

## Research Paper

## Preparation and Characterization of pH-sensitive Doxorubicin Grafted Hyaluronic Acid Nano-micelles


Babak Tajani<sup>1</sup> , Arash Mahboubi<sup>1</sup>, Seyyed Mostafa Ebrahimi<sup>1</sup>, Seyed Alireza Mortazavi<sup>1\*</sup>
<sup>1</sup>. Department of Pharmaceutics and Pharmaceutical Nanotechnology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.


**Citation:** Tajani B, Mahboubi A, Ebrahimi SM, Mortazavi SA. Preparation and Characterization of pH-sensitive Doxorubicin Grafted Hyaluronic Acid Nano-micelles. *International Journal of Medical Toxicology and Forensic Medicine*. 2024; 14(2):E40145. <https://doi.org/10.32598/ijmtfm.v14i2.40145>

<https://doi.org/10.32598/ijmtfm.v14i2.40145>

## Article info:

Received: 04 Dec 2022

First Revision: 07 Dec 2022

Accepted: 25 Dec 2022

Published: 29 May 2024

## ABSTRACT

**Background:** This study was done to achieve a new drug delivery system delivering two different chemotherapy molecules to the target tissue simultaneously.

**Significance:** Co-delivery of chemotherapy medicines provides synergistic effects leading to lowering the dose of administered drugs and side effects.

**Methods:** Doxorubicin (DOX) was introduced to water-soluble hyaluronic acid (Hyal) using 1-amino-3,3-diethoxy-propane (ADEP), as a pH-sensitive linker, to develop a new hydrophobic structure, i.e. Hyal-ADEP-DOX. The conjugates were grafted in three ratios (7.5%, 12.5%, and 20%) to Hyal and characterized by Fourier transform infrared (FT-IR) and proton nuclear magnetic resonance (1HNMR). Cannabidiol (CBD) was physically loaded in the core of nano-micelles.

**Results:** Prepared nano-micelles were analyzed for critical micelle concentration (CMC) particle size stability and drug release before and after loading the CBD. Hyal-ADEP-DOX-12.5 was the optimized ratio and had a mean diameter of 50 nm before loading the CBD and 120 nm after loading (observed by transmission electron microscopy). The release results showed that DOX releases slowly in physiological pH, and CBD has a burst release at acidic pH from Hyal-ADEP-DOX-12.5. These properties altogether make Hyal-ADEP-DOX nano-micelles, as a stimuli-sensitive nano-carrier, a potential candidate for hydrophobic anticancer agents' co-delivery.

**Conclusion:** The grafted DOX exhibited pH-sensitive release behavior, i.e. while 22.8% of the drug was released after 24 h at pH 5.5, and 5% was released after 24 h at pH 7.4. Hyal-ADEP-DOX due to its low CMC value, colloidal stability, slow drug release in physiological pH, and burst release in acidic pH Hyal-ADEP-DOX, is an excellent candidate as a carrier to co-deliver hydrophobic therapeutic agents to tumor tissues.

## Keywords:

Doxorubicin, Cannabidiol,  
Nano-micelle, pH-sensitive,  
Co-delivery

## \* Corresponding Author:

Seyed Alireza Mortazavi, Professor.

Address: Department of Pharmaceutics and Pharmaceutical Nanotechnology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Tel: +98 (21) 88209623

E-mail: [mortazavisar@yahoo.com](mailto:mortazavisar@yahoo.com)


Copyright © 2024 The Author(s);  
This is an open access article distributed under the terms of the Creative Commons Attribution License (CC-BY-NC; <https://creativecommons.org/licenses/by-nc/4.0/legalcode.en>), which permits use, distribution, and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

## 1. Introduction

**B**reast cancers are the most common cancers diagnosed in women and the second cause of cancer-related deaths in the world [1]. Treatment regimens usually consist of the simultaneous use of various types of anti-cancer molecules in reducing the dose used by increasing the effectiveness synergistically and consequently reducing the severe side effects of these drugs [2, 1]. Doxorubicin (DOX) is among the chemotherapy agents used in the treatment regimens of all types of breast cancer. It has shown effectiveness in the past, but resistance to DOX and side effects have limited its use [1]. DOX is used as a water-soluble salt with a solubility problem, but due to the potency of the drug and serious side effects, it requires a new drug delivery system to reduce the dose of the drug used [3, 4]. Cannabinoids (CBDs) are the molecules found in the extract of the flowering branches of the marijuana plant. CBDs have shown a good capacity in regulating the proliferation of cancer cells and tumor growth [5, 6]. CBD as one of the cannabinoid molecules found in the plant has a high concentration in the extract, and in past studies, various mechanisms of action on cancer cells have been proven to play a role in reducing the possibility of metastasis of cancer cells [5]. Also, the effectiveness of CBD has been proven in many studies on breast cancer cells through different mechanisms [7-10]. The CBD molecule has also been investigated for adjunctive drug regimens to radiotherapy treatment with acceptable results [11, 12].

The reduction of therapeutic success following the use of a drug molecule for cancer treatment often does not lead to good results because most of the molecules used in chemotherapy regimens are not ideal in terms of physicochemical characteristics and are rejected by the body's various defense systems [13, 14]. They cannot create a suitable concentration to destroy cancer cells in the tumor tissue. Delivering the drug to the target tissue simultaneously is a wise method to solve this problem, which reduces the dose of the used drug and therefore reduces the side effects [15]. The use of nano-micelles to deliver the drug to the target tissue in new drug delivery systems has many convincing reasons, and the small size (<200 nm) of these drug delivery systems, which is intrinsically one of its characteristics, is the most attractive of these reasons [13, 16]. The small size of the drug delivery system makes the drug delivery system benefit from the phenomenon of enhanced permeability and retention effect (EPR), which according to previous studies, increases the accumulation of the drug deliv-

ery system in the tumor tissue passively [17, 18]. This structure, which has a hydrophilic part, can avoid the defense system of the reticuloendothelial system (RES) and maximize the duration of its presence in blood circulation [19].

In drug delivery systems that need to be made from various components to modify the surface of hydrophilic polymers by synthetic routes with relatively low efficiency, choosing a polymer that is itself a ligand for receptors with a high expression on the surface of tumor cells seems to be a smarter choice [20]. Therefore, in this study, 10 kDa hyaluronic acid polymer was chosen as the main polymer, which solves the challenge of targeting the drug delivery system by targeting CD-44 receptors that are increasingly expressed on the surface of tumor cells [15, 21]. Carboxylic acid on its monomers can create a hydrophobic part on the main chain structure so that it can form self-assembly micellar structures. Many molecules can be used to create the hydrophobic part in the micelle system, but one way to increase the amount of drug loading in these systems is to use the main chemotherapy agent molecules, which are mostly hydrophobic, as the hydrophobic part of the micelle structure [22]. In this way, the drug molecule is bonded to the main chain structure using a chemical bond, and a linker with different properties can be used between them to make the release of the drug in the target tissue more controllable. This method allows us to have additional space for the physical loading of another molecule in our micellar drug delivery system. Therefore, the drug delivery system can carry two types of molecules at the same time, which is desirable for creating a synergistic effect of different molecules at the same time.

