Saudi Pharmaceutical Journal 27 (2019) 49–55

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

# Formulation and evaluation of docetaxel nanosuspensions: In-vitro evaluation and cytotoxicity

Mohamed A. Ibrahim [a,b], Gamal A. Shazly [a,c], Fadilah S. Aleanizy [a,*], Fulwah Y. Alqahtani [a], Gehan M. Elosaily [d,e]

[a] Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia
[b] Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt
[c] Department of Industrial Pharmacy, Faculty of Pharmacy, Assiut University, Assiut, Egypt
[d] Department of Pathology, Faculty of Medicine, Almareffa College for Science and Technology, Riyadh, Saudi Arabia
[e] Department of Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt

ARTICLE INFO

*Article history:*
Received 22 April 2018
Accepted 19 July 2018
Available online 20 July 2018

*Keywords:*
Docetaxel
Nanoparticles
Stabilizers
In-vitro release
Cytotoxicity

ABSTRACT

*Objective:* The aim of the present study was to formulate the anticancer drug; docetaxel (DOX) as nanoparticles to enhance its biological activity.
*Methodology:* Solvent precipitation method was used to prepare DOX-loaded nanoparticles and was stabilized by different concentrations of hydroxypropyl methylcellulose (HPMC, E5) and sodium deoxycholate (SDC).
*Results:* The results showed that the particle size of the prepared DOX nanoparticles stabilized by SDC was small in comparison to those stabilized by the corresponding HPMC concentrations. The smallest particle size (83.97 nm) was obtained by using SDC as stabilizer at 5% level with zeta potential of −13.6 mV. It was concluded that increasing the stabilizer concentration resulted in increase in both initial and overall cumulative drug release. The release rate in case of nanoparticles stabilized by 5% SDC was 33% and 87% after 1 and 24 h respectively. The results showed that a significant reduction in the viability of FRO cells was observed at all tested time intervals in case of nanoparticles stabilized by 5% SDC at concentrations of 100 and 1000 μM/ml. In contrast, no signs of cytotoxicity was observed for nanoparticles stabilized by 5% HPMC at 10 and 100 μM/ml concentrations.


## 1. Introduction

The World AS mortality rates in males vary more than two-fold across the different world regions, ranging from 173 per 100,000 in Central and Eastern Europe to 68 per 100,000 in Western Africa. In females, rates vary more than three-fold, ranging from 119 per 100,000 in Melanesia to 65 per 100,000 in South-Central Asia (Ferlay et al., 2015). Due to the multiple cellular physiological systems e.g. apoptosis and cell signaling arising from cancer, the most common treatments of cancer are restricted to chemotherapy, radiation, and surgery. These restrictions are due to existing challenges including inadequate drug concentrations, lack of early disease detection, systemic distribution, nonspecific reaching the tumor, and inability to monitor therapeutic responses (Jemal et al., 2006).

Docetaxel (DOX) is a chemotherapeutic drug used mostly for the treatment a number of cancer (Hanahan and Weingberg, 2000). This comprises head and neck cancer, breast cancer, prostate cancer, stomach cancer, and non small-cell lung cancer (Ganesh, 2007). It is a semi-synthetic analogue of paclitaxel but with high cytotoxic activity. Docetaxel is believed to have a two-fold mechanism of antineoplastic activity: (Greenlee et al., 2001) inhibition of microtubular depolymerization, and (Oh and Kantoff, 1998) attenuation of the effects of bcl-2 and bcl-xL gene expression. Like all chemotherapeutic drugs, side effects are collective and many varying side-effects have been known. DOC is cytotoxic to all dividing cells in the body because it is a cell cycle

* Corresponding author.
*E-mail address:* faleanizy@ksu.edu.sa (F.S. Aleanizy).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2018.07.018
1319-0164/

specific agent. This includes tumour cells as well as hair follicles, bone marrow and other germ cells. For this reason, common chemotherapy side effects such as hair loss occur; sometimes this can be permanent. Thus, DOX does not only target the cancer cells but also affects healthy tissues causing toxicity and side effects (Momparler et al., 1976).

Due to the low aqueous solubility, low bioavailability and high toxicity of DOC, it was formulated in the market for intravenous infusion using tween 80 and ethanol (Taxotere®). However, tween 80 can cause hemolysis and consequently hypersensitivity to patients after administration (Yin et al., 2009). Therefore, improving patient's convenience, and facilitate the use of the drug can be done by formulation of DOC in nanoparticles (NPs).

The clinical efficacy of Docetaxel is limited due to its poor solubility, low selective distribution, fast elimination *in vivo*, etc. (Sanna et al., 2011). In addition, despite the recently reported promising outcome of docetaxel, the drug is associated with systemic toxicity that limits the dose and duration of therapy, particularly in the elderly patients (Freitas and Muller, 1999).

Formulation of nanoparticles is one of the nanotechnology science that has rapidly increased an established record within the pharmaceutical sciences. NPs play an important role in cancer therapy by releasing the drugs in cancer cells rather than in normal cells (Maeda, 2001). Moreover, NPs have huge potential in both prevention, diagnosis, detection and treatment of cancer (Sun et al., 2011). However. the formed nanoparticles might tend to adhere together. Thus a stabilizer was added to avoid the aggregation of high-energy nanoparticles (Lou et al., 2011). The type and amount of stabilizer also have an important influence on the physical stability and *in vivo* behaviors of the nanoparticles (Gao et al., 2012). Moreover, possible toxicity concerns about the long-term use of large quantities of stabilizers have also limited the therapeutic application of drug nanoparticles (Wang et al., 2013a). For instance, as motioned above, Tween® 80, a commercial surfactant that is extensively used in the formulation of nanosuspensions, can result in severe neuro and nephrotoxicity as well as acute hypersensitivity reactions (Merisko-Liversidge et al., 2003).

