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Chol-Dex nanomicelles: Synthesis, characterization and evaluation as efficient drug carriers for colon targeting

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ABSTRACT

Core-shell structures obtained from amphiphilic molecules on self-assembly in a medium have emerged as an important tool in the area of biomedical sciences. Here, we have synthesized cholesteryl-dextran (Chol-Dex) amphiphiles in sufficiently high yields via conjugation of cholesteryl hemisuccinate to dextran in two different concentrations (5 and 10%). After physicochemical and spectral analysis, the nanomicelles were subjected to size measurements. DLS and TEM confirmed the formation of core-shell type of nanomicelles. Hydrophobic drug-entrapped formulations (Metronidazole and Rifampicin) displayed sustained release behaviour of drugs from them. Sustained release at neutral pH demonstrated usefulness of the non-toxic delivery system for colon specific diseases.

1. Introduction

Recently, supramolecular self-assembly has extensively been exploited to develop various types of drug delivery systems. It is the most prominent process, governed by inter- and intramolecular forces of attraction, through which amphiphilic molecules are spontaneously aggregated in aqueous media and give rise to diverse form of structures, varying in their shape and sizes [1–3] and are responsible for absorption of nutrients [4]. Basically, micelles are made up of amphiphilic molecules, having unique hydrophobic inner core shielded via outermost hydrophilic shell [5–7]. Currently, micelles are emerging as efficient nanocarriers due to the fact that their hydrophobic core easily solubilises hydrophobic drugs and enhances their efficacy. Besides, the hydrophilic outer shell of the micelles not only minimizes non-specific interactions with the tissues or cells but also increases the circulation time as well as stability [8,9]. Moreover, these structures provide multifunctional activity such as biocompatibility and amenability to modifications to improve the bioavailability of the drug molecules at the desired site [10–13].

Using this strategy, targeted drug delivery systems have also been developed which helped in obviating harmful side effects, improving the drug stability, maintaining the required concentration of the drug at the site of action. For this purpose, low molecular weight amphiphiles have been shown to undergo self-assembly and generate nanomicelles [14,15], however, their variable stability over a period of time and low solubility are the major bottlenecks. In order to bypass such concerns, polymer-based nanomicelles have been proposed with several advantages and for biomedical applications [16–20]. These nanomicelles are formed via self-assembly of amphiphilic substrates and offer several advantages, viz., provide increased solubility to hydrophobic drugs, improve the drug bioavailability, enhance permeability, uptake and retention effects, minimize toxic effects, etc. [21] and evaluated for their promising potential as drug delivery systems. Among the various polymeric systems, hydrophilic polymers with stealth properties are preferred ones, viz., polyethylene glycol (PEG), polyvinyl alcohol (PVA), alginate, galactomannan, hyaluronic acid (HA), pullulan, dextran, etc. [22–24]. Of these polymers, PEGs of various molecular weights have extensively been used to conjugate with hydrophobic drugs/ligands [7]. The resulting amphiphilic conjugates, bearing PEG as hydrophilic head group, self-assemble and form micellar structures which not only facilitate entrapment of hydrophobic drugs but also show a long-term stability even after dilution. Following this methodology, several hydrophobic ligands, viz., cholesterol, cholic acid, palmitoyl, squalene, naproxene, retinoic acid, etc. [25,26], have been conjugated and

