HA-PEG-PCL Nanoparticles for Targeted Delivery of Paclitaxel to Colon Cancer

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Abstract

For improved treatment efficacy with no or few adverse effects and higher patient compliance, novel delivery techniques with selective dispersion are required. The biodegradable and biocompatible polymers hyaluronic acid (HA), polyethylene glycol (PEG), and polycaprolactone (PCL) were used to create paclitaxel-loaded nanoparticles. The development and characterisation of a Paclitaxel-loaded HA-PEG-PCL polymeric nanoparticulate drug delivery system were the goals of the current study work in order to increase the therapeutic efficacy and targeted Paclitaxel distribution. The copolymers were chemically created, and IR spectroscopies were used to identify them. Transmission electron microscopy, particle size, percentage of drug entrapment, cell viability studies, stability, and in vitro drug release profile were used to describe the nanoparticles' structure and morphology. To determine whether PTX was crystalline or amorphous inside the polymer matrix, experiments using differential scanning calorimetry and X-ray diffraction were also carried out. Zeta potentials for blank NPs and PTX-HA-PEG-PCL NPs were respectively -15.4±1.23 mV and -18±2.35 mV. In 144 hours, PTX-HA-PEG-PCL NPs demonstrated a 58.08±3.8 release of PTX at pH 7.4. Because HA functions as a ligand for the CD44 receptors that are over expressed on HT-29 cells, the PTX-HA-PEG-PCL NPs demonstrated noticeably more cytotoxicity as a result of better formulation internalization. The proposed nanotechnology's-controlled drug release behavior showed its potential for colon cancer cell lines.

Keywords: Biodegradable polymer, Colon cancer, Paclitaxel, Hyaluronic acid, Nanoparticles, Targeted drug delivery.

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Introduction

The World Health Organization estimates that 9.6 million cancer deaths and 18.1 million new cancer diagnoses worldwide in 2018, making cancer the most lethal disease on earth by 2020. Out of more than 100 forms of cancer, colorectal cancer is one of the top five with the highest mortality rate. The 5-year overall survival rate for patients with advanced colon cancer, such as those in the metastatic stage, might be as low as 13% [1]. One of the most common malignant tumours originating from the digestive tract is colon cancer. The outcome of localised colon cancer has successfully improved as a result of recent advancements in chemotherapy, surgical technique, and the use of molecularly targeted medications. The prognosis of colon cancer patients with metastases or surgical recurrence, however, continues to be a major source of worry [2]. Due to its exceptional biodegradability, biocompatibility, and low toxicity, hyaluronic acid (HA), a natural macromolecule, is frequently used in biomedical applications, particularly in cosmetics, arthritis treatments, and drug delivery. Recent studies have revealed that the CD44 receptors, to which HA strongly binds, are overexpressed on the surface of cancer cells. As a result, HA can be employed as a target ligand in the administration of drugs that target tumours. A variety of derivatives can be made by changing the structure of HA, which not only maintains its stability but also increases its biocompatibility. The several hydroxyl and carboxyl groups that make up HA stand for different chemical modification sites. The US Food and Drug Administration (FDA) have

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cleared the common glue polyethylene glycol (PEG), which is non-toxic and highly water-soluble, for use in food and a variety of therapeutic applications. When combined with organic molecules, PEG can enhance biocompatibility and boost the solubility of compounds that are only marginally soluble in water. The adsorption of transaminase proteins can be hindered by PEG modification, which prevents their clearance from systemic circulation, extends their half-life in blood, and affects drug delivery system transport. As a result, PEG has been extensively used in research on controlled drug release, wound healing, and pharmaceutical delivery [3]. The Pacific yew tree, Taxus brevifolia, produces paclitaxel (PTX), a potent anticancer drug [1]. The manner of action of PTX is unique. It alters the dynamic equilibrium of the microtubule system, inhibiting cell replication and stopping cells in the late G2 and M phases of the cell cycle [4]. Chemotherapy has been and is one of the most effective cancer therapies, despite the fact that many of the drugs used are cytotoxic and have serious side effects. One of the most popular and effective chemotherapy drugs is paclitaxel. It can also cause vasodilation, heavy breathing, lethargy, and hypotension [4]. It can also cause neurotoxicity, nephrotoxicity, endothelium, and vascular muscle effects. Only a few of the major adverse effects include alopecia, nausea and vomiting, myelosuppression, and peripheral neuropathy. Paclitaxel can move outside of the tumour location since it is given intravenously, which increases the likelihood that it will have negative effects [5]. Although paclitaxel is a first-line chemotherapeutic medication for solid tumours, its use has been made challenging by factors such as limited solubility, toxicity, and the development of resistance during the course of treatment. Paclitaxel is often used with cisplatin and other antineoplastic drugs by doctors to increase effectiveness and prevent the development of resistance. As nanotechnology has advanced, numerous kinds of paclitaxel nanoparticles (NPs) have been created to modulate drug release, promote drug encapsulation, enhance bioavailability, and target cancer cells [6]. These NPs use polyethylene glycol, polylactic acid, polyglycolin acid, or liposomes as frame materials. This study aims to investigate the therapeutic potential of PTX-loaded HA-PEG-PCL nanoparticles (PTX-HA-PEG-PCL NPs) for colon cancer. This was accomplished by ionic cross-linking of PTX-HA-PEG-PCL NPs. The plain and drug-loaded NPs were characterised using TEM, zeta potential, FT-IR, and DSC. The created NPs were then examined for anticancer activity, cellular uptake, and in vitro drug release.