In this study, we designed a targeted drug delivery system that includes surface-modified hyaluronic acid with DOX drug molecules [23]. To create stimuli-responsive properties, we used the ADEP molecule as a pH-sensitive linker between DOX molecules and carboxylic acid functional groups on the polymer chain so that the synthesized copolymer can have self-assembly properties and form a micellar structure [24]. The CBD molecule is physically loaded inside the core of the nano-micelles so that both drugs can be delivered to the tumor tissue in a targeted manner and the drug can be released intact in the endosomal acidic environment.

## 2. Materials and Methods

The current original research was conducted at the Pharmacy Faculty of [Shahid Beheshti University of Medical Sciences](#).

## Materials

Doxorubicin HCl, hyaluronic acid (molecular weight=10 kDa), 1-amino-3,3-diethoxy-propane (ADEP), dimethyl aminopyridine (DMAP), 1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), and cannabidiol reference standard were purchased from Sigma-Aldrich, United States of America. Dialysate (3.5 kDa and 12 kDa) was obtained from Orange, United States of America. Dichloromethane (DCM) and other solvents were supplied from Merck, Germany.

## Synthesis of Hyal-ADEP

Hyal-ADEP was prepared by carbodiimide-mediated reaction using EDC and NHS. After solving hyaluronic acid (20 mg, 0.002 mmol) in 20 mL of 0.1 M PBS (pH:7.4) at room temperature, EDC (10 mg, 0.05 mmol), NHS (6 mg, 0.05 mmol), and ADEP (10 mg, 0.0675 mmol) were added in five steps (every 2 hours). Then, the reaction was allowed to continue for 72 hours. The obtained solution was precipitated and washed with 50 mL of acetone three times to eliminate any impurities along with unreacted ADEP. Following centrifugation at 8500 rpm for 10 min remained precipitant was dialyzed by a 3.5 kDa cut-off dialysis bag for 24 hours. The final solution was freeze-dried and kept at -20°C for the next synthesis step (Figure 1a). The purified product was re-dissolved in distilled water and the pH of the solution was adjusted to two using 1 M HCl and the temperature increased to 50°C for the aldehyde process. After 24 h, the pH was changed to 6–7 before dialysis (24 h) and freeze-drying (Figure 1b) [21, 25].

## Synthesis of Hyal-ADEP-DOX

Hyal-ADEP-DOX was produced by conjugating the aldehyde group of Hyal-ADEP with the amine group of DOX at DOX/Hyal-ADEP molar ratios of 7.5%, 12.5%, and 20% (Hyal-ADEP-DOX-7.5, Hyal-ADEP-DOX-12.5, and Hyal-ADEP-DOX-20) at room temperature and purified by a 12 kDa dialysis bag for 24 h before freeze drying (Figure 1c) [21, 26].

## Physicochemical characterization of Hyal-ADEP-DOX conjugates

Proton nuclear magnetic resonance (1H NMR) analysis was performed using Bruker Biospin (AC400, Germany). Then, 5 mg/mL of Hyal-ADEP and Hyal-ADEP-DOX, and DOX.hcl dissolved in D<sub>2</sub>O, were measured using an NMR spectrometer. FT-IR spectra were record-

ed on a Fourier-transform infrared spectrometer (WQS-510/520, Raleigh, China) using KBr discs.

The substitution degree was measured by the HPLC-UV method. Briefly, 1 mg of each conjugated copolymer was exposed to an acidic environment (HCl, pH:1) containing 2% w/w tween 80. The mobile phase (ACT: D<sub>2</sub>O, 80:20, formic acid: 0.1 w/w, pH:3) flow rate was 0.5 mL/min and samples were measured by UV detector at 225 nm. All measurements were carried out in triplicate.

## Preparation of nano-micelles

Nano-micelles were prepared using a probe sonicator (Bandelin, Germany) at 90W for 2 minutes. Hyal-ADEP-DOX was protected from heat by performing sonication in an ice bath with the pulse function on for 7 seconds and off for 3 seconds. The nano-micelles solution was passed through a 0.45-μm filter (millipore) and stored in the dark for further studies.

## Critical micelle concentration

CMC was measured by fluorescence spectroscopy using Nile red as a fluorescence probe. After preparing a Nile red solution of  $1 \times 10^{-5}$  M in methanol, some 15 mL tubes were added with 100 mL of the prepared solution. Methanol was then allowed to evaporate and 4 mL of the colloidal dispersion in water was added to each tube (concentration range:  $4.88 \times 10^{-4}$ -0.125 mg/mL). The tubes were sonicated at ambient temperature for 1 hour, and then, the tubes were allowed to rest for 2 hours to help the micelles stabilize. The maximum fluorescence emission of solutions was determined at excitation and emission wavelengths: 520 and 620–680 nm, respectively. Finally, the maximum fluorescence intensity vs. logarithmic concentration of the DOX-ADEP-Hyal copolymers curve was drawn, and the intersection of the curve's two linear portions was considered the CMC [27].

## Preparation of CBD-loaded nano-micelles

We added 120 μg/mL (0.06 mg) and 140 μg/mL (0.07 mg) CBD in 0.5 mL acetone to Hyal-ADEP-DOX-12.5 and Hyal-ADEP-DOX-20 copolymers (1 mg) in 10 mL of deionized water respectively and then was sonicated for 2 minutes performing probe sonication 7 seconds on and three seconds off, three cycles of 2 minutes for cooling between each cycle, in an ice bath. Then, the colloidal solution was freeze-dried and the remained powder was stored in a sealed and dark place [28].

### Particle size, zeta potential, and stability of sizes

The Z-average and poly-dispersity index (PDI) of nano-micelles were determined by dynamic light scattering (DLS) using the photon correlation spectroscopy (PCS) technique, and the zeta potential of nano-micelles was measured by the laser Doppler electrophoresis technique for both CBD-unloaded and CBD-loaded nano-particles. The measurements were performed on samples ( $n=3$ ) at ambient temperature using a Malvern Nano ZS (Malvern Instruments Ltd., Worcestershire, UK) equipped with a 633-nm laser and 173° detection optics.

### Transmission electron microscopy (TEM)

The optimum ratios of conjugated co-polymers (CBD-loaded and CBD-unloaded) were dropped on a 300-mesh carbon-coated copper grid. After air-drying, the sample was stained with 1 w/w% uranyl acetate solution. TEM observation was carried out on a ZEISS EM10C (Germany) operating at an accelerating voltage of 80 kV.

### In vitro release

Release of DOX molecule from the Hyal-ADEP-DOX conjugate micelles was assessed in the phosphate buffer and acetate buffer; 7.4 and 5.5 to mimic the pH of physiologic and endosome media, respectively, using a dialysis bag with a cut-off pore size of 12 kDa. All measurements were performed in triplicates. Either phosphate buffer or acetate buffer (10 mL for CBD relate ease profile and 30 mL for DOX release profile, 37°C) was used as the medium, and stirring was conducted constantly. After pouring 1 mL of Hyal-ADEP-DOX micelle dispersion (1 mg/mL) into a dialysis bag, the bag was placed in the medium. HPLC-UV method is used for analyzing sample solutions [18].