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demonstrated as efficient drug delivery systems in vitro and in vivo. Using this hydrophobic-hydrophilic polymer-ligand combination, several stimuli-responsive amphiphiles have been created which are responsive to pH, enzyme, light, temperature, etc. Recently, Zhang et al. have fabricated a reduction sensitive drug delivery system for tumor sites using pluronic F68 and cholesterol linked through a disulfide linkage. By taking advantage of high concentration of glutathione at tumor sites, the entrapped doxorubicin (DOX) in the micelles was released in a sustained manner over a longer period of time. The projected system was not only fairly stable but also found to be almost non-toxic to cells [27]. For target-specific delivery of therapeutics, these conjugates require further derivatization to incorporate such properties, which becomes cumbersome and a time consuming step. In order to avoid such tedious multi-step processes, several polysaccharides have been shown to possess targeting ability. A single-step coupling of hydrophobic drugs/ligands with such polysaccharides results in amphiphilic conjugates capable of entrapping therapeutics and delivering them in a site-specific manner. Dextran is one of the most commonly used polymers, which possesses colon-targeting ability [28,29]. By taking advantage of this property of dextran, we anticipated that an amphiphilic conjugate of dextran-cholesterol would self-assemble in aqueous medium entrapping a drug molecule of choice and deliver it at a particular site in a controlled manner thereby improving the bioavailability of the drug at that site. The resulting formulation would be biocompatible, biodegradable and remain in the system for longer duration due to hydrophilic shell comprising of glucose chains. Therefore, in the present study, we have selected dextran and conjugated it with cholesteryl hemisuccinate, prepared by the reaction of cholesterol with succinic anhydride, using a condensing reagent, dicyclohexylcarbodiimide (DCC), in the presence of 4-dimethylaminopyridine (DMAP), to obtain cholesteryl-dextran (Chol-Dex) amphiphiles. Two formulations (5 and 10% substitution of cholesteryl units, Chol-Dex-5 and Chol-Dex-10) were subjected to self-assembly followed by entrapment of two drugs, metronidazole and rifampicin. Under different conditions, the release profiles of the drugs were obtained and cytocompatibility of the formulations was evaluated in vitro.

2. Materials and methods

Dextran (MW 40,000), N,N-diisopropylethylamine (DIPEA), 4-dimethylaminopyridine (DMAP), dicyclohexylcarbodiimide (DCC), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Dulbecco's Modified Eagle's medium (DMEM), metronidazole (MZ) and rifampicin (RF) were purchased from Sigma-Aldrich Chemical Co. (USA). Dialysis membrane (MW = 500–1000 Da) was procured from Spectrum Labs, USA. Other chemicals and reagents used in the present study were obtained from local vendors.

^{1}H NMR spectra of cholesterol hemisuccinate and cholesteryl-dextran conjugates, dissolved in DMSO-d_6, were carried out on JEOL-EXCP 400 MHz spectrometer, Japan. Chemical shifts (δ) are expressed in ppm. FTIR spectra of the synthesized conjugates (~2.0 mg) were recorded using PerkinElmer BX Series spectrophotometer, USA with the following scan parameters: scan range, 4000 to 500 cm^{-1}; number of scans, 16; resolution, 4.0 cm^{-1}; interval, 1.0 cm^{-1}; unit %T.

2.1. Synthesis of cholesterol hemisuccinate

Cholesterol (2.0 mmol, 775 mg), succinic anhydride (3.0 mmol, 300 mg), DIPEA (3.0 mmol, 510 μl) and DMAP (1.0 mmol, 122 mg) were dissolved in 1,2-dichloroethane (8 ml) and stirred for 24 h at room temperature. The progress of the reaction was monitored on TLC using chloroform: methanol (9:1, v/v). After completion of the reaction, the reaction mixture was further diluted with dichloroethane (30 ml) and washed with 10% aqueous citric acid (2 × 20 ml) followed by washings with distilled water (2 × 20 ml). The organic layer was collected, dried over anhydrous sodium sulphate, filtered and concentrated on a rotary evaporator. Finally, the syrupy residue of cholesterol hemisuccinate was left in a vacuum desiccator overnight (Yield = ~92%).

2.2. Synthesis of cholesteryl-dextran (Chol-Dex) conjugate

The cholesterol hemisuccinate (CS) was conjugated with dextran in two different ratios. Dextran (1 g, 6.17 mmol) was dissolved in dry DMF (25 ml) and added cholesterol hemisuccinate (149 mg, 0.3 mmol for 5% substitution) followed by dicyclohexylcarbodiimide (93 mg, 0.45 mmol) and DMAP (55 mg, 0.45 mmol). The reaction was allowed to stir overnight at an ambient temperature and then kept in a refrigerator for 2h. The precipitate was removed by filtration after washing with DMF (2 ml). The filtrate was concentrated on a rotary evaporator to one-third of the volume and diethyl ether (100 ml) was added to precipitate the cholesteryl-dextran (Chol-Dex). The solid was recovered, dispersed in water and subjected to dialysis in a dialysis bag (MWCO 12 kDa) for 24 h with intermittent change of water (4 x 6h). The dialyzed material was concentrated to obtain the Chol-Dex-5 in ~82%. Similarly, Chol-Dex-10 with 10% substitution was prepared. Both the preparations were characterized spectroscopically and degree of substitution was determined.