Materials and Methods

The supplier of paclitaxel was Hi Media Lab in Mumbai, India. From Sigma-Aldrich, we bought polycaprolactone, hyaluronic acid, and poly (ethylene glycol) diamine (USA). Pluronic F-68, 1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride, and DMSO (dimethyl sulfoxide) were bought from Hi Media Lab in Mumbai, India. From Qualigen Fine Chemical Ltd in Mumbai, India, acetone was acquired.

Copolymer synthesis and characterization

Diamine PEG (0.125 mg) was dissolved in 10 ml of anhydrous DMSO, and then agitated at room temperature (RT) for the duration of the next day. Transferred to the reaction flask was activated HA (0.574 g of HA in distilled water with 230 mg of EDC added at a 1.2-fold molar concentration). The reaction was permitted to run at RT for 48 hours. By using a dialysis molecular weight cutoff (MWCO) of 12,000–14,000 Da against water for 24 hours, the product was separated from the organic (DMSO) phase and vacuum dried.

Preparation of nanoparticles (NPs) formulations

With a few minor adjustments, HA-PEG-PCL NPs were created in accordance with the already published procedure [7]. 10 ml of organic solvent (such as water and dichloromethane) were used to dissolve 10 mg of the HA-PEG-PCL copolymer while it was held at room temperature for 15 minutes. Drop-by-drop, under magnetic stirring at 5000 rpm for approximately 6 hours, organic solution was transferred to the aqueous phase with varying quantities of surfactant (Pluronic F-68) solution and medication (1 mg). The suspension that resulted was then passed through a 0.45 m membrane filter. NPs formed right away and evaporated the solvent overnight at room temperature using a Bucchi Rotavapor R-300 rotary evaporator made by Thermo Scientific in the US.

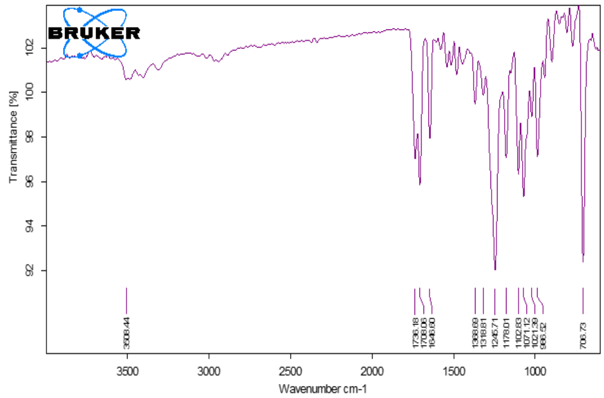

Figure 1 FTIR of Paclitaxel

Characterization of NPs

Morphological observations

Negative staining with phosphotungstic acid (PTA, 1.0% w/v) was utilised to analyse the form and morphology of the produced NP using transmission electron microscopy (TEM). Briefly, 20µl of NP (1.0 mg/ml) solutions were applied to a 200-mesh copper grid and placed on the formvar/carbon side (TED PELLA, Redding, CA, USA). The zeta potential of the nanoparticles was ascertained using a zetasizer Nano ZS90 (Malvern Instruments, UK, Malvern) [8]. The DSC 1 STAR System (Mettler Toledo, USA) was used to perform a differential scanning colorimetry (DSC) analysis with ceramic: FRS6 serving as the primary sensor material. In the temperature range of 0 to 350 °C, the nitrogen gas flow rate was 100 mL/min, and the scanning rate was 10 K/min [1].