Polymers and surfactants are routinely used in stabilization of nanosuspension formulations by several stabilization mechanisms, leading to the formation of a stereospecific blockade between the nanoparticles inhibiting particle aggregation (Kim et al., 2011).

Thus, the aim of the present work is to enhance the anticancer activity of DOX by formulating the drug in nanoparticles that will be stabilized by different concentrations of HPMC and SDC. Nanoparticles' particle sizes, zeta potential values, *in vitro* release rate of DOX were evaluated. In addition, Cytotoxicity assay of selected DOX nanoparticles formulation has been carried out.

## 2. Materials and methods

### 2.1. Materials

Docetaxel (DOX) anhydrous (MWt = 807.9) was purchased from Knowshine (Shanghai) Pharmachemicals Inc. Hydroxypropyl methylcellulose (HPMC) E5 was purchased from Dow Chemical Co. (Midland, Michigan, USA). Sodium deoxycholate (SDC) was purchased from BDH Chemical Co. (Poole, England). Other chemicals were of reagent grade and were used as received. Undifferentiated/anaplastic thyroid cancer cell line (FRO) was kindly gifted from (King Faisal Specialist Hospital & Research Centre) KFSH & RC. The cells were cultured in humidified air with 5% CO2 at 37 °C in RPMI-1640 Medium with L-glutamine and sodium bicarbonate (R8758, Sigma Aldrich, St. Louis, MO, USA), and supplemented with 10% fetal bovine serum (FBS), and 1% penicillin-streptomycin, both from (Gibco).

### 2.2. Methods

#### 2.2.1. Preparation of DOX nanosuspensions

DOX nanosuspensions were prepared by solvent precipitation method, which is one of the bottom-up techniques. In brief, 50 mg of DOX was dissolved in 5 mL (chloroform). The drug solution in the organic solvent was dropped slowly to 20 mL of an aqueous solution containing a specific concentration of the stabilizer, and stirred at 800 rpm (Multipoint stirrer, IKA R05, USA). The dispersion was allowed to stir for 4 h to ensure complete removal of the organic solvent. The resulting nanosuspension was then characterized for its particle size and zeta potential.

The prepared DOX nanosuspension was freeze-dried at −60 °C and the vacuum pressure was less than 1 mbar (Alpha 1–4 LD Plus, Martin Christ Gefriertrocknugs anlagen GmbH, Osterode am Harz, Germany) and stored in tightly closed containers protected from light until further studies.

#### 2.2.2. Particle size determination

The mean particle sizes of freshly prepared DOX nanosuspensions as well as the freeze-dried nanoparticles were measured using photon correlation spectroscopy (PCS) using zetasizer (Nano ZS, Malvern Instruments, UK) at room temperature. The tested samples were diluted with deionized water to a suitable dilution, and added to the electrophoretic measuring cuvette. The particle size experiments carried out in triplicates, and the polydispersity index (PDI) was measured.

#### 2.2.3. Zeta potential

Zeta potential of the prepared DOX nanosuspensions were measured by zetasizer (Nano ZS, Malvern Instruments, UK). All measurement were carried out at room temperature in triplicates.

#### 2.2.4. DOX content in nanoparticles

In 50 mL volumetric flask, an accurately weighed 10 mg of lyophilized nanoparticle powder was dissolved in 5 mL methanol, and volume was complete by phosphate buffer pH 6.8 then 4 mL of solution will be taken and transferred to 10 mL volumetric flask and volume will be adjusted with phosphate buffer 6.8 pH. The absorbance at $\lambda_{max}$ 230 nm was measured (UV-2800 spectrophotometer, Labomed Inc., USA) against a suitable blank made of the aqueous solution containing the corresponding amount of the stabilizer. In addition, the % yield of the freeze-dried nanoparticles was calculated.

#### 2.2.5. X-ray powder diffraction (XRPD)

The X-ray diffraction spectral analysis of some selected DOX nanoparticles stabilized by HPMC E5 and SDC as well as the individual components was carried out by RIGAKU diffractometer (Japan) which is equipped with curved graphite crystal monochromator, automatic divergence slit and automatic controller PW/1710. The target used was Cu Kα radiation operating at 40 KV and 40 mA (λkα = 1.5418 Å). The X-ray diffraction profiles were obtained using continuous scan mode with 2θ° ranging from 4° to 60°.

#### 2.2.6. In-vitro release studies

The *in vitro* release of DOX from its nanoparticles was determined by using a modified method described by zur Mühlen et al. (1998). An amount of the freeze dried nanoparticles equivalent to 5 mg DOX was accurately weighed into 50-mL falcon tubes, then, 25 mL of phoshate buffer was added, and the dispersion was shaken for a minute to disperse the particles homogeneously in the release medium. The tubes were allowed to be shaken in a thermostatically controlled shaking water bath maintained at 37 °C. At predetermined time intervals, 5 mL aliquot sample was withdrawn

by using 0.22 um Millipore filter, and diluted to an appropriate volume with the release medium. The absorbance was then measured at 230 nm (Yousef et al., 2009) and the amount of DOX released was calculated.