2.3. Self-assembly of Chol-Dex conjugates

The process of self-assembly of amphiphilic Chol-Dex (5 and 10% substituted) was carried out by dialysis method. Chol-Dex (~2 mg) was dissolved in DMSO (100 μl) and water (900 μl) was added gradually with continuous stirring. The solution was vortexed for 5 min and left for 4 h at RT. Then the solution was poured in dialysis tubing (MWCO 1.0 kDa) and dialyzed against water for 6 h to get rid of organic solvent. The dialyzed solution was lyophilized to obtain Chol-Dex nanocomposites as solid material.

2.4. DLS measurements

To measure the average size and polydispersity index (PDI) of Chol-Dex nanocomposites and drug-embedded nanocomposites, ~1.0 mg of the material was dispersed in 1.0 ml of Milli Q water and vortexed for 2 min. The dispersion was sonicated for 5 min and subjected to measurement of size on Zetasizer Nano-ZS (Malvern Instruments, UK). The values are mean of three independent measurements in triplicates. Stability of Chol-Dex nanomicelles, dispersed in Milli Q water, was also examined by measuring the variation in the hydrodynamic diameter up to 48 h.

2.5. Transmission electron microscopy (TEM)

This technique was used to find out the actual size of the self-assembled micellar nanocomposites in dryform. The dispersion solution (~5 μl of 1.0 mg/ml) was dropped on carbon-coated copper grids and negatively stained with uranyl acetate (1%, 5 μl). These grids were initially air-dried and then kept in a desiccator for vacuum drying. Subsequently, the grids were subjected to investigation under HR-TEM (200 kV) and images were captured.

2.6. Drug loaded Chol-Dex nanomicelles

In this study, two hydrophobic drugs, metronidazole and rifampicin, were used to entrap in the nanomicelles. The drugs have been encapsulated in amphiphilic molecules of both the formulations, Chol-Dex-5 and Chol-Dex-10, to check their entrapment efficiency. To achieve the encapsulation of the drugs, the projected formulations and drugs were taken in a w/w ratio of 10:2. Briefly, Chol-Dex-5 (~20 mg) and the drug (~4 mg) were dissolved in DMSO (2.4 ml) to make a clear solution and then Milli Q water (~21.6 ml) was added dropwise with continuous vortexing over the period of 5 min. The homogenous solution was incubated for 2 h and then subjected to dialysis against water in 1.0 kDa

dialysis tubing for 4 h. The dialyzed solution was lyophilized and used for percent entrapment and drug loading studies. Similarly, Chol-Dex-10 amphiphile was used to entrap both the drugs separately. To determine the entrapment efficiency and drug loading for each drug, calibration curves were drawn. UV-VIS spectrophotometer (Cary 60, Agilent Inc., USA) was used to carry out the absorbance measurements. Metronidazole (MZ) and rifampicin (RF) were spectrophotometrically measured at wavelength of 326 and 341 nm, respectively. Following formulae were used to determine the entrapment efficiency and drug loading:

Entrapment efficiency (%) = Amt. of drug in nanostructures x 100 / Amt. of drug used

Drug loading (%) = Amt. of drug in nanostructures x 100 / Amt. of nanostructures used

2.7. Drug release kinetics

Dialysis method was used for monitoring the in vitro drug release from both the formulations. The release of drugs from the nanomicelles (nanocomposites) was investigated at two different pHs (7.2 and 4.2) in 1x PBS. In this method, drug (metronidazole) loaded nanocomposites (~3 mg) were dispersed in 1x PBS of pH 7.2 and poured in dialysis membrane of molecular weight cut-off of 1.0 kDa. Then the bag was placed in a reservoir of 1x PBS (50 ml) of pH 7.2. Release of the drug was measured spectrophotometrically by withdrawing aliquots of 1 ml at regular interval of time and pouring back to the solution after measurement. Likewise, release of drugs from other formulations at pH 7.2 and 4.2 was measured. The amount of drugs released from the nanomicelles was finally calculated from the pre-drawn calibration curves.