Entrapment efficiency and loading efficiency

Centrifuging PTX-HA-PEG-PCL NPs allowed researchers to measure the PTX's entrapment effectiveness (EE) (20,000 rpm, 45 min). The pellet was treated in DMSO and sonicated to extract PTX from NPs. After being stirred up all night, the mixture was centrifuged, and the supernatant was analysed spectrophotometrically. The final medication concentration in the NPs was compared to the initial concentration employed in the encapsulation assays (n = 3). The weight of the NPs obtained after centrifugation (n = 3) was used to calculate the system's loading efficiency (LE) [8, 9].

$$\text{EE \%} = \frac{\text{Total amount ofPTX entrapped inside the pellet}}{\text{Initial amount of PTX taken for drug loading}} \times 100$$

$$\text{LE \%} = \frac{\text{Total amount ofPTX entrapped inside the pellet}}{\text{Total amount of NPs obtained}} \times 100$$

In vitro drug release study

Under sink circumstances, PTX in vitro release profile from HA-PEG-PCL NPs was evaluated at pH 7.4 and 37 °C. The drug-loaded pellet was resuspended in 3 mL of PBS and put in a 10 kDa dialysis bag in a beaker with 50 mL of PBS. The shaker incubator that housed the complete arrangement was adjusted to 37 °C. 1 mL of PBS was withdrawn and new PBS was added at regular intervals. Spectrophotometric analysis was used to calculate release [10]. Three distinct release media with varying pH values were used for the release investigation (pH 2, 5, and 7.4). Plotting against time showed how much of each medication was released from the HA-PEG-PCL NPs.

Cell viability study

The cytotoxic potential of free PTX and drug-loaded HA-PEG-PCL NPs on HT-29 colon cancer cells was evaluated using the MTT test. In a 96-well plate, cells were sown and left 24 hours to adhere. The cells were treated with fresh media and exposed to different dosages of the drugs as well as an equivalent concentration of NP. After 24 and 48 hours, the medium was replaced with 100 ml of MTT solutions (1.25 mg/ml) and incubated for 3 hours at 37 °C. After applying DMSO to remove the formazan crystals, the absorbance was measured at 227 nm with a microplate spectrophotometer (UVM 340, Biochrom Asys, Italy) [11]. The cell viability was determined using:

$$\text{Cell viability (\%)} = A_{sample}/A_{control}\text{-}100$$

The absorbance value is which A. In order to determine the IC50 a value, GraphPad Prism 6.0 was used (La Jolla, CA, USA).

Hemolytic toxicity

HiAnticlot blood collection jars were used to collect whole human blood (Himedia Labs). Centrifugation was used to separate the red blood cells (RBC), which were then resuspended in a normal saline solution. After preparing the volume to 10 ml with normal saline, one millilitre of red blood cell (RBC) suspension was incubated separately with pure PTX, distilled water (taken as 100% hemolytic standard), normal saline (taken as blank for spectrophotometric estimation), and HA-PEG-PCL and PCL nanoparticles containing equivalent amounts of PTX (200 mg/ml). With intermittent shaking, the sample tubes were allowed to stand at 378°C for one hour. The tubes were then centrifuged for 15 minutes at 3000 rpm, and the supernatant's absorbance at 227 nm was used to calculate the percentage of hemolysis in comparison to the absorbance of distilled water, a standard that is 100% hemolytic and is diluted identically, for comparison [12].

Stability study

The lyophilized PTX-loaded HA-PEG-PCL powders were stored at 25 °C and 4 °C in a sealed container for six months. After six months of storage, the particle size and surface charge were evaluated using the ZetaPALS® analyzer. The quantity of PTX that was still present was assessed using HPLC. Briefly, 100 mg of the sample were dissolved in 1 mL of pure EtOH, sonicated for 30 seconds at an amplitude of 40%, and then centrifuged for 30 minutes at 31,514 g. To determine the amount of PTX in the supernatant, a standard curve with a concentration range of 3.125-200 g/mL, y = 25,628x-1452.7, was employed. The stationary phase was an ACE Generix 5, C18 column (4.6 mm, 150 mm, 5 m), the mobile phase was water:acetonitrile (11:9 v/v), and the HPLC conditions were created using USP and ICH validated standards. The system was run at a temperature of 25 °C, 1.5 mL/min, with a detecting wavelength of 227 nm [1].