*2.2.7. Kinetic modeling of the in-vitro release data*

In order to describe the release model that best correlates the profile of drug release from its-loaded nanoparticles, the in-vitro release data were fitted to zero order, first order and diffusion controlled release mechanisms according to the simplified Higuchi model (Higuchi, 1963) as follows:

(a) Zero-order kinetic model:

$$C = Co - Kot$$

(b) First order kinetic model:

$$\log C = \log Co - Kt/2.303$$

(c) Higuchi diffusion model:

$$Q = 2Co(Dt/\pi)1/2$$

where

Co = initial drug concentration
C = drug concentration (remaining) at time t.
t = time of release
Q = amount of drug released/unit area

Ko = zero order rate constant, K = first order rate constant and D = diffusion Coefficient and it was calculated according to the following equation.

$$D = (Slope/2Co)2\pi$$

The favored mechanism was grounded on the correlation coefficient (r) for the studied parameters, where the highest correlation coefficient is preferred for the selection of mechanism of release.

Sequential proof of the relative validity of diffusion model was acquired by analyzing the data using the following equation (Korsmeyer and Peppas, 1983; Korsmeyer et al., 1983)

$$Mt/M_{\infty} = K \cdot t^{1/2}$$

where $Mt/M\infty$ is the fraction of drug released at time t, K is a constant including structural and geometric characteristic and n is the release exponent characteristic for the drug transport mechanism. When n = 0.5 fickian diffusion is observed and the release rate independent on t, while 0.5 < n < 1.0 indicate anamalous (non fickian) transport and when n = 1, the release is zero order.

*2.2.8. Cytotoxicity assay*

The undifferentiated/anaplastic thyroid cancer cell line (FRO) were maintained in RPMI-1640 that included L-glutamine (GIBCO) with 10% FBS (GIBCO) and 1% penicillin–streptomycin (GIBCO). Cells were cultured at 37 °C in a 5% CO2 incubator. The effect of Na Deox. NPs, HPMEC docetaxel NPs, and docetaxel on cellular viability was evaluated using Alamar Blue assay (BUF012B; AbD Serotec, Langford Ln, Kidlington OX5 1GE, United Kingdom). The Alamar Blue assay is used to assess cell viability based on the reduction potential of metabolically active cells. Viable cells were seeded in the growth medium into 96-well microtiter plates ($1 \times 10^4$ cells/well) and were incubated at 37 °C in a 5% CO2 incubator for 24 h. The Na Deox. NPs, HPMEC docetaxel NPs, and docetaxel were adjusted to final 10, 100, and 1000 μM/ml concentrations by diluting with the growth medium. After standing for 24 h, media were removed and the test sample was added to each well. Control wells consisted of cells alone. After 24, 48, and 72 h of addition of test sample, 10 μL of Alamar Blue reagent was added to each well (final concentration, 10 μg/ml) and the plates were incubated at 37 °C for 4 h. After incubation, plates were read using a spectrophotometric microplate reader (Biotek Synergy 2; Biotek Instruments, Highland Park, Winooski, Vermont, NE, USA) and the relative fluorescence unit was recorded. Results were expressed as percentage cell viability versus the control.

## 3. Results and discussion

### 3.1. Particle size of nanosuspensions and freeze-dried nanoparticles

Particle size analysis of DOX nanosuspensions and freeze-dried nanoparticles stabilized by different concentrations of sodium deoxycholate and HPMC E5 is displayed in Table 1. The results obtained showed that by increasing the stabilizer concentration, a pronounced reduction of the sizes of DOX in its nanosuspension and nanoparticle forms has been observed. For example, upon using 1% concentration of HPMC and SDC, the recorded particle sizes of nanosuspensions were 714.0 and 295.8 nm, respectively, while the measured particles sizes in case of 5% concentration of the stabilizer were 375 nm and 83.97 nm, respectively.

Ma et al. (2016) showed that the particle size of Azilsartan nanosuspension was reduced from 370 nm to about 310 nm by using 1% SDC instead of 0.5%. They concluded that SDC can help intensely decrease the particle size of nanosuspension.

After lyophilization of the nanosuspensions, an increase in the particle size was observed for all samples. The measured particle size of nanoparticles stabilized by 5% SDC was found to be 83.97 nm and 108 nm before and after lyophilization, respectively, Fig. 1. Lee et al. (2004) showed that the particle size of lyophilized chitosan nanoparticles was increased after freeze-drying, and they explained this phenomenon to particle aggregation as a result of the strong inter- and intramolecular hydrogen bonding.

### 3.2. Zeta potential

The measured zeta potential value of DOX suspension was −7.9 mV, Table 1. In case of nanoparticles stabilized by SDC, the value of zeta potential increased with increasing the concentration of SDC as stabilizers. A highest zeta potential value (−13.6 mV) of nanosuspension prepared by using 5% concentration of SDC was

**Table 1**
Particle size analysis of DOX nanosuspensions and freeze-dried nanoparticles stabilized by different concentrations of sodim deoxycholate and HPMC E5.

| Sample | Nanosuspension | | | Freeze-dried nanoparticles | | |
|---|---|---|---|---|---|---|
| | Particle size (nm) | PDI | Zeta potential (mV) | Particle size (nm) | PDI | % Yield |
| Docetaxel untreated | $1.115 \times 10^5$ | 0.34 | −7.9 | – | 0.35 | 92.3 |
| 0.5% Sodim deoxycholate | 601.3 | 0.23 | −9.99 | 674.5 | 0.41 | 85.7 |
| 1% Sodim deoxycholate | 295.8 | 0.51 | −11.85 | 302.1 | 0.29 | 89.0 |
| 5% Sodim deoxycholate | 83.97 | 0.48 | −13.6 | 107.8 | 0.47 | 94.1 |
| 0.5% HPMC | 1038 | 0.61 | −8.9 | 1210 | 0.52 | 87.6 |
| 1% HPMC | 714 | 0.53 | −8.5 | 810 | 0.46 | 84.7 |
| 5% HPMC | 375 | 0.47 | −7.24 | 425 | 0.37 | 90.7 |

