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Research Article

Development and Characterization of Biodegradable Polymeric Nanoparticles for Colon Cancer Treatment

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ABSTRACT

The current study aimed to assess the cytotoxic potential of doxorubicin (DOX) loaded nanoparticles (NPs) for effective colon cancer targeting. Poly-caprolactone (PCL) was used to make the NPs, and PCL was then conjugated with hyaluronic acid (HA) and polyethylene glycol (PEG) (HA-PEG-PCL NPs). The developed NPs were used to encapsulate the DOX, which was then tested for stability, *in-vitro* drug release, cell viability, and entrapment effectiveness. The NPs were manufactured with care, and TEM examinations showed that they were spherical. Zeta potential measurements for HA-PEG-PCL and PCL NPs were 16.4 ± 0.84 mV, -4.9 ± 0.3 , and PDI 0.648 and 0.553, respectively, for both formulations. HA-PEG-PCL NPs and PCL NPs were found to have particle sizes of 267 ± 0.5 and 142 ± 1.5 nm, respectively. The NPs made using HA-PEG-PCL copolymers demonstrated their ability to continue the release of DOX. While DOX-HA-PEG-PCL NPs released $93.1 \pm 2.4\%$ of DOX in 96 hrs, DOX-PCL NPs released nearly 100% of the medication in the same time frame. When the stability was assessed in terms of particle size and %EE, it was discovered that the NPs formulations were more stable at $4 \pm 2^\circ\text{C}$ and then $28 \pm 2^\circ\text{C}$. Because HA binds to the overexpressed CD44 receptors on HT-29 cells, the DOX-HA-PEG-PCL NPs demonstrated noticeably more cytotoxicity as a result of better formulation internalization. The controlled drug release behavior of the proposed nanotechnology showed their potential for colon cancer cell lines.

INTRODUCTION

According to the American Cancer Society, colon cancer is the third leading cause of cancer-related death in the country. In 2022, the United States have diagnosed 1.9 million new cancer cases and have seen 609,360 cancer deaths.^[1] Colon cancer can be treated with a range of techniques, including surgery, radiation, chemotherapy, and targeted medication delivery.^[2,3] The main drawbacks of anticancer medications are their negative side effects on target locations other than the intended target and their difficulty in delivering the right dose to that target.^[4,5] Because conventional techniques do not specifically target colon cancer cells, they are ineffective for providing anticancer medications. In addition to the risks of side effects, chemotherapy has not produced adequate results,

and anticancer medicine cannot tell healthy cells from cancerous ones. The drug concentration at the target site is maximized *via* a site-specific drug delivery system to the colon, which reduces adverse effects by only requiring small dosages. The concentration of the medicine at the target site will be raised by specifically targeting drug delivery to the colon. As a result, less medication will be used to lessen adverse effects.^[6] New and secure methods to treat colon cancer have been made possible by the amazing advancements in nanomedicine. This does not, however, provide us with a thorough and trustworthy remedy. Materials, including metal, carbon, lipids, and synthetic and biodegradable polymers are used to create nano-formulations.^[7] The unique characteristics of NPs, such as their low toxicity, targeting ability, enhanced surface area, conjugation with ligands and polymers,

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biodegradability in physiological conditions, surface modification, etc. have drawn attention.^[8,9] A well-known biodegradable and biocompatible polymer, HA is an aglycosaminoglycan polysaccharide with a molecular mass of 80 to 250 kDa and is made up of N-acetylglucosamine and D-glucuronic acid disaccharide units. Since HA has the ability to bind to CD44 receptors and colon cancer cells overexpress the CD44 receptor, it is chosen as a ligand for targeting colon cancer cells.^[10,11] Colon cancer has been treated with a variety of anticancer medications, including paclitaxel, epirubicin, gemcitabine, and DOX^[12-14] drug called anthracycline. Numerous cancers have responded well to treatment with DOX. DOX is most frequently used to treat solid tumors in pediatric and adult patients. The drug's propensity to intercalate inside DNA base pairs, which causes DNA fragmentation and prevents the creation of both DNA and RNA, is the underlying mechanism of DOX. By inhibiting topoisomerase II, DOX causes DNA damage and activates apoptosis.^[15] In this study, PCL NPs and HA-PEG-PCL NPs, whose composition was further improved, were loaded with DOX. The new formulation was tested on the HT-29 cell line to see if it could cause cell cytotoxicity, and the *in-vivo* and *in-vitro* outcomes of this method were compared to free DOX. The planned study aims to create a safe and efficient method of treating colon cancer with few negative effects.