Statistical analysis

The results of every test were given as mean standard deviation after being run three times in duplicate. The standard deviation data were represented by error bars in the pertinent plots. The student's t-test was used to evaluate whether these values were statistically significant. A probability of $p < 0.05$ was used to define statistical significance

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Result and Discussion

Table 1 Particle size and zeta potential of blank NPs and PTX loaded HA-PEG-PCL

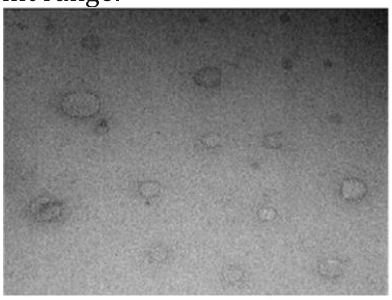
Formulation	**Particles Size (nm)**	**Zeta Potential (mV)**
Blank NPs	294±12.3	-15±1.23
PTX- loaded HA-PEG-PCL NPs	321±08.21	-18±2.35

Table 2 Properties of PTX loaded HA-PEG-PCL NPs at various initial amount of PTX (1 mg, 0.5 mg, and 0.25 mg). The results are expressed in terms of mean ± SD (n = 3)

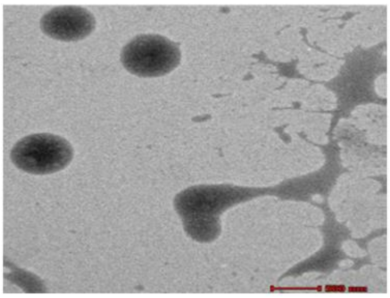
Formulation	**PTX amount (mg)**	**Particle size (nm)**	**Entrapment Efficiency (%)**
PTX-loaded PCL NPs	1	390.8 ± 14.2	71.20 ± 3.49
	0.5	372.1 ± 10.2	65.43 ± 4.65
	0.25	330.5 ± 2.2	57.30 ± 2.16
PTX-loaded HA-PEG-PCL NPs	1	379.2 ± 15.2	93.32 ± 3.27
	0.5	345.3 ± 10.1	92.55 ± 2.98
	0.25	346.7 ± 6.5	90.34 ± 1.50

Morphological observations

This section looked at how the initial PTX dosage affected the parameters of the HA-PEG-PCL NPs (Particle size, in-vitro release, and EE %). First, we contrasted the particle populations with and without PTX. When compared to their blank counterparts, the PTX-loaded HA-PEG-PCL NPs' mean size rose considerably while maintaining a similar zeta potential. All of these peaks vanished in the PTX-loaded HA-PEG-PCL NPs but appeared once more in the PTX-and-blank HA-PEG-PCL NP physical combination. Similar peak patterns were also seen in the FT-IR spectra for the free PTX and the physical combination, particularly in the 400-1000 cm^{-1} fingerprint range.