2.8. Cell viability assay

In vitro cytotoxicity of the nanomicelles, drug entrapped formulations and bulk drugs (MZ and RF) was determined by MTT assay on HEK 293 cells. In this study, HEK 293 cells were seeded at the concentration of ~10^4 cells/well in a 96-well plate followed by 24 h of incubation in a humidified incubator having an atmosphere of 5% CO_2 at 37 °C for adherence of cells. After achieving confluency of ~70%, the media was aspirated and the cells washed with 1x PBS (200 μl). Solutions of void nanomicelles, drug entrapped formulations and bulk drugs were prepared at the concentration of (10–100 μg/ml) and added gently onto the cells. The plate was kept in the incubator for 24 h. Subsequently, the media was aspirated and MTT solution (100 μl, 1 mg/ml) was added in each well followed by incubation for 3 h at 37 °C. The solution was removed and DMSO (100μl/well) was added, kept for 5–10 min and read on ELISA Plate reader at 540 nm (μQuant, Biotek Instruments, USA). The cell viability of each sample was calculated by the following formula:

Cell viability (%) = $Abs_{treated}$ / $Abs_{control}$ x 100

3. Results and discussion

Supramolecular self-assembly is an advanced concept for the nanostructure developments in the field of material science, particularly, in the area of drug and gene delivery [30,31]. Recently, polymer-based drugs and drug delivery systems have been shown to possess promising potential to treat various human diseases. Several such formulations are either at developing stage or are undergoing clinical trials for several deadly diseases including cancers. During developmental stage, the low molecular weight hydrophobic drug molecules are attached with the polymers, which rearrange their structural features and enhance the bioavailability of the drug as well as help in maintaining the sustained release to avoid side effects. These new generation nanomedicines (nanotherapeutics) would not only address the issues such as adsorption, distribution, stability of the drug molecules but also prove to be wonder formulations for the treatment of wide array of human diseases. In the present study, we have attempted to develop nanomicellar drug delivery systems derived from amphiphilic cholesteryl-dextran (Chol-Dex) via self-assembly in aqueous medium.

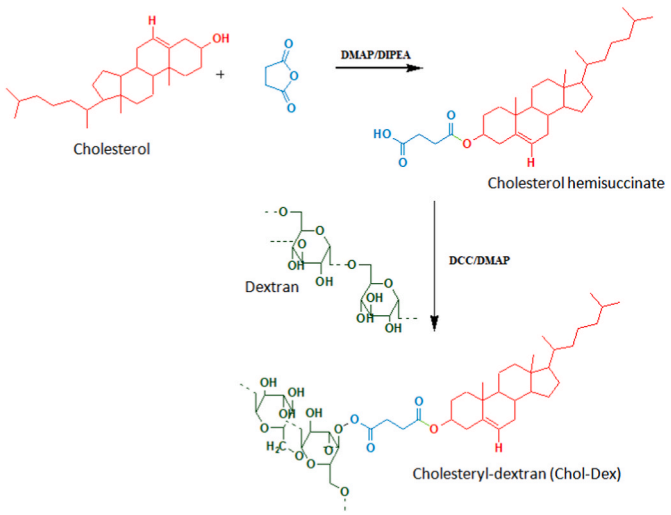

Fig. 1. Synthesis of (a) Cholesterol hemisuccinate, and (b) amphiphilic Cholesteryl-Dextran (Chol-Dex) conjugates.