### Statistical analysis

All data were presented as Mean $\pm$ SD. Parametric variables were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. All statistical analyses were performed by SPSS software, version 18 (SPSS Inc., Chicago, IL, United States of America).  $P<0.05$  were considered significant for all statistical tests.

## 3. Results

### Hyal-ADEP-DOX characterization

According to FT-IR analysis (Figure 2), Hyal-ADEP-DOX was successfully produced. The newly appeared

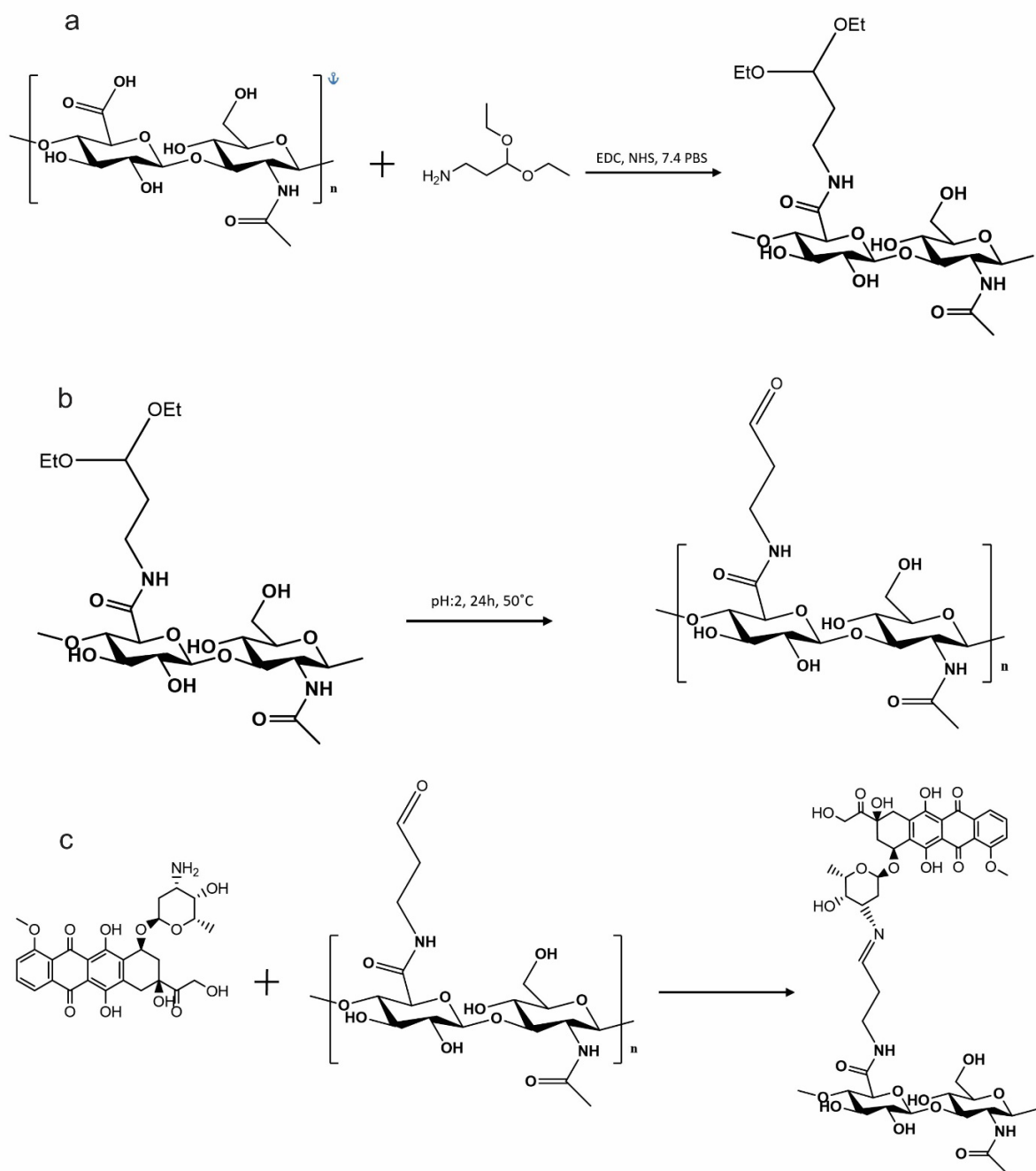
characteristic peaks at 1728  $\text{cm}^{-1}$  and 2808  $\text{cm}^{-1}$  were associated with CH=O stretch vibration and C-H stretching of the aldehyde group on HA-ADEP. These two peaks became invisible, attributing to the consumption of aldehyde to form an imine bond between HA-ADEP and DOX. FTIR spectrum of pure DOX was due to N-H stretching vibrations for the primary amine structure and at 3355  $\text{cm}^{-1}$  due to O-H stretching vibrations. However, in the case of DOX-conjugated, the peak due to N-H stretching vibrations and O-H stretching vibrations overlap, are broadened and shifted to the lower frequency range (3284  $\text{cm}^{-1}$ ). The peak at 1207  $\text{cm}^{-1}$  (C-O-C asymmetric stretching vibration) in DOX was observed in the spectrum of Hyal-ADEP-DOX with a little shift to 1213  $\text{cm}^{-1}$  (Figure 2a). Characteristic peaks of ADEP were observed at 1.15 and 2.6 ppm of  $^1\text{H}$ NMR spectra of Hyal-ADEP. Following the performed procedures, ADEP was present in the conjugate. These two peaks almost vanished in the aldehyde process and there was a shift from 2.6 to 2.95 ppm. Since the peak at (8.3, d) ppm was related to the DOX (Figure 2b, c, and d), the molecule was present in the conjugate after washing the impurities out.

### Degree of substitution

The total substitute degree of carboxyl groups (SD%) of Hyal-ADEP-DOX-7.5, Hyal-ADEP-DOX-12.5, and Hyal-ADEP-DOX-20 was measured by the HPLC-UV method. The structure of conjugated copolymers in a harsh acidic environment and high concentration of tween 80 was destructed and drug molecule (DOX) was released in solution. The SD% of Hyal-ADEP-DOX-7.5, Hyal-ADEP-DOX-12.5, and Hyal-ADEP-DOX-20 was 6.1%, 10.2%, and 15.9%, respectively.

### Critical micelle concentration

CMC was measured by fluorescence spectroscopy and using Nile red for each of the ratios of grafted copolymers. In this method, the maximum emission peak of Nile red against the copolymer concentration is used as an index to measure the CMC. The advantage of this method compared to the CMC measurement method using the emission peak of Nile red is that the possible turbidity in the samples does not interfere with the results. Also, the excitation and emission wavelengths of Nile red do not interfere with the DOX molecule. To determine the CMC of grafted copolymers in water, the values obtained from Nile red are plotted against the logarithm of copolymer concentrations and the CMC point is the concentration, at which Nile red emission has a mutation. Therefore, the CMC of Hyal-ADEP-DOX-7.5,



International Journal of  
Medical Toxicology & Forensic Medicine

**Figure 1.** Schematic structure of synthesis procedures:

a) Synthesis of Hyal-ADEP conjugate, b) Aldehyde process of Hyal-ADEP, c) Synthesis of Hyal-ADEP-DOX

Hyal-ADEP-DOX-12.5, and Hyal-ADEP-DOX-20 copolymers was  $1.56 \times 10^{-2}$ ,  $3.9 \times 10^{-3}$ , and  $1.9 \times 10^{-3}$  g/l, respectively (Figure 3). The CMC of these three ratios was in the usual range of the CMC of polymer micelles ( $10^{-5}$  to  $10^{-7}$  M), which is four orders of magnitude lower than the CMC of low molecular weight surfactants [29].