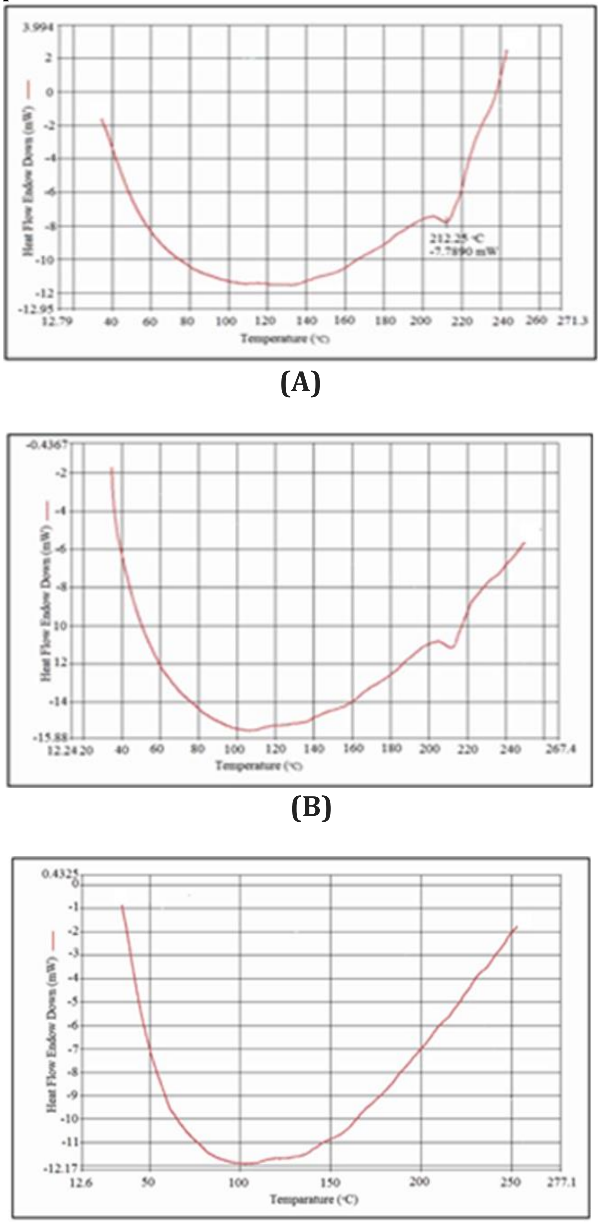
(A)



(B)

Figure 2 TEM images of (a) HA-PEG-PCL nanoparticles (b) PTX-loaded HA-PEG-PCL nanoparticles DSC analysis

PTX's physical condition in the formulation and its interactions with other excipients were confirmed using DSC. The melting endothermic peak on the PTX DSC thermogram, which is attributed to the compound's melting temperature of 212.25°C, was visible. The formulation of the drug-loaded nanoparticles also had an endothermic peak at the same location, indicating that the formulation's drug and the free drug were in the same physical state. Blank formulation, however, lacked a peak.



(A)

(B)

(C)

Figure 3 Differential scanning calorimetry thermogram of (A) PTX (B) PTX-loaded HA-PEG-PCL NPs (C) blank formulation.

In vitro drug release

Behaviors of the HA-PEG-PCL copolymer's PTX release. The PTX release profile showed a biphasic pattern, with a quick initial release and a significantly slower final release. The PTX was adsorbed on and/or enclosed close to the surface of the polymersomes, which caused the first fast release. The subsequent sustained and protracted release way may have been caused by the diffusion of PTX across the thick polymer some membrane. The ambient acidity had an impact on the PTX release, with a faster release rate at pH 5.3 than at pH 7.4. The enhanced solubility of PTX and the weaker contacts between PTX and the polymersomes in acidic circumstances may be the cause of this pH dependent PTX release. Similar quicker PTX release was seen by other research teams at lower pH levels than those associated with normal physiological pH. Without an initial burst, the release of PTX from polymersomes was sluggish and prolonged. The release rates at pH 7.4 and 5.5 revealed modest pH dependence. PTX-PCL-NPs and PTX-HA-PEG-PCL-NPs released 65.34±3.4 and 58.08±3.8 at pH 7.4 in 144 hours, respectively, in comparison. The strong hydrophobic contact between PTX and the hydrophobic PCL membrane of the polymersomes may be the cause of the gradual and continuous release of PTX from the HA-PEG-PCL [7]. For boosting drug accumulation at the tumour site and attaining intracellular release of anticancer medicines, the prolonged and pH-dependent release of PTX is crucial. 5437