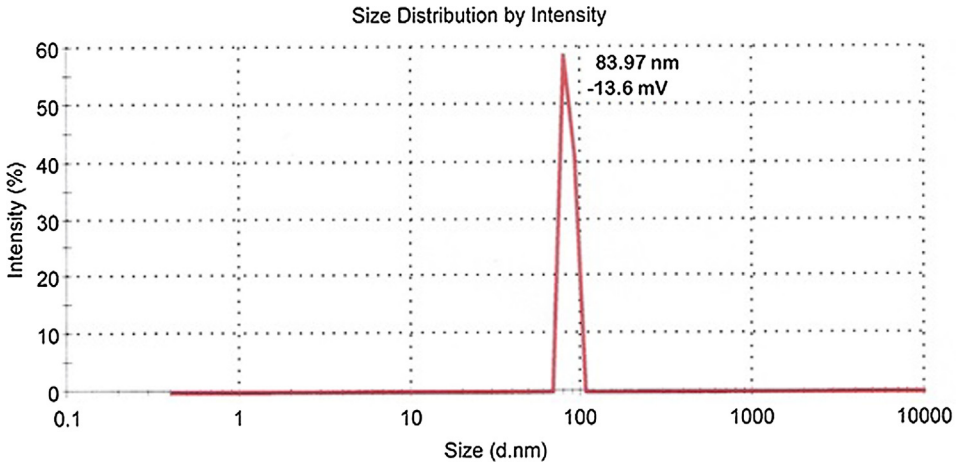


**Fig. 1.** Particle size analysis and zeta potential of DOX nanosuspension stabilized by 5% sodium deoxycholate.

observed. However, nanosuspensions stabilized by different concentrations f HPMC did not show significant changes of their zeta potential values compared to the DOZ suspension.

The anionic surfactant (SDC) exhibited best stabilizing effect in comparison with HPMC E5 for nanosuspension preparation. This can be attributed to a fact that the anionic surfactant has excellent dispersion properties, and therefore, its molecules could diffuse more rapidly to the particle surfaces (Azad et al., 2015; Pawar et al., 2014). Du et al. (2016) showed that anionic surfactant adsorption onto the surface of the nanoparticle causes a raise in the zeta potential value, and the stabilization of the dispersed nanoparticles can be then attained by surface modification. Similarly, the use of ionic or nonionic surfactants causes electrostatic repulsion between the dispersed nanoparticles.

Stabilization of nanosuspensions by using polymers such as HPMC can be achieved by the polymer adsorption to the surface of the dispersed nanoparticles providing a kind of steric hindrance (Azad et al., 2015).

### 3.3. X-ray powder diffraction (XRPD)

XRD analysis was achieved to know the crystalline structure of DOX in the prepared nanoparticles. Figs. 2 and 3 show the X-ray diffraction spectra for DOX nanoparticles stabilized by 5% SDC and 5% HPMC, respectively, compared to the individual components. DOX exhibited significant diffraction peaks from at the 2-theta range of 3 to 30, indicating high crystallinity. The crystalline characteristics of DOX were changed in its-loaded nanoparticles (stabilized by SDC and HPMC) to amorphous form.

### 3.4. Stabilization of DOX loaded nanosuspensions

Several stabilizers are incorporated during the preparation of DOX nanosuspension so as to prevent particles aggregation and agglomeration (Wang et al., 2013b). The frequently used stabilizers used to stabilize nanosuspensions and nanoparticles include polymers such as hydroxypropyl methylcellulose; HPMC (Azad et al., 2015) and surfactants (Wang et al., 2013b). In the present study, HPMC E5 and SDC were used as stabilizers for DOX nanosuspensions at different concentrations (0.5, 1 and 5% w/v).

### 3.5. In-vitro release

The in-vitro release profiles of DOX from its nanoparticles stabilized by HPMC E5 are displayed in Fig. 4. Untreated DOX showed very slow release rate, in which with an initial release of 11% has