**Determination of particle size and stability of sizes**

Particle size and polydispersity index were plotted against 30 days period of time to choose the most stable ratio of grafted copolymer (Hyal-ADEP-DOX) before and after the physical loading of CBD molecules. As shown in Figure 4, the size of Hyal-ADEP-DOX-7.5



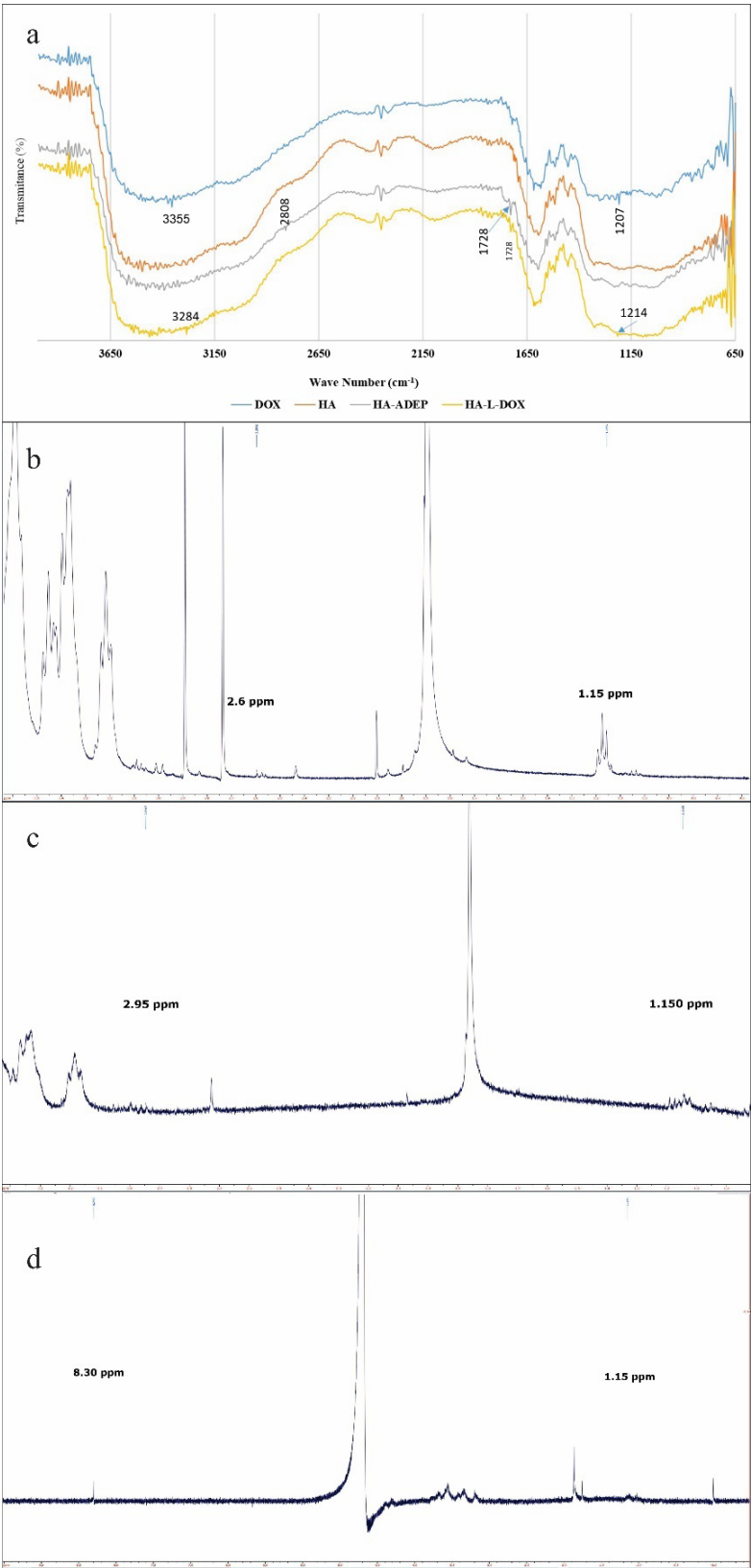
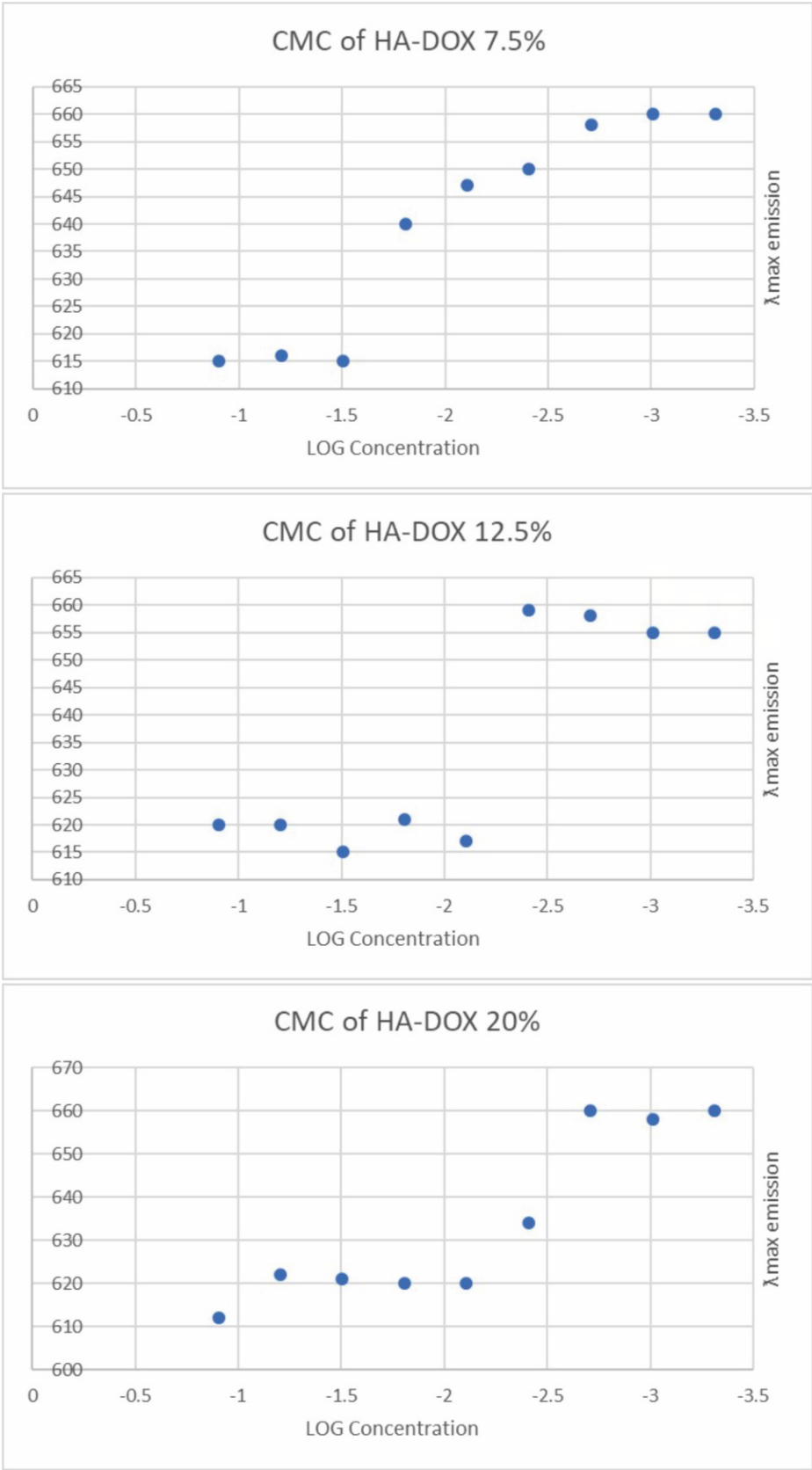