MATERIALS AND METHODS

Materials

Sigma-Aldrich provided the HA and PCL (Bangalore, India). Fresenius Kabi Ltd. provided a free sample of doxorubicin. The Pluronic F-68 was bought in Gurgaon (Haryana, India) from Himedia Lab in Mumbai. From Merck Limited in Mumbai, India, we obtained N-hydroxysuccinimide (NHS), NaOH, DCM (Dichloromethane), DMSO, acetonitrile, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), isopropyl alcohol, and ethylene diamine (EDA). Sigma Aldrich supplied PEG and dialysis sacs (MW cut-off 12000–15000 Da) (New Delhi, India). The highest analytical grade was chosen for all additional compounds.

Methodology

Copolymer HA-PEG-PCL Synthesis and Characterization

Weighed and dissolved in 10 mL of DMSO, HA (0.2 g) and EDC (0.18 g) were then stirred at room temperature (RT) for 12 hours. By doing this, the carboxyl group in HA is activated. The PEG solution was then added to the above solution, consisting of PEG (12.5 g), NHS (0.1 g), and DMSO (10 mL). The mixture was then ultrasound-mixed for 5 minutes, and the reaction was allowed to finish for 1 day. The excess byproduct, DMSO, and other reactants were then eliminated by dialysis using PBS and water. The HA-PEG conjugate was then obtained using lyophilization. The following step involved dissolving HA-PEG (0.2 g)

and EDC (0.18 g) in DMSO (10 mL) before stirring the mixture for 12 hours at RT. The PCL solution was then added, consisting of PCL (0.27 g), NHS (0.1 g), and DMSO (10 mL). The mixture was then ultrasound-mixed for 5 minutes, and the reaction was allowed to finish for 1 day. The DMSO and other reactants were eliminated using a dialysis membrane and water five times over the course of three days. The solution was lyophilized to produce the HA-PEG-PCL conjugate.^[16,17]

Preparation of DOX-loaded HA-PEG-PCL Nanoparticles

Through the process of nanoprecipitation, the DOX-loaded NPs were created. In a nutshell, DOX (100 mg) was dissolved in 6.2 mL of NaOH and stirred at RT for 24 hours. Following that, HA-PEG-PCL copolymer (5 mg) was dissolved in DCM (5 mL), then added while being stirred to the DOX solution. The NPs were made using Pluronic F-68 (0-0.1 w/v) as a nonionic surfactant. Pluronic F-68 was produced as a 10 mL solution in distilled water. Following that, an organic solution containing the medication and polymer was added to the Pluronic F-68 solution, and it was agitated for 24 hours at a speed of 800 rpm. NPs were created and let to dry. In this experiment, the Pluronic F-68 concentration (0.05% w/v) was held constant while the copolymer concentration was changed from 10 to 30 mg. Similar to this; variable Pluronic F-68 concentrations were employed to create NPs while preserving a constant copolymer level (10 mg).

Optimization

The polymer concentration, surfactant concentration, stirring speed, and duration are the different process variables that have an impact on the stability and characteristics of NPs. In order to achieve homogeneous preparation with ideal particle size and optimum entrapment effectiveness, these parameters were all found and improved.

