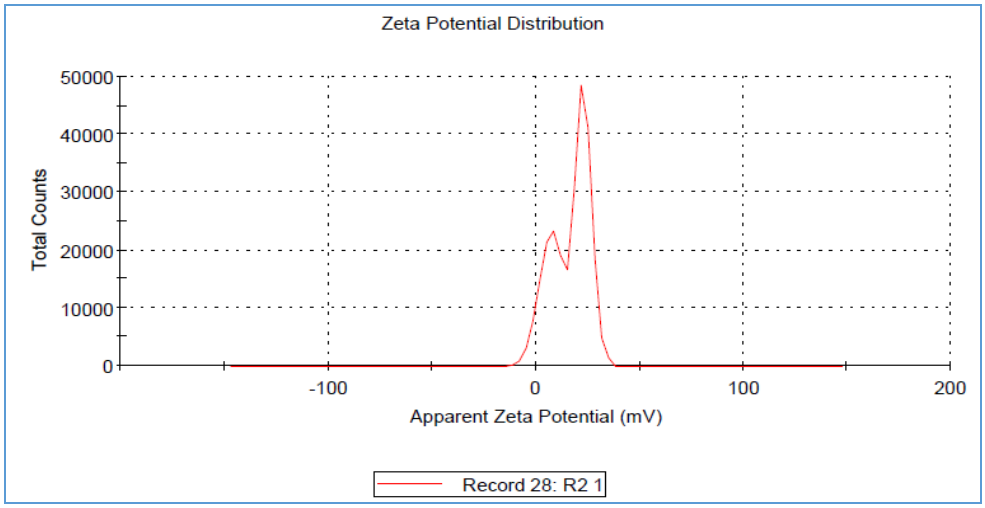


Figure No.8: Zeta Potential of Formulation DPF2

***In vitro* Drug Release Profile:**

The findings of in vitro dissolution of pure paclitaxel nanoparticles were given in Figure No.9 to see if the aim of enhancing the rate of paclitaxel dissolution was met. Raw micro-sized paclitaxel dissolved more quicker than nanosized paclitaxel. The diffusion technique was used to test the in-vitro release of all generated dendrimeric nanocrystal compositions. The percentage of drug entrapment efficiency is related to the rate of drug release, according to the research. Paclitaxel nanoparticles were dispersed in phosphate buffer for in vitro disintegration. After 24 hours, the percent CDR of paclitaxel nanoparticles DPFI, DPF2, DPF3, and DPF4 was determined to be 77.45, 82.01, 89.76, and 78.22 percent, respectively.

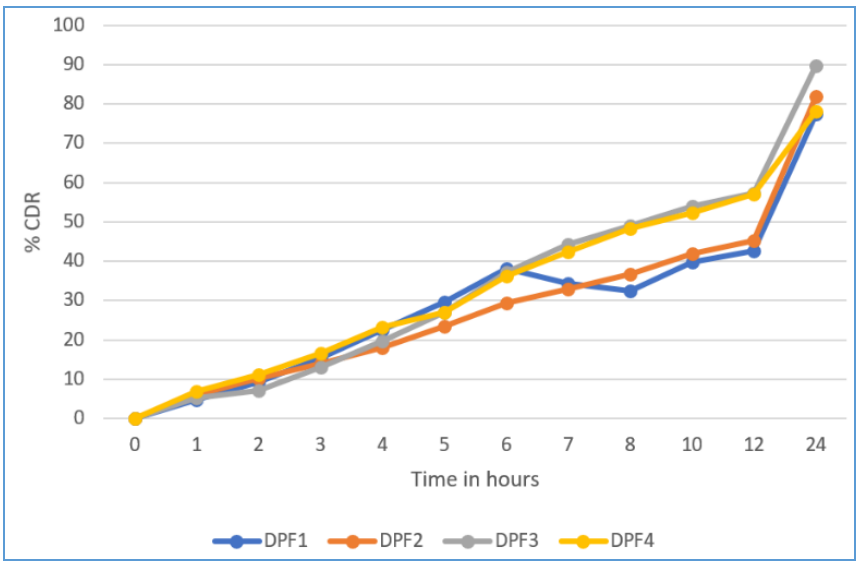


Figure No.9: % Cumulative Drug Release of Formulation DPF1-DPF4

CONCLUSION

This study has shown that Paclitaxel dendrimeric nanoparticles can be produced using the anti-solvent method. The homogenization process and stabilisers were carefully chosen to achieve stability during controlled crystallisation and to improve the wettability of hydrophobic medicines in dissolving media. The stability of paclitaxel with the different excipients used in our inquiry has been validated by FT-IR NMR, Mass, and UV studies, showing that the formulation approach is appropriate for the production of weakly water-soluble medicines in nanoscale.

- Preparation of paclitaxel nanoparticles using anti-solvent method technique by the use of methanol, ethanol, ether diethyl sulphoxide hydrophilic colloidal carrier, tween 80 as stabilizers is found to feasible, economical and more productive.

- Paclitaxel to Dendrimer 70:2240 has fond to be the optimum formulation with reference to various physicochemical characteristics such a dissolution profile. encapsulation efficiency etc.

- The dissolution profile revealed that nanoparticles significantly improved the efficacy of the paclitaxel dendrimer formulation.

- Extensive investigations are required prior to formulation optimization in order to achieve a safer, more effective, and cost-effective product for human consumption.

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