Figure 2. FT-IR analysis and 1H NMR

a) FT-IR spectra of DOX, hyaluronic acid (HA), Hyal-ADEP, and Hyal-ADEP-DOX. b) Hyal-ADEP, c) Aldehyde process of Hyal-ADEP, d) Hyal-ADEP-DOX.



**Figure 3.** Plots of the intensity of Nile red (NIR) from fluorescence spectra against logarithm concentration of nano-micelles: Hyal-ADEP-DOX-7.5%, Hyal-ADEP-DOX-12.5 %, and Hyal-ADEP-DOX-20%. NIR was used as a fluorescent probe.

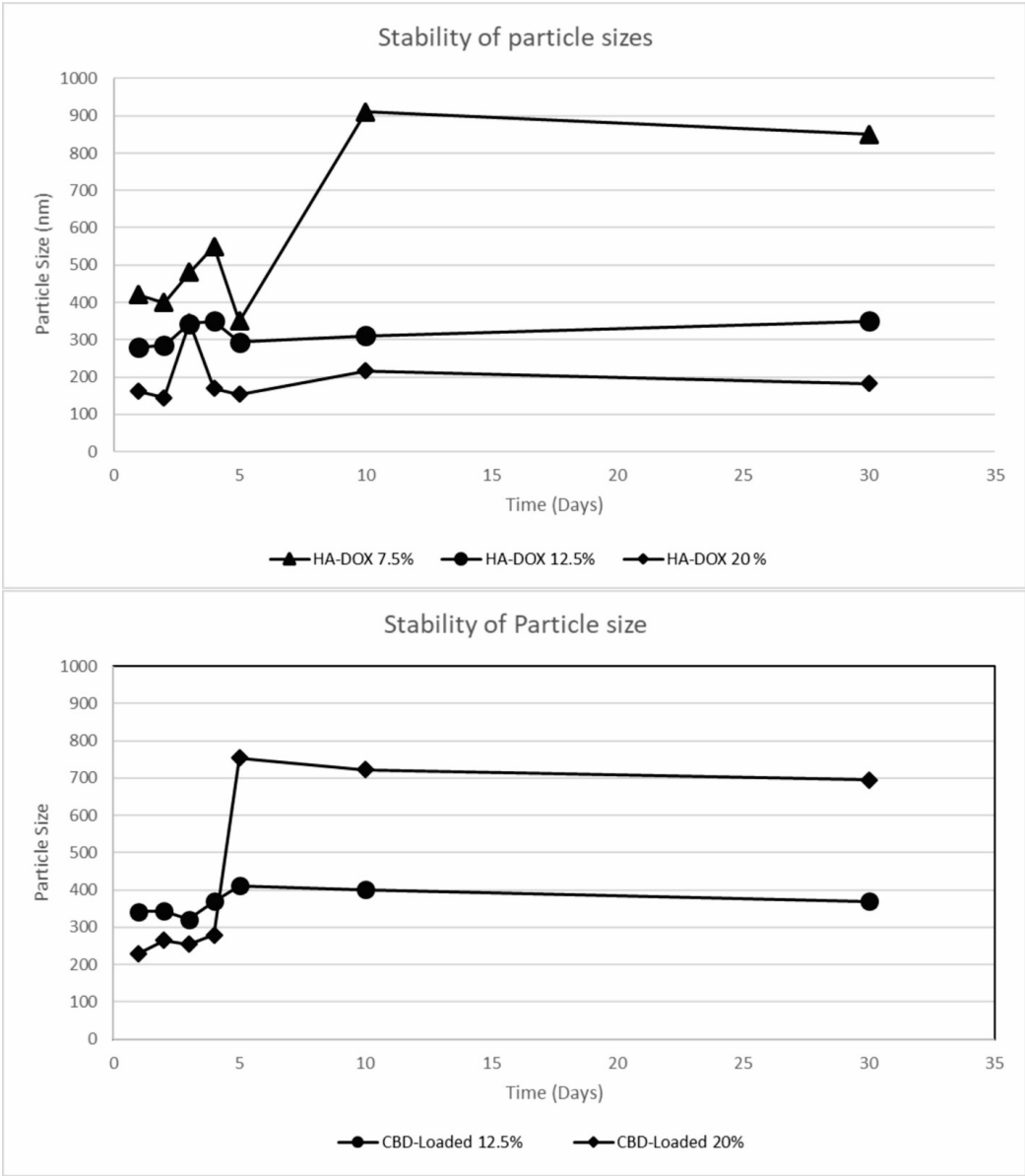


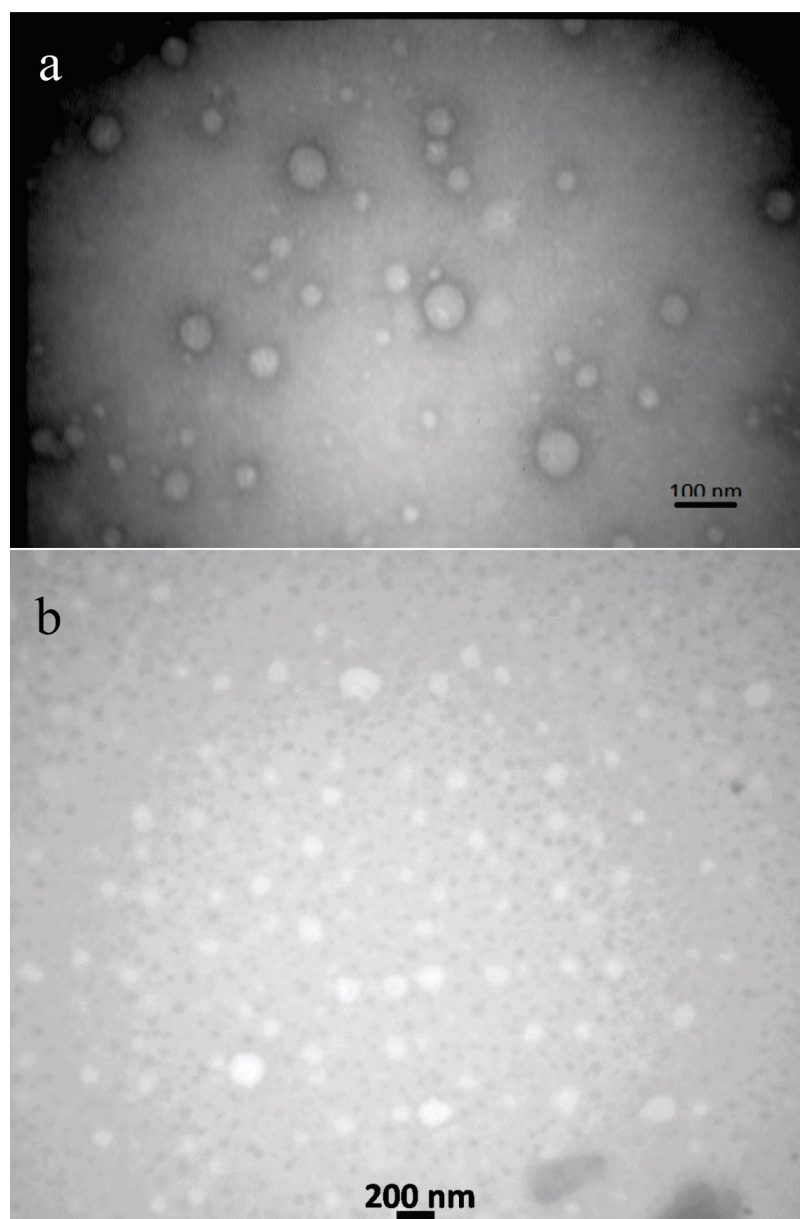
Figure 4. Particle size determined during 30 days of hollow and loaded nano-micelles.

International Journal of  
Medical Toxicology & Forensic Medicine

micelles was gradually increased. Despite its higher zeta potential, this ratio was unstable after day five and also there was some instability in size during the first five days because of the lower CMC of this ratio. In the case of Hyal-ADEP-DOC-20, there was instability in size after loading the CBD. In addition, there was the lowest zeta potential between ratios, and an increase was observed in size due to aggregation after four days. The most suitable drug-polymer ratio was Hyal-ADEP-DOX-12.5 with a mean particle size of  $280\pm19$  nm (Figure 4). The particle size and polydispersity of Hyal-ADEP-DOX particles were significantly higher than two higher ratios. Consequently, an increase in the degree of substitution of hydrophobic content in the backbone of