Characterization of Nanoparticles

Morphological Observation

Particle size, polydispersity index, and zeta potential calculations were performed using Zetasizer (Malvern Instruments Ltd., UK). A transmission electron microscope (TEM) operating at a 200 kV accelerating voltage was used to observe the form of NPs. Samples for TEM analysis were created by dropping the sample onto a copper grid that had been coated with carbon and allowing the excess solvent to evaporate in ambient light.^[18]

Atomic force microscopy (AFM) (Alpha 300RA AFM, WITec, Germany) was utilized to assess the morphological properties of produced NPs. On a copper grid with a carbon coating and a mesh size of 300, a little drop of NPs solution was used, and the grid was air dried. AFM experiments of DOX-HA-PEG-PCL were performed utilizing a glass substrate in AC mode at RT and at various magnifications.



With the use of some channel paper, the additional sample was eliminated.

Differential Scanning Colorimetry (DSC) Study

The crystal form was determined by DSC analysis of pure PCL, pure DOX, physical mixture PCL-NPs, and DOX-loaded HA-PEG-PCL NPs using the TA (Instrument 2910 MDSC V4.4E, Shin, Osaka, Japan). The samples were heated at a 5°C/minute rate from 40 to 400°C. A blank pan served as a guide. Nitrogen purging was used to maintain an inert atmosphere.

X-ray Diffraction (XRD) Study

Utilizing Ni-filtered Cu-K α radiation, the Rigaku D/Max 1200 X-ray diffractometer (Rigaku Denki Co. Ltd, Tokyo, Japan) examined the diffraction pattern of the formulations HA-PEG-PCL NPs and DOX-HA-PEG-PCL NPs (35kV, 15mA). The temperature range of the samples was 0 to 50°C. The pattern was captured at a 40 kV voltage.

In-vitro Drug Release Study

Utilizing a dialysis tube for the *in-vitro* release evaluation, PBS was used as the release medium (pH 7.4). Briefly, 100 mL of PBS was used to dissolve 2 mL of NPs formulation in dialysis tubing (M.W cut-off: 12000–15000 Da). The stirring speed was then set to 100 rpm and the magnetic stirrer was kept at a constant 37°C. Total of 1-mL of the release medium's solution was drawn out at regular intervals and immediately replaced with an equal volume of the medium. The amount of released DOX was calculated using a UV spectrophotometer (λ_{max} 476 nm, Thermo Scientific, Mumbai, India).

Cell Viability Study

We used the Sulforhodamine B (SRB) assay to determine the viability of cells. A medium containing 10% fetal bovine serum and L-glutamine was used to grow the cells (HT-29 colon cancer cells). Plates of 96-well mL were inoculated and then incubated for 24 hours in a carefully controlled environment (37°C, 5% CO₂, 95% air, 100% relative humidity). Following the addition of the drug DOX, the formulations DOX-PCL NPs and DOX-HA-PEG-PCL NPs were incubated for 48 hours at various drug doses of 20, 40, 60, and 80 $\mu\text{g/mL}$. After gently adding cold TCA (10% TCA, 50 l) to the cells, they were fixed in place for further 60 minutes at 4°C. After removing the supernatant and five water rinses, the plates were dried by air. Following that, each well received 50 l of SRB solution (0.4% w/v) dissolved in acetic acid (1%), which was then incubated in the plates for 20 minutes at RT. After staining was finished, the cells were rinsed five times with acetic vinegar (1%) to remove any remaining color before being allowed to air dry. Elution of the bound stain with 10 mM trizma base was followed by the measurement of the sample's absorbance at 540 nm with a reference wavelength of 690 nm using

a plate reader (Bio-Rad, Model 550-Microplate Reader, Hercules, California).