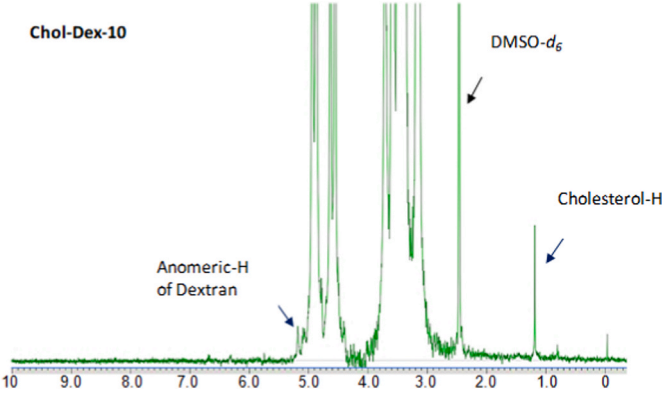

Fig. 2. ^{1}H NMR spectrum of Chol-Dex-10 carried out in DMSO-d_6

3.1. Synthesis and characterization of Chol-Dex nanomicelles

Amphiphilic Chol-Dex was prepared in a two-step synthesis (Fig. 1). In the first step, cholesterol hemisuccinate was synthesized in ~92% yield from cholesterol and succinic anhydride in the presence of an acylating catalyst, 4,4-dimethylaminopyridine. In the subsequent step, chemical conjugation of cholesterol hemisuccinate was achieved on to dextran in two different concentrations (5 and 10%) using DCC/DMAP coupling reagent to obtain Chol-Dex in ~82% yield. The products with different degree of substitution of cholesterol were analyzed by ^{1}H NMR and FTIR spectra. ^{1}H NMR spectroscopy was also used to determine the degree of substitution of cholesterol on to dextran. We attempted 5 and 10% substitution of cholesterol on dextran, however, the actual substitution was found to be ~1.9 and 4.7%, for Chol-Dex-5 and Chol-Dex-10, respectively. It was calculated from the integration values of protons of anomeric hydrogen of dextran (δ = 4.9 ppm) and methyl protons of cholesterol succinate (δ = 1.2 ppm) (Fig. 2). Similarly, the formation of Chol-Dex conjugate was confirmed by infrared spectroscopy. It was

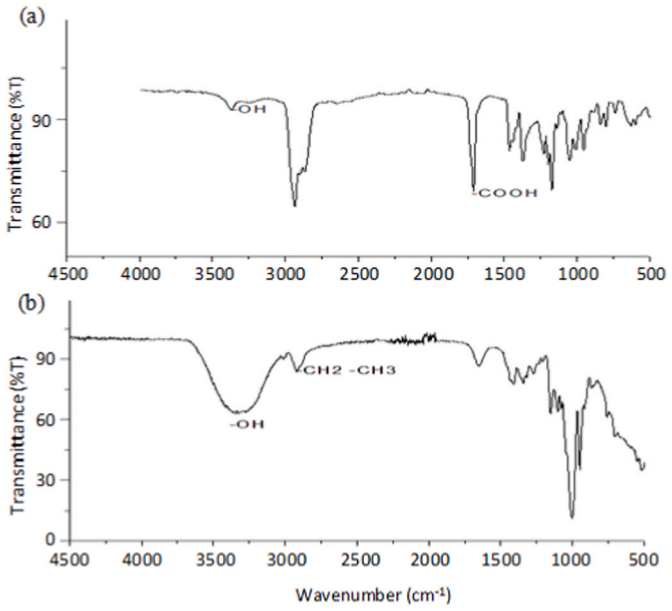

Fig. 3. FTIR spectra of cholesterol hemisuccinate (a) and amphiphilic Chol-Dex-10 conjugate (b).

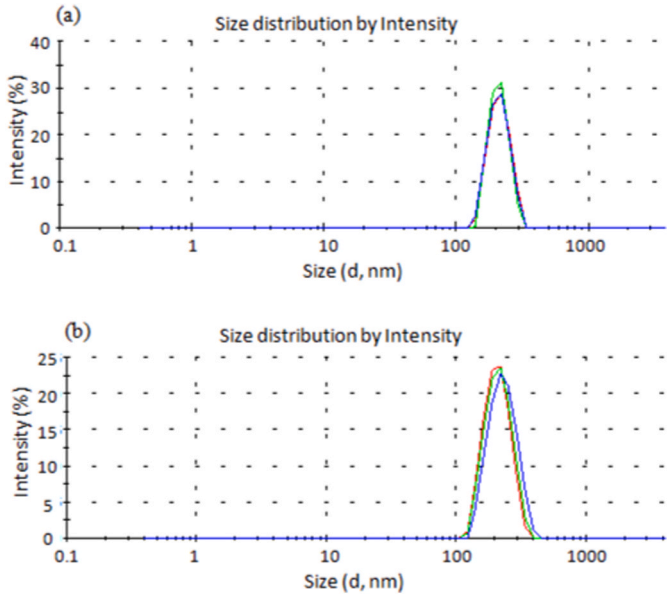

Fig. 4. DLS measurement of size distribution graph of (a) Chol-Dex-5, and (b) Chol-Dex-10. Average hydrodynamic diameter of Chol-Dex-5 and Chol-Dex-10 nanostructures was found to be ~305 and 292 nm, respectively.

observed that a major band at 1700 cm^{-1} due to C═O stretching of carboxyl group in cholesterol succinate disappeared after the conjugation with dextran and a smaller band appeared at 1625 cm^{-1} which could be due to formation of ester group (Fig. 3). Another band at ~3280 cm^{-1} due to abundant –OH functions in dextran appeared in the spectrum of Chol-Dex conjugate, which further established the successful formation of the conjugate.