the copolymer encouraged hydrophobic interactions and decreased the size of the micelles. Previous studies have also reported similar findings [30]. Zeta potential analysis of ratios showed that a higher degree of substitution was associated with a lower zeta potential. The result of zeta potential confirmed successful grafting on polymer structure. Since the high negative charge of hyaluronic acid-derived nano-micelles is caused by the carboxyl groups of the hyaluronic acid monomers in the polymer structure, the involvement of free carboxyl functional groups in substitution causes a reduction in zeta potential and leads to aggregation [31]. According to the results of this part, further experiments were hence not performed on Hyal-ADEP-DOX-7.5 and Hyal-ADEP-DOX-20.





International Journal of  
Medical Toxicology & Forensic Medicine

**Figure 5.** Transmission electron microscopy image of Hyal-ADEP-DOX in the ratio of 12.5%

a) Hollow nano-micelles, b) CBD-loaded nano-micelles

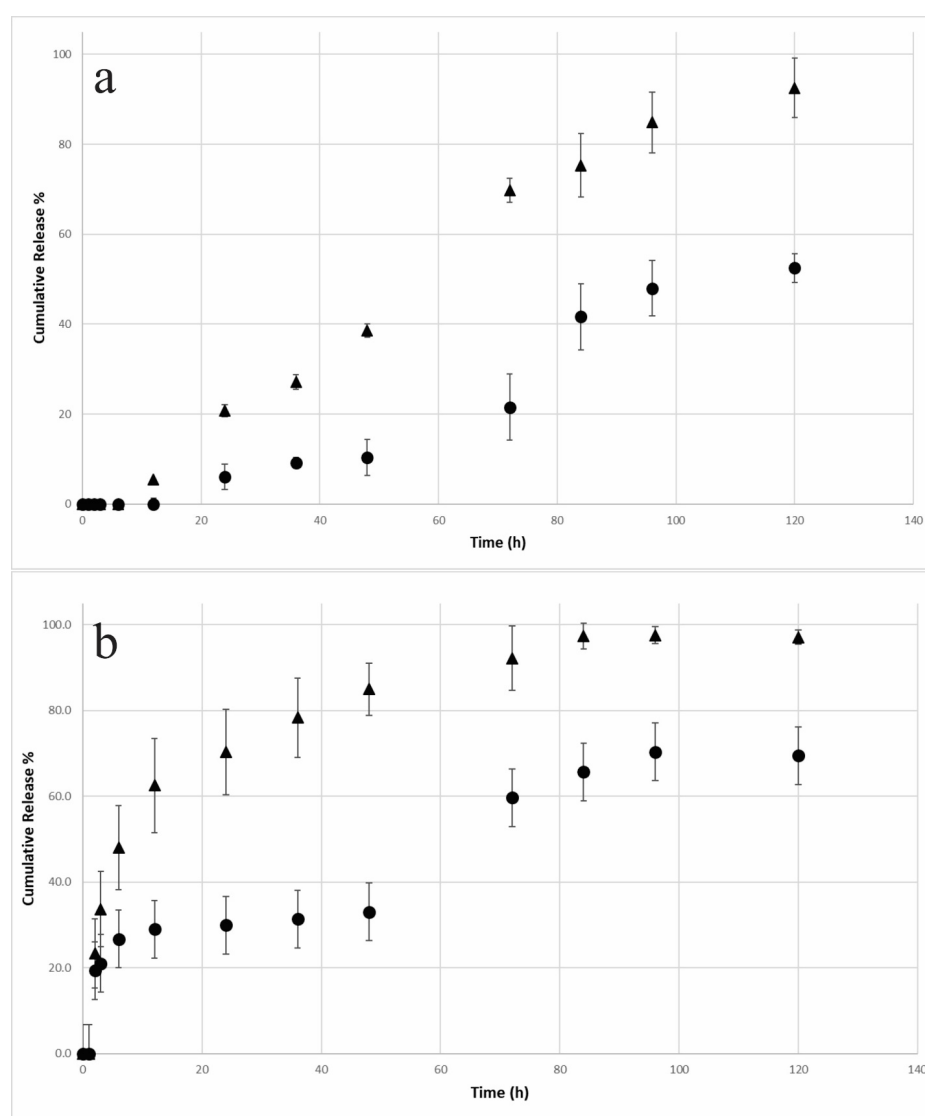
### TEM

TEM was used to observe the morphology of nano-micelles before and after the loading of CBD molecules. The spherical shape of the particles is shown in Figure 5 and the mean diameter of unloaded and loaded nano-micelles was about 50 nm and 120 nm, respectively. Both hollow and CBD-loaded nano-micelles had spherical shapes and were smaller than the light scattering method. Different states of the nano-micelles (the dried state in the TEM method and the hydrated state in the light scatter-

ing method) were responsible for the difference observed between the results of the sizes of methods [32].