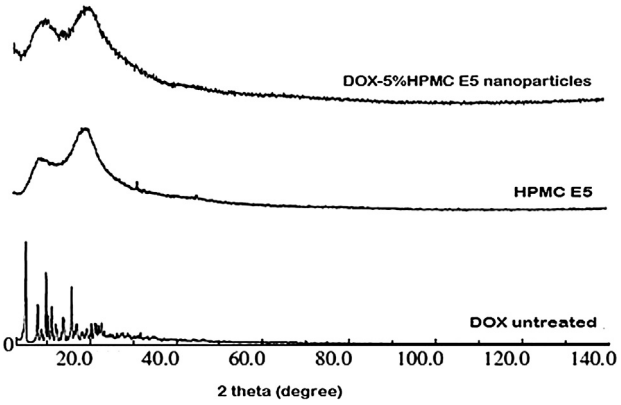


**Fig. 2.** XRPD spectra of DOX nanoparticles stabilized by 5% HPMC E5 compared to the individual components.

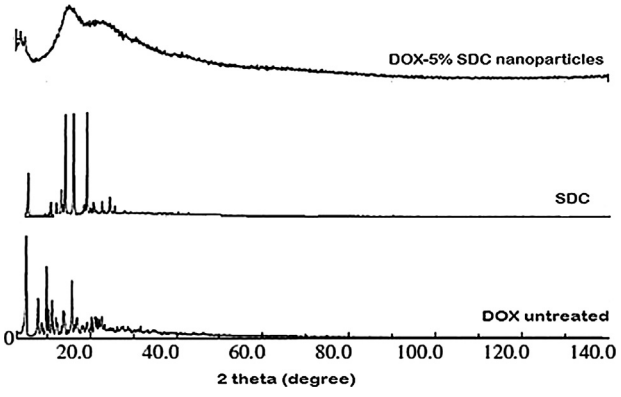


**Fig. 3.** XRPD spectra of DOX nanoparticles stabilized by 5% SDC compared to the individual components.

been recorded, and only 25% release has been observed after 24 h (the period of release study). The very slow release of DOX in the release medium (pH 7.4) is due to the basic nature of DOX that results in unionization of the drug in such release pH (Ibrahim et al., 2012). Formulation of drug nanoparticles stabilized by different concentrations of HPMC E5 caused a noticeable enhancement of drug release rate as a function of HPMC concentration. DOX nanoparticles stabilized with 5% HPMC exhibited an initial release rate of 64.4% after 0.25 h, and after 4 h, a complete release was

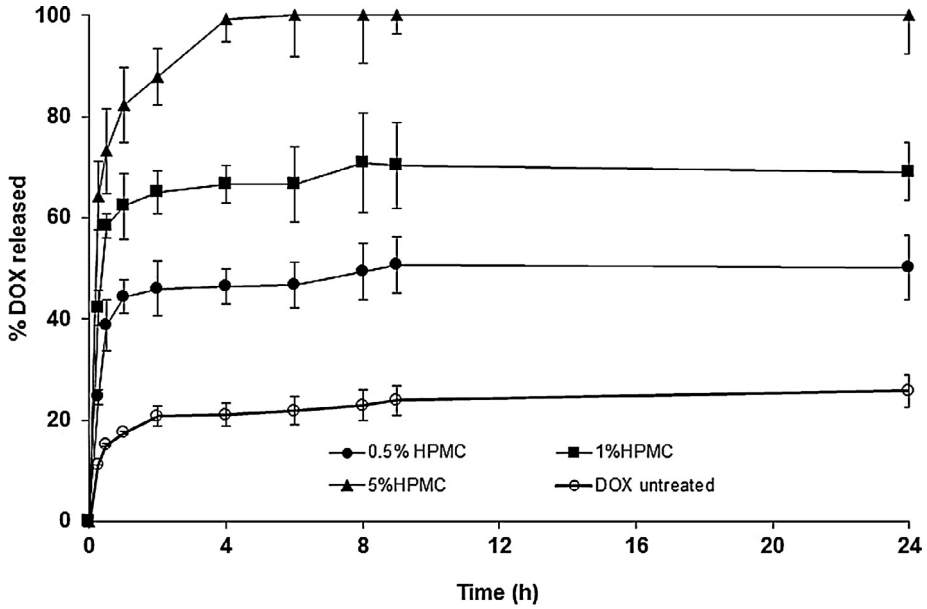


**Fig. 4.** In-vitro release profiles of DOX nanoparticles stabilized by different concentrations of HPMC E5.

attained. In case of using 0.5% and 1% concentrations of HPMC, the drug exhibited initial release rates of 24.7 and 42.19%, respectively, and only 46.44 and 66.69% release were observed, respectively, after 4 h.

Similar pattern of drug release has been found in case of nanoparticles stabilized by different SDC concentrations. At the lowest level of SDC, nanoparticle release was found too slow, that is not different from the untreated drug. By increasing SD concentration an increase in both initial and overall release rate was exhibited; reaching its highest rate in case of nanoparticles stabilized by 5% SDC, in which an initial rate of 33% after 1 h, and a release rate of 87% after 24 h was observed, Fig. 5.

At a high concentration of the low viscous grade HPMC (5%), a noticeable enhancement of drug dissolution rate was detected in comparison to the lower concentrations used (0.5% and 1%). This might be due to decreasing particle size of the prepared nanoparticles (425 nm) because of increasing HPMC concentration to 5%, Table 1. Moreover, the hydrophilic layer aligning around, coating and stabilizing nanoparticles caused by polymer molecules is expected to be thicker at this high HPMC concentration, which, in turn, might result in enhancing drug dissolution. This could be supported by decreasing zeta potential value as the HPMC concentration increased, Table 1. Vesrma et al. (2009) showed that the hydrophobic interactions between HPMC and ibuprofen surface were contributing to the massive and tough adsorption of the polymer molecules onto the nanoparticle surface, which resulted in the adsorption of the molecules in an open-chain-like way rather than a compact/coiled form.