Biodistribution Study

Free DOX, DOX-PCL NPs, and DOX-HA-PEG-PCL NPs were administered intravenously *via* tail veins into groups I, II, and III, respectively. Each rat was sacrificed after 1, 6, and 24 hours during the experiment. The visceral organs, such as the lungs, liver, kidney, colon and tumour, were then meticulously retrieved, twice cleansed to remove any adherent cellular debris or tissues, and then preserved in a frozen state. These tissue specimen were homogenized and vortexed (Superfit, Mumbai, India) in PBS at a pH of 7.4. The supernatant was decanted into a sterile container following a 15 minute centrifugation of the homogenates at 20,000 rpm. The contents were mixed thoroughly, centrifuged, and filtered after being treated with a 10% TCA solution (Millipore, Billerica, MA). Without using an internal standard, the resultant clear supernatant was examined using the reverse phase HPLC technique.

Stability Study

The produced PCL-NPs and HA-PEG-PCL NPs were tested for stability during a 180-day period at RT (28°C) and under refrigeration ($4 \pm 2^\circ\text{C}$; R-26SVND, Hitachi, Tokyo, Japan). At various times, the samples were examined for particle size and %EE (15, 30, 60, 90, 150 and 180 days). After three months, look for any changes in the sample's colour, amount of remaining medicine, or size of the globules.

Statistical Analysis

The statistical analysis was done using Graph Pad Instat Software (Version 3.00, Graph Pad Software, San Diego, CA). Multiple comparisons were assessed using Tukey-Kramer tests after one-way ANOVA. *p-values* of less or equal to 0.05 were considered statistically significant. The experiments' findings were presented as mean $\pm$ standard deviation (S.D.).

RESULT AND DISCUSSION

Cancer treatment has always been difficult. Since they are routinely treated with traditional procedures, but their use has various negative side effects. With fewer adverse effects, techniques based on nanotechnology are gaining popularity. In the current study, the biodegradable polymers HA, PCL, and PEG were conjugated as well as PCL alone to create the nanoparticles. These polymers have a reputation for being biodegradable and biocompatible as well as having the ability to target cancer cells.^[19,20]

To assess the NPs potential for treating HT-29 colon cancer cell lines, DOX was injected into them. In order to characterize the generated NPs, such as their particle size, surface morphology, and entrapment, effective DOX-loaded NPs were then tuned based on copolymer concentration, surfactant concentration, stirring time, and speed. By

changing the copolymer concentration while holding the other factors constant, the NPs were created. On particle size and %EE, the impact of polymer concentration was clearly seen. When 10 mg of copolymer was employed, the maximum %EE (84.5 ± 2.7) and optimal size (132 ± 2.1 nm) were noted. This could be because the particles aggregated; increasing the concentration had no effect on the %EE. At the lowest copolymer concentration (5 mg), the average particle size was found to be 154 ± 2.4 nm and $74.3 \pm 1.5\%$ EE. While all other factors remained constant, the surfactant concentration significantly impacted the percentage of EE and particle size. The particle size fell from 182 ± 2.3 to 149 ± 2.8 nm when it was raised from 0.5 to 1.0%. At a surfactant concentration of 1.5%, the ideal size of 149 ± 2.8 nm and $86.3 \pm 2.4\%$ EE was attained. The ideal size and %EE were discovered to be 189 ± 1.8 nm and $87.10 \pm 1.1\%$, respectively, at the stirring speed of 500 rpm. Due to the particles' aggregation, the size grew when the rpm was raised from 200 to 700. The maximum %EE was determined to be $91.16 \pm 2.3\%$ and particle size 269 ± 3.0 nm when the formulation was held for stirring for 12 hours, marking the successful optimization of the stirring period. The optimized data is compiled in Table 1 for easier understanding.

Determination of Particle Size, Zeta Potential and Polydispersity Index (PDI)

With PDI 0.553, the average size of the PCL-NPs was discovered to be approximately 142 ± 1.5 nm. Although the HA-PEG-PCL NPs had a size of 267 ± 0.5 nm and a PDI of 0.648, the size increase may be related to the employment of polymer conjugate in the NPs generation process. PCL

NPs and HA-PEG-PCL NPs were discovered to have zeta potentials of -4.9 ± 0.3 and -16.4 ± 0.8 mV, respectively (Table 2, Fig. 1).