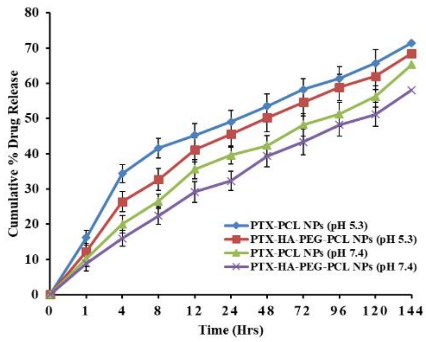


Figure 4 PTX release from HA-PEG-PCL NPs

Cell viability

Furthermore, there was no evidence of cell death from the blank formulation, indicating that the excipients utilized in the formulation had no impact on the cytotoxicity of PTX (without drug). HT-29 cells were used in an MTT assay to test the anti-proliferative effects of the free medication, HA-PEG-PCL NPs, and blank formulation. Following a 72-hour incubation period, the rate of cell death rose as NP concentration increased and was comparable to that of PTX and free medication. At a concentration of 0.01 mg/mL, the free PTX exhibited minimal cytotoxicity, inhibiting the proliferation of cells by 38.01±0.6%. This might be because cells aren't absorbing the drugs as well. Additionally, PTX-PCL NPs demonstrated a 30.3±1.8% decrease of cell growth, which may have been caused by passive diffusion, minimal intracellular dispersion, or drug concentration. The fact that PTX-HA-PEG-PCL NPs showed a 28.6±1.3% decrease of cell growth could be attributed to the formulation's internalisation through HA receptor-mediated endocytosis. Observations revealed that cell viability was higher at lower concentrations, and considerable cytotoxicity was seen as concentrations were raised. Because HA acts as a ligand for the CD44 receptors that are over expressed on HT-29 cells, the NPs with the copolymer of HA (PTX-HA-PEG-PCL NPs) demonstrated much higher cytotoxicity as a result of better internalisation of formulation.

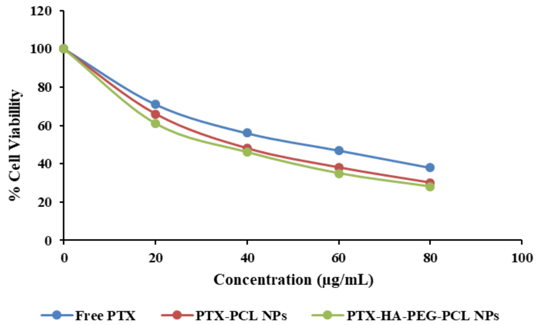


Figure 5 Percent cell growth inhibition by PTX, PTX-PCL NPs and PTX-HA-PEG-PCL NPs

Hemolytic toxicity

The concentration of soluble PTX in solution determines its hemolytic toxicity. Hemolysis was not seen at doses lower than 100 mg/ml, however it was seen at 200 mg/ml in 11% of cases [13]. RBCs were subjected to the hemolytic activities of free PTX and PTX-loaded HA-PEG-PCL and PCL nanoparticles. For the 200 mg/ml PTX concentration utilized in the studies, PTX-loaded HA-PEG-PCL and PCL nanoparticulate formulations showed low hemolytic toxicity in the RBC. It was discovered that PTX-loaded HA-PEG-PCL nanoparticles were more hem suitable for drug delivery applications.

Stability studies

Over time, the formulation maintained a decent physical look. The initial particle size at day zero was 172 nm, and the final particle size after 6 months was 185 nm. This slight increase in particle size may have been caused by the weak van der waals force that holds the particles together and causes the formation of soft agglomerates. The drug content after 6 months was similar, indicating that there was no drug leakage during storage. In Table 3, the evaluation criteria are listed.

Table 3 Stability studies of PTX-loaded HA-PEF-PCL nanoparticles

Temperature	Evaluation parameter	Observation (months)				
		0	1	2	4	6
30± 2°C RH = 65± 5%	Physical appearance	Cream Colour	No variation	No variation	No variation	No variation
	Particle size (nm)	172 ±8	179 ±2	180± 5	184± 7	185 ±4
	Drug loading %	88± 0.6	88.2 ±0.4	88.4 ±0.5	88.5 ±0.1	88.2 ±0.2

Conclusion

By using nanoprecipitation, PTX-loaded nanoparticles were created. All of these polymers are desirable for targeted drug delivery due to their biodegradability and biocompatibility. The morphology of the NPs as evaluated by TEM showed them to be sphere-shaped. The DSC analysis revealed each compound's own peak from the formulation. The presence of HA and PEG may be the cause of the NPs' sustained release and temporal release. The addition of HA, which serves as a ligand to target the receptor found on colon cancer cells, resulted in the PTX-HA-PEG-PCL NPs having the highest level of cytotoxicity in comparison to other groups (HT-29). PTX can be delivered to tumor tissue utilizing HA-PEG-PCL nanoparticles. According to in-vitro experiments, the medication would release slowly at physiological pH and quickly in the more acidic endolysosomal compartments following endocytosis. To distribute Paclitaxel safely and effectively, improved delivery mechanisms are therefore required.

Declaration of interest

No conflicts of interest.

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