Regarding nanoparticles stabilized by SDC, The hydrocarbon (hydrophobic) moiety of the surfactant is crucial for the stabilization of nanosuspension. The hydrophobic moieties of the surfactant is considered as the driving force for the adsorption onto the

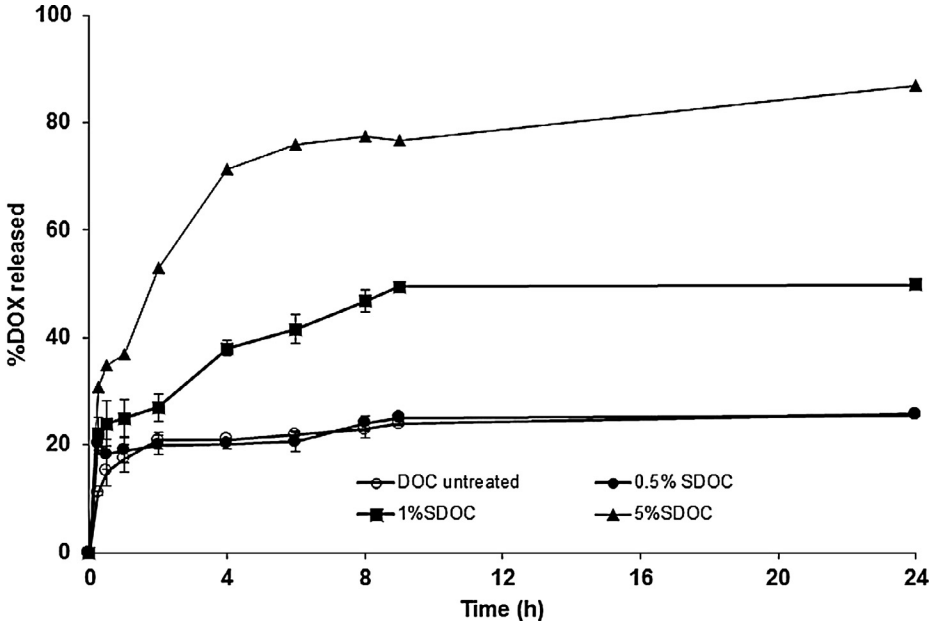


**Fig. 5.** In-vitro release profiles of DOX nanoparticles stabilized by different concentrations of sodium deoxycholate.

hydrophobic drug particle surface; which causes anchoring of the particle surface, then provide steric or ionic stabilization (Nakarani et al., 2010). The increase in zeta potential value by increasing SDC concentration, Table 1 may support this mechanism. In addition, due to the presence of both hydrophilic and hydrophobic regions in the same surfactant molecule, orientation of surfactant hydrophilic part toward water instead of the hydrophobic drug surface may prevail, thereby resulting in a further effective steric or ionic stabilization for drug nanosuspensions (Nakarani et al., 2010).

### 3.6. Kinetic modeling of the in-vitro release data

The data of the *in vitro* release profiles of DOX from its nanoparticles were analyzed using zero order, first order and Higuchi diffusion models as well as Korsmeyer-Peppas equation so as to define the mechanism that fits the drug release, Table 2. Preference of the release model is centered on the value of correlation coefficient of each model with lower n values of Korsmeyer-Peppas. The data of DOX release from nanoparticle formulations stabilized by SDC showed best fit to Higuchi diffusion model based on the correlation coefficients (r) of the release data fit to the model equation, however, lower r values were recorded in case of the drug release from nanoparticles stabilized by 0.5% SDC. This might be due to the very slow release of the drug from nanoparticle formulations stabilized by 0.5% SDC after 2 h. Similarly, the data of release DOX from nanoparticles stabilized by HPMC exhibited good fit to Higuchi model, but with lower r values, which can be also attributed to the slow release period after 2 h after initial fast release.

The value of release exponent n derived from Korsmeyer-Peppas mode, for polydisperse systems lower values are possible (Ritger and Peppas, 1987). Moreover, the deviation from 0.45 may be the consequence of the existence of porosity in these nanoparticle formulations (Saur et al., 2014).

### 3.7. Cytotoxicity assay

Cytotoxicity of nanoparticle formulations stabilized by 5% SDC and 5% HPMC compared to the untreated drug was evaluated using Alamar Blue assay. The Alamar Blue assay was used to assess cell viability and cell proliferation and is based on the reduction potential of metabolically active cells. The effect of different DOX formulations on FRO cells viability at different concentrations (10, 100, 1000 μM/ml) at different time intervals 24, 48 and 72 h was demonstrated in Fig. 6. Samples treated with untreated DOX (dissolved in 0.5% carboxymethyl cellulose solution) exhibited high

**Table 2**
Kinetic modeling of DOX release from different nanoparticle formulations.

| Formulation | Zero order model r | First order model r | Higuchi diffusion model r | Korsmeyer-Peppas model r | n* |
|---|---|---|---|---|---|
| 0.5% Sodim deoxycholate | 0.536 | 0.555 | 0.684 | 0.647 | 0.043 |
| 1% Sodim deoxycholate | 0.864 | 0.912 | 0.945 | 0.952 | 0.220 |
| 5% Sodim deoxycholate | 0.877 | 0.948 | 0.966 | 0.979 | 0.298 |
| 0.5% HPMC | 0.618 | 0.662 | 0.788 | 0.837 | 0.155 |
| 1% HPMC | 0.591 | 0.685 | 0.764 | 0.867 | 0.115 |
| 5% HPMC | 0.579 | – | 0.761 | 0.859 | 0.125 |

r = correlation coefficient, and n is the release exponent.
* Obtained from Korsmeyer-Peppas equation.