TEM and AFM Analysis of DOX-loaded NPs

The close to spherical shape of the NPs with the tight size distribution was confirmed by TEM examinations of the produced formulations (Fig. 2). The TEM picture shows that the particles are 200 nm in size. The average particle size constrained by zeta sizer was somewhat larger than the size obtained by TEM methods. Since the hydrodynamic diameter of solid structures, including the hydrodynamic layer surrounding them, is measured using the zeta sizer approach. The morphological properties of these nanoparticles were observed using atomic force microscopy (AFM), and it was discovered that they were uniform, spherical in shape, free of aggregation, and smooth on the surface with a nanometric range. The particle size is revealed to be in the nanometric range by line analysis of AFM images.

DSC Analysis

The examination of NPs thermal properties and the provision of qualitative and quantitative data regarding the physicochemical state of drugs and drug combinations were both greatly aided by DSC analysis. Fig. 3 displays a DSC Thermogram for HA-PEG-PCL NPs and PCL NPs that have been loaded with DOX, HA, PCL, diamine PEG, and PCL. At 105°C , DOX displayed an endothermic peak. Diamine PEG displays a strong peak at 69°C , and PCL showed an endothermic peak at 61°C , both of which are exactly the same temperature as its melting point.

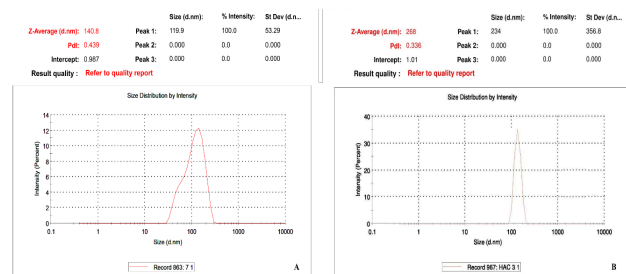
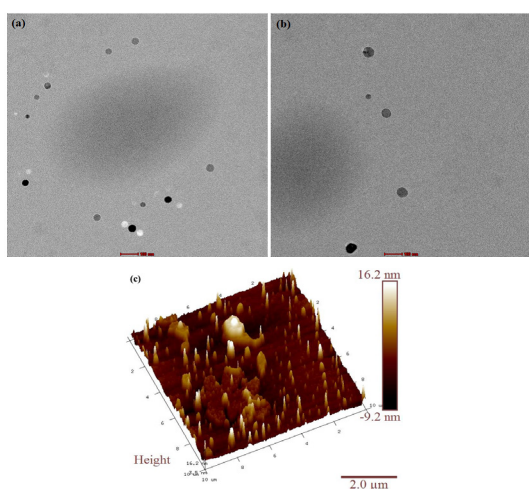
Table 1: Optimization of process and formulation variables in preparation of Nanoparticles

Formulation Code	Copolymer Concentration (%)	Drug Concentration (mg)	Surfactant concentration (%)	Stirring Speed	Stirring Time (hours)	EE (%)	Average Particle Size* (nm)
Copolymer Concentration							
DF1	5	100	0.5 %	500	6	74.3 ± 1.5	154 ± 2.4
DF2	10	100	0.5 %	500	6	84.5 ± 2.7	132 ± 2.1
DF3	15	100	0.5 %	500	6	83.3 ± 1.2	178 ± 6.1
Surfactant concentration							
DF1S1	10	100	0.5 %	500	6	73.3 ± 1.6	182 ± 2.3
DF1S2	10	100	1 %	500	6	81.5 ± 2.8	162 ± 2.5
DF1S3	10	100	1.5 %	500	6	86.3 ± 2.4	149 ± 2.8
Stirring Speed							
DF1S3R1	10	100	1.5 %	200	6	70.05 ± 1.1	173 ± 2.2
DF1S3R2	10	100	1.5 %	500	6	87.10 ± 1.6	189 ± 1.8
DF1S3R3	10	100	1.5 %	700	6	89.13 ± 1.3	204 ± 2.5
Stirring Time							
DF1S3R2T1	10	100	1.5 %	500	6	71.02 ± 1.3	272 ± 2.0
DF1S3R2T2	10	100	1.5 %	500	12	91.16 ± 2.3	269 ± 3.0
DF1S3R2T2	10	100	1.5 %	500	24	76.41 ± 1.6	191 ± 1.4