### In vitro drug release behavior

Micelle stability, long circulation time, and controlled drug release are required characteristics of a suitable active targeted micellar drug delivery system. Although the conjugation of DOX to polymers could solve water solubility, control release was not addressed as well in these systems. In our previous study on CPT-succinic acid-CHO conjugation at pH 7.4 and 37°C, not more



**Figure 6.** In vitro release behavior

International Journal of  
Medical Toxicology & Forensic Medicine

a) DOX from Hyal-ADEP-DOX 12.5%, b) CBD from loaded Hyal-ADEP-DOX 12.5% at two different pH values (7.4 and 5.5) that represented physiological and endosomal/tumor tissue environments, respectively.

Note: Each point is the mean of three replicates. Data are presented as Mean $\pm$ SD.

than 0.831% of the drug was released from CPT-succinic acid-CHO micelles after 52 h. The rate was as low as 0.691% in 26 h at pH 5.5 and 37°C [25, 32]. Drug release from Hyal-ADEP-DOX micelles was faster than from the CPT-succinic acid-CHO conjugate at pH 5.5. The obtained results showed that in the case of Hyal-ADEP-DOX-12.5, only 6% of the loaded drug was released after 24 h at pH 7.4 and 37°C. At pH 5.5 and 37°C, 20.8% of the loaded drug was released over 24 h (Figure 6). Whole grafted DOX was approximately released at the end of five days of release test at pH 5.5 but there

was about 52.5% release at pH 7.4. Also, we observed the same profile for physically-loaded CBD into the core of the micelles. There was 69.5% release at pH 7.4 and complete release at pH 5.5. This remarkable difference in release profile showed the sensitivity of the ADEP linker to endosomal pH (acidic). Comparing the drug release behavior of the two conjugates reveals that the designed system worked correctly and Hyal-ADEP-DOX may become a fascinating candidate for stimuli-responsive co-delivery systems.

## 4. Conclusions

A new nano-micellar drug co-delivery system for DOX was designed in the present study. Because of the presence of ADEP between DOX and Hyal, this formulation was responsive to low pH (<5.5). Nano-micelles were prepared in three ratios (7.5%, 12.5%, and 20%) and the 12.5% ratio was chosen as the optimum formulation after physiochemical analyses. CBD as an adjuvant therapeutic agent was loaded physically into the core of micelles. Self-assembled nano-micelles had a small size of around 50 nm and 120 nm after loading the CBD and spherical shapes observed by TEM. The grafted DOX exhibited pH-sensitive release behavior, i.e. while 22.8% of the drug was released after 24 h at pH 5.5, and 5% was released after 24 h at pH 7.4. Hyal-ADEP-DOX due to its low CMC value, colloidal stability, slow drug release in physiological pH, and burst release in acidic pH Hyal-ADEP-DOX, is an excellent candidate as a carrier to co-deliver hydrophobic therapeutic agents to tumor tissues.

## Ethical Considerations

### Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

### Funding

The financial support was received from [Shahid Beheshti University of Medical Sciences](#).

### Authors' contributions

Conceptualization, investigation, and writing the original draft: Babak Tajani; Supervision, review, and editing: Seyed Alireza Mortazavi; Methodology and resources: Arash Mahboubi.

### Conflict of interest

The authors declared no conflict of interest.

### Acknowledgements

This study is part of the PhD. thesis of Babak Tajani, proposed and approved by the School of Pharmacy, [Shahid Beheshti University of Medical Sciences \(SBMU\)](#), Tehran, Iran. The authors gratefully acknowledge the financial support received from [SBMU](#) for carrying out this project.

## References

- [1] Greish K, Mathur A, Al Zahrani R, Elkaissi S, Al Jishi M, Nazzal O, et al. Synthetic cannabinoids nano-micelles for the management of triple negative breast cancer. *Journal of Controlled Release*. 2018; 291:184-95. [DOI:10.1016/j.jconrel.2018.10.030] [PMID]
- [2] Brannon-Peppas L, Blanchette JO. Nanoparticle and targeted systems for cancer therapy. *Advanced Drug Delivery Reviews*. 2004; 56(11):1649-59. [DOI:10.1016/j.addr.2004.02.014] [PMID]
- [3] Shukla S, Wu CP, Ambudkar SV. Development of inhibitors of ATP-binding cassette drug transporters: Present status and challenges. *Expert Opinion on Drug Metabolism & Toxicology*. 2008; 4(2):205-23. [DOI:10.1517/17425255.4.2.205] [PMID]
- [4] Stierlé V, Laigle A, Jollès B. Modulation of MDR1 gene expression in multidrug resistant MCF7 cells by low concentrations of small interfering RNAs. *Biochemical Pharmacology*. 2005; 70(10):1424-30. [DOI:10.1016/j.bcp.2005.08.007] [PMID]
- [5] Fraguas-Sánchez AI, Fernández-Carballido A, Simancas-Herbada R, Martín-Sabroso C, Torres-Suárez AI. CBD loaded microparticles as a potential formulation to improve paclitaxel and doxorubicin-based chemotherapy in breast cancer. *International Journal of Pharmaceutics*. 2020; 574:118916. [DOI:10.1016/j.ijpharm.2019.118916] [PMID]
- [6] Grifoni L, Vanti G, Donato R, Sacco C, Bilia AR. Promising nanocarriers to enhance solubility and bioavailability of cannabidiol for a plethora of therapeutic opportunities. *Molecules*. 2022; 27(18):6070. [DOI:10.3390/molecules27186070] [PMID] [PMCID]
- [7] Holland ML, Panetta JA, Hoskins JM, Bebawy M, Roufogalis BD, Allen JD, et al. The effects of cannabinoids on P-glycoprotein transport and expression in multidrug resistant cells. *Biochemical Pharmacology*. 2006; 71(8):1146-54. [DOI:10.1016/j.bcp.2005.12.033] [PMID]
- [8] Ślędzinski P, Zeyland J, Słomski R, Nowak A. The current state and future perspectives of cannabinoids in cancer biology. *Cancer Medicine*. 2018; 7(3):765-75. [DOI:10.1002/cam4.1312] [PMID] [PMCID]
- [9] McAllister SD, Soroceanu L, Desprez PY. The antitumor activity of plant-derived non-psychoactive cannabinoids. *Journal of Neuroimmune Pharmacology*. 2015; 10(2):255-67. [DOI:10.1007/s11481-015-9608-y] [PMID] [PMCID]
- [10] Tomko A, O'Leary L, Trask H, Achenbach JC, Hall SR, Goralski KB, et al. Antitumor activity of abnormal cannabidiol and its analog O-1602 in taxol-resistant preclinical models of breast cancer. *Frontiers in Pharmacology*. 2019; 10:1124. [DOI:10.3389/fphar.2019.01124] [PMID] [PMCID]
- [11] D'Aloia A, Ceriani M, Tisi R, Stucchi S, Sacco E, Costa B. Cannabidiol antiproliferative effect in triple-negative breast cancer MDA-MB-231 cells is modulated by its physical state and by IGF-1. *International Journal of Molecular Sciences*. 2022; 23(13):7145. [DOI:10.3390/ijms23137145] [PMID] [PMCID]
- [12] Scott KA, Dalglish AG, Liu WM. The combination of cannabidiol and  $\Delta^9$ -tetrahydrocannabinol enhances the anticancer effects of radiation in an orthotopic murine glioma model. *Molecular Cancer Therapeutics*. 2014; 13(12):2955-67. [DOI:10.1158/1535-7163.MCT-14-0402] [PMID]