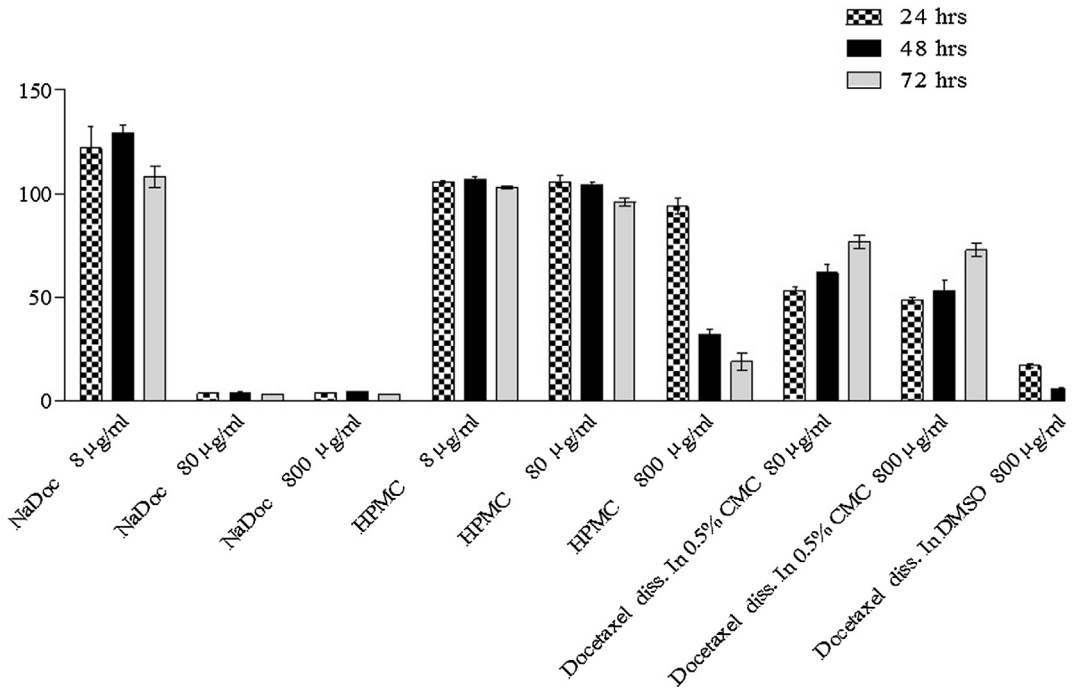


**Fig. 6.** Cell viability percentage of FRO cells after incubation with different DOX nanoparticle formulations after 24, 48, and 72 h incubation with three concentrations of each formulation (10, 100, 1000 μM/ml) presented as mean ± SD.

level of cell viability in both the tested concentrations (80 μg/mL and 800 μg/mL), but a slight, but insignificant reduction in the cell viability ($p > 0.05$) was observed after 48 and 72 h in case of the cell line treated with 800 μg/mL of DOX. In case of SDC-stabilized nanoparticles, there was no significant alteration in cells viability at concentration of 10 μM/ml after incubation with the cells for 24, 48, and 72 h. In contrast, highly significant reduction in the viability of FRO cells was observed at all tested time intervals treated with higher concentrations (100 and 1000 μM /ml) of nanoparticles stabilized by 5% SDC. The calculated p-values were less than 0.00001 in both cases. Moreover, there were no significant signs of cytotoxicity were observed for DOX nanoparticles stabilized by 5% HPMC at 10 and 100 μM/ml concentrations. However, 1000 μM/ml concentration of this nanoparticle formulation resulted in significant reduction of 32% and 19% in cell viability only after 48 and 72 h respectively (the calculated p values were 0.00017 and 0.00013, respectively).

## 4. Conclusion

Nanonization of the anticancer drug docetaxel in presence of the polymeric stabilizer (HPMC) and surfactant stabilizer (SDC) resulted in enhanced the drug dissolution rate *in vitro*, which might be due to several effects including particle size reduction and the increased particles wettability caused by stabilizers. In addition, cytotoxic activity of docetaxel has been significantly improved in its nanoparticle formulations, especially nanoparticle formulation stabilized by 5% SDC at all concentrations, and this cytotoxic activity extended to 72 h.

## Acknowledgment

The authors extend his appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGP – VPP – 139.

## References

Azad, M., Afolabi, A., Bhakay, A., Leonardi, J., Dave, R., Bilgili, E., 2015. Enhanced physical stabilization of fenofibrate nanosuspension via wet co-milling with a superdisintegrant and an adsorbing polymer. Eur. J. Pharm. Biopharm. 94, 372–385.

Du, J., Zhou, Y., Wang, L., Wang, Y., 2016. Effect of PEGylated chitosan as multifunctional stabilizer for deacetyl mycoepoxydience nanosuspension design and stability evaluation. Carbohyd. Polym. 153, 471–481.

Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D.M., Forman, D., Bray, F., 2015. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int. J. Cancer 136 (5), E359–E386.

Freitas, C., Muller, R.H., 1999. Correlation between long-term stability of solid lipid nanoparticles (SLN) and crystallinity of the lipid phase. Eur. J. Pharm. Biopharm. 47 (2), 125–132.

Ganesh, T., 2007. Improved biochemical strategies for targeted delivery of taxoids. Bioorg. Med. Chem. 15, 3597–3623.

Gao, L., Liu, G.Y., Ma, J.L., Wang, X.Q., Zhou, L., Li, X., 2012. Drug nanocrystals: *in vivo* performances. J. Control Release 160, 418–430.

Greenlee, R.T., Hill-Harmon, M.B., Murray, Thun M., 2001. Cancer statistics. CA Cancer J. Clin. 51, 15–36.

Hanahan, D., Weingberg, R.A., 2000. The Hallmarks of Cancer. Cell 100, 57–70.

Higuchi, T., 1963. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J. Pharm. Sci. 52, 1145–1149.

Ibrahim, M.A., El-Toni, A.M., Khan, A., Labis, J.P., Al-Hoshan, M., 2012. Impact of textural properties of double mesoporous coreshell silica nanospheres on drug loading and *in vitro* release. Digest J. Nanomat. Biostruc. 7, 447–458.