Table 2: Particle Size, zeta potential and PDI

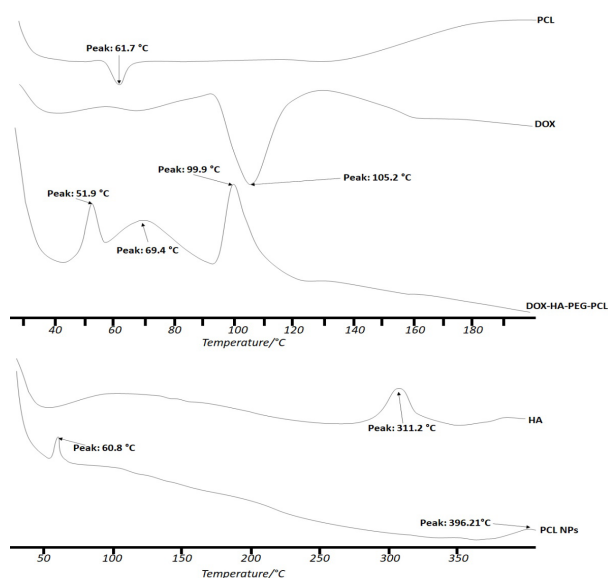
Formulation Code	Particle Size (nm)	Zeta Potential (mV)	PDI
HA-PEG-PCL NPs	267 ± 0.5	-16.4 ± 0.8	0.648
PCL NPs	142 ± 1.5	-4.9 ± 0.3	0.553

**Fig. 1:** Particle size of PCL NPs and HA-PEG-PCL NPs measured by zeta sizer.**Fig. 2:** (A) TEM microphotograph of PCL NPs (B) TEM microphotograph of HA-PEG-PCL NPs (C) Atomic Force Microscopy (AFM) images of DOX-HA-PEG-PCL NPs.

At 311.2°C, HA displayed a large peak. The excipients crystalline composition was confirmed by the strong peak. Sharp endothermic peaks were seen in the DOX-loaded HA-PEG-PCL NPs at 99, 510, and 69°C. PCL in DOX-loaded PCL NPs demonstrates a peak at 60°C.^[17,21,22]

XRD Analysis

The XRD patterns of HA-PEG-PCL and DOX-HA-PEG-PCL NPs are depicted in Fig. 4. The extended *in-vivo* breakdown durations of PCL, a semi-crystalline polymer with hydrophobic qualities, could have been managed by HA-PEG-PCL copolymers, which may also have contributed to the achievement of better physicochemical attributes and process ability. Peaks discovered by XRD examination of HA-PEG-PCL NPs verified the copolymer's crystalline composition. In contrast to blank NPs, which had relatively fewer peaks, the XRD patterns of DOX-HA-PEG-PCL NPs revealed the usual sharp peaks found in the drug crystal. The XRD patterns for the DOX-HA-PEG-PCL NPs showed distinct drug peaks.

**Fig. 3:** DSC thermogram of PCL, DOX, HA, DOX-HA-PEG-PCL NPs PCL NPs.

In-vitro Drug Release

The copolymer-prepared NPs, such as HA-PEG-PCL, demonstrated their capacity to maintain the release of DOX (Fig. 5). At first, there was a burst of DOX release, which may have been caused by medication that had been surface-adsorbed. When compared to DOX-PCL NPs, DOX-HA-PEG-NPs released 93.1 ± 2.4% of the medication in 144 hours while DOX-PCL NPs released about 97.5 ± 2.8% in 96 hours. Due to their hydrophobicity and trapping in the inner core of copolymeric NPs, the medication may release slowly over time. It was anticipated that these NPs would evade reticuloendothelial system (RES) uptake, enabling them to stay in circulation for longer.