- [13] Hyung Park J, Kwon S, Lee M, Chung H, Kim JH, Kim YS, et al. Self-assembled nanoparticles based on glycol chitosan bearing hydrophobic moieties as carriers for doxorubicin: in vivo biodistribution and anti-tumor activity. *Biomaterials*. 2006; 27(1):119-26. [DOI:10.1016/j.biomaterials.2005.05.028] [PMID]
- [14] Zhou YY, Du YZ, Wang L, Yuan H, Zhou JP, Hu FQ. Preparation and pharmacodynamics of stearic acid and poly (lactic-co-glycolic acid) grafted chitosan oligosaccharide micelles for 10-hydroxycamptothecin. *International Journal of Pharmaceutics*. 2010; 393(1-2):143-51. [DOI:10.1016/j.ijpharm.2010.04.025] [PMID]
- [15] Shi J, Guobao W, Chen H, Zhong W, Qiu X, Xing MMQ. Schiff based injectable hydrogel for in situ pH-triggered delivery of doxorubicin for breast tumor treatment. *Polymer Chemistry*. 2014; 5(21):6180-9. [DOI:10.1039/C4PY00631C]
- [16] Xiong XB, Ma Z, Lai R, Lavasanifar A. The therapeutic response to multifunctional polymeric nano-conjugates in the targeted cellular and subcellular delivery of doxorubicin. *Biomaterials*. 2010; 31(4):757-68. [DOI:10.1016/j.biomaterials.2009.09.080] [PMID]
- [17] Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: A review. *Journal of Controlled Release*. 2000; 65(1-2):271-84. [DOI:10.1016/S0168-3659(99)00248-5] [PMID]
- [18] Opanasopit P, Yokoyama M, Watanabe M, Kawano K, Maitani Y, Okano T. Block copolymer design for camptothecin incorporation into polymeric micelles for passive tumor targeting. *Pharmaceutical Research*. 2004; 21(11):2001-8. [DOI:10.1023/B:PHAM.0000048190.53439.eb] [PMID]
- [19] Maeda H. The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. *Advances in Enzyme Regulation*. 2001; 41:189-207. [DOI:10.1016/S0065-2571(00)00013-3] [PMID]
- [20] Zhang Y, Yang C, Wang W, Liu J, Liu Q, Huang F, et al. Co-delivery of doxorubicin and curcumin by pH-sensitive prodrug nanoparticle for combination therapy of cancer. *Scientific Reports*. 2016; 6:21225. [DOI:10.1038/srep21225] [PMID] [PMCID]
- [21] Chang Q, Gao H, Bu S, Zhong W, Lu F, Xing M. An injectable aldehyde 1-amino-3,3-diethoxy-propane hyaluronic acid-chitosan hydrogel as a carrier of adipose derived stem cells to enhance angiogenesis and promote skin regeneration. *Journal of Materials Chemistry*. 2015; 3(22):4503-13. [DOI:10.1039/C5TB00027K] [PMID]
- [22] Liu J, Li H, Jiang X, Zhang C, Ping Q. Novel pH-sensitive chitosan-derived micelles loaded with paclitaxel. *Carbohydrate Polymers*. 2010; 82(2):432-9. [DOI:10.1016/j.carbpol.2010.04.084]
- [23] Delplace V, Couvreur P, Nicolas J. Recent trends in the design of anticancer polymer prodrug nanocarriers. *Polymer Chemistry*. 2014; 5(5):1529-44. [DOI:10.1039/C3PY01384G]
- [24] She W, Li N, Luo K, Guo C, Wang G, Geng Y, et al. Dendronized heparin-doxorubicin conjugate based nanoparticle as pH-responsive drug delivery system for cancer therapy. *Biomaterials*. 2013; 34(9):2252-64. [DOI:10.1016/j.biomaterials.2012.12.017] [PMID]
- [25] Tajani B, Tahvilian R, Khazaei S, Javadi KS, Fattahi A. Preparation and characterization of camptothecin grafted chitosan oligosaccharide nanomicelles. *Journal of Reports in Pharmaceutical Sciences*. 2015; 4(1):1-11. [Link]
- [26] Tahvilian R, Tajani B, Sadrjavadi K, Fattahi A. Preparation and characterization of pH-sensitive camptothecin-cis-aconityl grafted chitosan oligosaccharide nanomicelles. *International Journal of Biological Macromolecules*. 2016; 92:795-802. [DOI:10.1016/j.ijbiomac.2016.07.100] [PMID]
- [27] Stuart MC, van de Pas JC, Engberts JB. The use of Nile Red to monitor the aggregation behavior in ternary surfactant-water-organic solvent systems. *Journal of Physical Organic Chemistry*. 2005; 18(9):929-34. [DOI:10.1002/poc.919]
- [28] Woraphatphadung T, Sajomsang W, Rojanarata T, Ngawhirunpat T, Tonglairoom P, Opanasopit P. Development of chitosan-based pH-sensitive polymeric micelles containing curcumin for colon-targeted drug delivery. *AAPS PharmSciTech*. 2018; 19(3):991-1000. [DOI:10.1208/s12249-017-0906-y] [PMID]
- [29] Oerlemans C, Bult W, Bos M, Storm G, Nijssen JF, Hennink WE. Polymeric micelles in anticancer therapy: Targeting, imaging and triggered release. *Pharmaceutical Research*. 2010; 27(12):2569-89. [DOI:10.1007/s11095-010-0233-4] [PMID] [PMCID]
- [30] Fattahi A, Golozar MA, Varshosaz J, Sadeghi HM, Fathi MH. Preparation and characterization of micelles of oligomeric chitosan linked to all-trans retinoic acid. *Carbohydrate Polymers*. 2012; 87(2):1176-84. [DOI:10.1016/j.carbpol.2011.08.093]
- [31] Du Y. A review of structural equation modeling and its use in library and information studies. *Library & Information Science Research*. 2009; 31(4):257-63. [DOI:10.1016/j.lisr.2009.07.007]
- [32] Giacomelli C, Schmidt V, Borsali R. Nanocontainers formed by self-assembly of poly(ethylene oxide)-b-poly(glycerol monomethacrylate)-drug conjugates. *Macromolecules*. 2007; 40(6):2148-57. [DOI:10.1021/ma062562u]