Jemal, A., Siegel, R., Ward, E., Murray, T., Xu, J., Smigal, C., Thun, M.J., 2006. Cancer statistics. CA Cancer J. Clin. 56, 106–130.

Kim, J.H., Jang, S.W., Han, S.D., Hwang, H.D., Choi, H.G., 2011. Development of a novel ophthalmic ciclosporin A-loaded nanosuspension using top-down media milling method. Pharmazie 66, 491–495.

Korsmeyer, R.W., Gurny, R., Docler, E., Buri, P., Peppas, N.A., 1983. Mechanism of solute release from porous hydrophilic polymers. Int. J. Pharm. 15, 25–35.

Korsmeyer, R.W., Peppas, N.A., 1983. Macromolecular and modeling aspects of swelling-controlled systems. In: Roseman, T.J., Mansdorf, S.Z. (Eds). Controlled Release Delivery Systems, Dekker, New York, N.Y., pp. 77–101.

Lee, D.W., Powers, K., Baney, R., 2004. Physicochemical properties and blood compatibility of acylated chitosan nanoparticles. Carbohydr. Polym. 58, 371–377.

Lou, H.Y., Gao, L., Wei, X.B., Zhang, Z., Zheng, D.D., Zhang, D.R., et al., 2011. Oridonin nanosuspension enhances anti-tumor efficacy in SMMC-7721 cells and H22 tumor bearing mice. Colloids Surf. B 87, 319–325.

Ma, J., Yang, Y., Sun, Y., Sun, J., 2016. Optimization, characterization and *in vitro*/*vivo* evaluation of azilsartan nanocrystals. Asian J. Pharm. Sci. 12, 344–352.

Maeda, H., 2001. The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. Adv. Enzyme Regul. 41, 189–207.

Merisko-Liversidge, E., Liversidge, G.G., Cooper, E.R., 2003. Nanosizing: a formulation approach for poorly-water-soluble compounds. Eur. J. Pharm. Sci. 18, 113–120.

Momparler, R.L., Karon, M., Siegel, S.E., Avila, F., 1976. Effect of adriamycin on DNA, RNA, and protein synthesis in cell-free systems and intact cells. Cancer Res. 36 (8), 2891–2928.

Nakarani, M., Patel, P., Patel, J., Murthy, R.S., Vaghani, S.S., 2010. Cyclosporine a-nanosuspension: formulation, characterization and *in vivo* comparison with a marketed formulation. Sci. Pharm. 78, 345–361.

Oh, W.K., Kantoff, P.W., 1998. Management of hormone refractory prostate cancer: Current standards and future prospects. J. Urol. 160, 1220–1229.

Pawar, V.K., Singh, Y., Meher, J.G., Gupta, S., Chourasia, M.K., 2014. Engineered nanocrystal technology: In-vivo fate, targeting and applications in drug delivery. J. Control. Release 183, 51–66.

Ritger, P.L., Peppas, N.A., 1987. A simple equation for description of solute release I. Fickian and non-fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. J. Control. Release 5, 23–36.

Sanna, V., Roggio, A.M., Posadino, A.M., Cossu, A., Marceddu, Mariani, A., et al., 2011. Novel docetaxel-loaded nanoparticles based on poly(lactide-co-caprolactone) and poly(lactide-co-glycolide-co-caprolactone) for prostate cancer treatment: formulation, characterization, and cytotoxicity studies. Nanoscale Res. Lett. 6 (1), 260–268.

Saur, J., Millán, D., Suñé-Negre, J.M., Colom, H., Ticó, J.R., Miñarro, M., 2014. Quality by Design approach to understand the physicochemical phenomena involved in controlled release of captopril SR matrix tablets. Int. J. Pharm. 477, 431–441.

Sun, W., Mao, S.R., Shi, Y., Li, L.C., Fang, L., 2011. Nanonization of Itraconazole by high pressure homogenization: stabilizer optimization and effect of particle size on oral absorption. J. Pharm. Sci. 100, 3365–3373.

Vesrma, S., Huey, B.D., Burgess, D.J., 2009. Scanning probe microscopy method for nanosuspension stabilizer selection. Langmuir 25, 12481–12487.

Wang, Y., Zheng, Y., Zhang, L., Wang, O., Zhang, D.B., 2013b. Stability of nanosuspensions in drug delivery. J. Control. Release 172, 1126–1141.

Wang, Y.C., Ma, Y.Y., Zheng, Y., Song, J., Yang, X., Bi, C., et al., 2013a. *In vitro* and *in vivo* anticancer activity of a novel puerarin nanosuspension against colon cancer, with high efficacy and low toxicity. Int. J. Pharm. 441, 728–735.

Yin, Y.-M., Cui, F.-D., Mu, C.-F., Cho, M.-K., Kim, J.S., Chung, S.J., et al., 2009. Docetaxel microemulsion for enhanced oral bioavailability: Preparation and in vitro and in vivo evaluation. J. Control. Release 140 (2), 86–94.

Yousef, A., Esmaeil, F., Rahman, S., Atayebi, F., Dinarrvand, R., 2009. Preparation and in vitro evaluation of a pegylated nano-liposomal formulation containing docetaxel. Sci. Pharm. 77, 453–464.

zur Mühlen, A., Schwarz, C., Mehnert, W., 1998. Solid lipid nanoparticles (SLN) for controlled drug delivery—drug release and release mechanism. Eur. J. Pharm. Biopharm. 45, 149–155.