Cell Viability

When treated with cells for 72 hours, it was shown that DOX-HA-PEG-PCL NPs revealed noticeably greater cytotoxicity than free DOX and DOX-PCL. At a dose level of 0.01 mg/mL, the free DOX demonstrated minimal cytotoxicity, inhibiting the proliferation of 59.5 ± 1.5% of the cells. This might be because cells aren't absorbing the drugs as well. Additionally, DOX-PCL NPs demonstrated 45.3 ± 1.4% cell growth inhibitions, which may have been brought on by passive diffusion, minimal intracellular dispersion, or drug concentration. The fact that 20.6 ± 1.2% of cell growth was inhibited by DOX-HA-PEG-PCL NPs could be attributed to the formulation's internalization 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 functions as a ligand for the CD44 receptors that are over expressed on HT-29 cells, the NPs with the HA copolymer (DOX-HA-PEG-PCL NPs) demonstrated much higher cytotoxicity as a result of better internalization of formulation (Fig. 6).^[22]

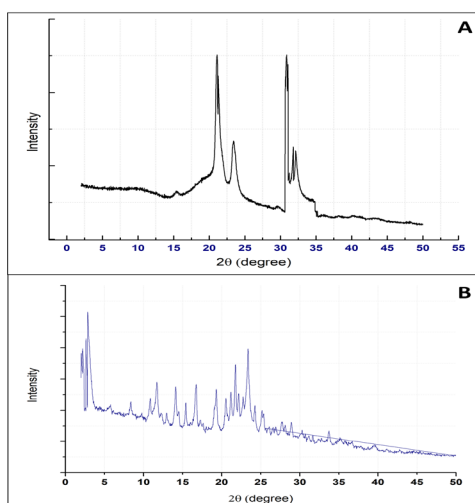


Fig. 4: (A) XRD of HA-PEG-PCL NPs, (B) DOX-HA-PEG-PCL NPs.

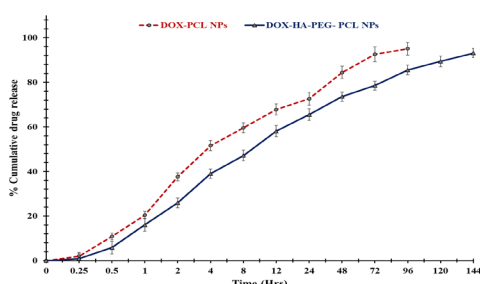


Fig. 5: *In-vitro* DOX release from DOX-PCL NPs and DOX-HA-PEG-PCL NPs.

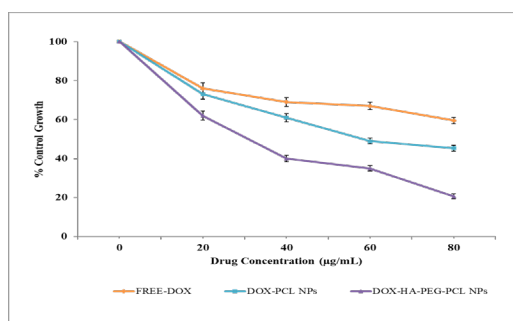


Fig. 6: Percent cell growth inhibition by DOX, DOX-PCL NPs and DOX-HA-PEG-PCL NPs.

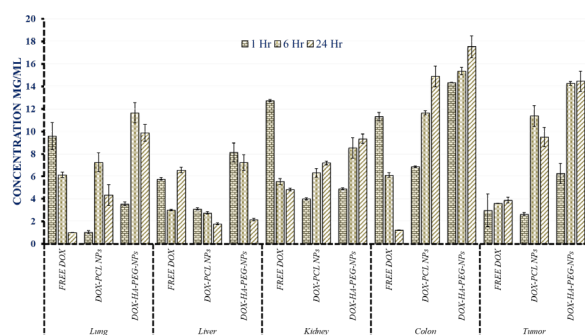


Fig. 7: Biodistribution study of DOX, DOX-PCL NPs and DOX-HA-PEG-PCL NPs after IV administration

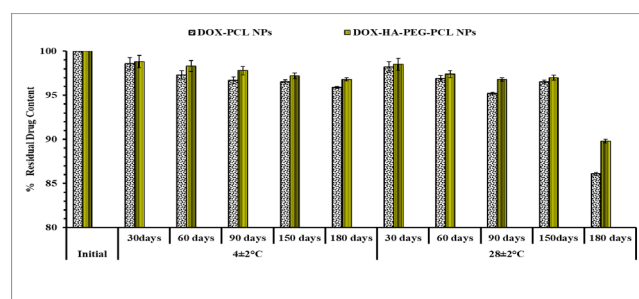


Fig. 8: Effect of storage at $4 \pm 2^\circ\text{C}$ and $28 \pm 2^\circ\text{C}$ on % Residual Drug Content

Biodistribution Study

Following the delivery of several formulations, including DOX, DOX-PCL NPs, and DOX-HA-PEG-PCL NPs, the DOX concentration was assessed until 24 hours. The concentration of DOX retrieved from DOX solution was determined to 1.01 ± 0.011 , 6.58 ± 0.25 , 9.35 ± 0.19 , 1.23 ± 0.005 , and $3.9 \pm 0.25\%$ in the lung, liver, kidney, colon, and tumor, respectively. Contrary to this, DOX concentration in case of DOX-PCL NPs was found to be $4.35 \pm 0.93\%$ in lungs, $1.8 \pm 0.096\%$ in liver, $4.85 \pm 0.17\%$ in kidney, $14.9 \pm 0.93\%$ in colon, and $14.74 \pm 0.89\%$ in tumor after 24 hours. Likewise, the concentration of DOX determined after DOX-HA-PEG-PCL NPs administration was $9.87 \pm 0.62\%$ in the lungs, $6.58 \pm 0.18\%$ in liver, $7.21 \pm 0.42\%$ in kidney and $17.54 \pm 0.85\%$ in colon, and $14.74 \pm 0.89\%$ in tumor, after 24 hours (Fig. 7). The amount of DOX determined from DOX-HA-PEG-PCL NPs to colon and tumor was much more than that of free DOX solution and DOX-PCL NPs. Likewise, both NPs formulation reduced the drug accumulation in the liver. This might be the result of colon cancer cells having too high a numbers of HA receptors. PEGylation on NPs significantly slowed down the liver's ability to absorb DOX-PCL NPs and DOX-HA-PEG-PCL while facilitating their blood circulation.

Stability

By keeping the NPs formulations at 4 ± 2 and $28 \pm 2^\circ\text{C}$, the stability of DOX-HA-PEG-PCL NPs was observed. The stability was assessed in terms of particle size and %EE. Storage stability studies were finished to assess the integrity of formulations under various environmental conditions. The stability was assessed after 180 days of formulation storage. The particle size was shown to be larger at $28 \pm 2^\circ\text{C}$ as a result of the polymer matrix aggregating, but it was not significantly changed at $4 \pm 2^\circ\text{C}$ (Fig. 8). The considerable change in %EE seen at $28 \pm 2^\circ\text{C}$ compared to the formulation when stored at $4 \pm 2^\circ\text{C}$ may be caused by medication leakage or degradation at this temperature.

CONCLUSION

Following the preparation of the HA, PEG, and PCL conjugate, the nanoprecipitation method was used to create the NPs. 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. Due to their greater targeting capacity due to the inclusion of HA, which functions as a ligand to target the receptor present in colon cancer cells, the DOX-HA-PEG-PCL NPs had the highest cytotoxicity in comparison to other groups (HT-29). At $4 \pm 2^\circ\text{C}$, the formulation was more stable. However, additional research is required to perhaps increase targeting efficiency and decrease toxicity.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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