3.2. Characterization of self-assembled Chol-Dex nanocomposites

To characterize the self-assembled nanocomposites, amphiphilic Chol-Dex nanocomposites (Chol-Dex-5 and Chol-Dex-10) were dispersed in water, vortexed and subjected to measurement by dynamic light scattering (DLS). In aqueous medium, self-assembly of these amphiphiles resulted in the formation of nanomicelles with hydrophobic core (cholesterol moieties) and hydrophilic shell (dextran residues). The results revealed that the size of these micelles were obtained in nanometer range. The size of Chol-Dex-5 and Chol-Dex-10 micelles was found to be ~305 and 292 nm, respectively (Fig. 4). Higher substituted Chol-Dex-10 nanomicelles showed more compact size which could be attributed to greater hydrophobic interactions which resulted in smaller sized micelles [32]. These nanocomposites were further analyzed by electron microscopy and the results revealed that average size of Chol-Dex-5 nanocomposite was found to be ~31.7 nm and Chol-Dex-10, ~23.2 nm (Fig. 5), which are in complete agreement with the results obtained by the DLS measurements. The difference in the size of the nanocomposites in DLS and TEM might be due the fact that DLS measures hydrodynamic diameter of the particles in wet state while TEM measures in dry state. Furthermore, the stability of the projected nanomicellar structures was also assessed in Milli Q water over a period of 48 h using DLS. It was observed that no significant variation in the size of the micelles noticed, advocating sufficient stability of the nanoscoposites. Moreover, due to hydrophilic shell comprising of dextran residues, the proposed carriers would be having prolonged half-life in the blood.

3.3. Determination of drug entrapment and loading efficiency

The usefulness of the Chol-Dex nanocomposites was established by entrapment of hydrophobic drug molecules such as rifampicin (RF) and metronidazole (MZ), mainly used for certain bacterial and parasitic infections as well as colonic diseases and bacterial infections including tuberculosis, respectively [33,34]. The main objective to entrap these drugs was to reduce their side effects and control their release so that the desired concentration of these drugs could be maintained for a longer duration of time. These drugs were entrapped at w/w ratio of 10:2 (nanocomposites:drug). Post-entrapment, the size of the nanocomposites was measured by DLS. Metronidazole-entrapped nanocomposites did not show much variation which could be due to smaller size of the drug molecule. However, rifampicin (RF)-loaded nanocomposites displayed increase in the size of the composites. The size increased from ~305 nm to ~348 nm in case of Chol-Dex-5 (RF) and ~292 nm to ~338 nm in case of Chol-Dex-10 (RF). The observed physical entrapment of metronidazole in Chol-Dex-5 and Chol-Dex-10 was found to be ~18 and 33%, respectively, with drug loading of ~4.7 and 7.3%, respectively. The results were found to be in accordance with the degree of hydrophobicity in the systems. Chol-Dex-10, having greater degree of substitution of cholesterol, showed higher entrapment efficiency as compared to Chol-Dex-5. Similar results were obtained in case of entrapment of rifampicin. Entrapment efficiency was found to be ~11 and 39% for Chol-Dex-5 and Chol-Dex-10, respectively, with drug loading of ~2.3 and 7.1%, respectively.

3.4. Drug kinetic study

In order to assess the suitability of the nanocomposites to deliver the drugs, in vitro drug release studies were performed via dialysis bag method. The study was carried out at two different pHs (4.2 and 7.2) in 1x PBS spectrophotometrically. The results revealed that release of metronidazole from Chol-Dex-10 nanocomposites was marginally higher (~68%) in neutral pH (7.2) as compared to pH 4.2 (~65%) over a period of 8 h. In case of Chol-Dex-5 nanocomposites, no differentiation in the release was observed up to 5h, however, after 5h, the rate of release increased in pH 7.2 and after 8h, it was found to be ~77% as compared to ~70% at pH 4.2 (Fig. 6). Higher percent release of metronidazole in lower substituted formulation, Chol-Dex-5 (MZ), could be due to lower degree of hydrophobic interactions between the drug and cholesterol moieties present in the core of nanomicellar structures.

Almost similar type of results were obtained in case of rifampicin

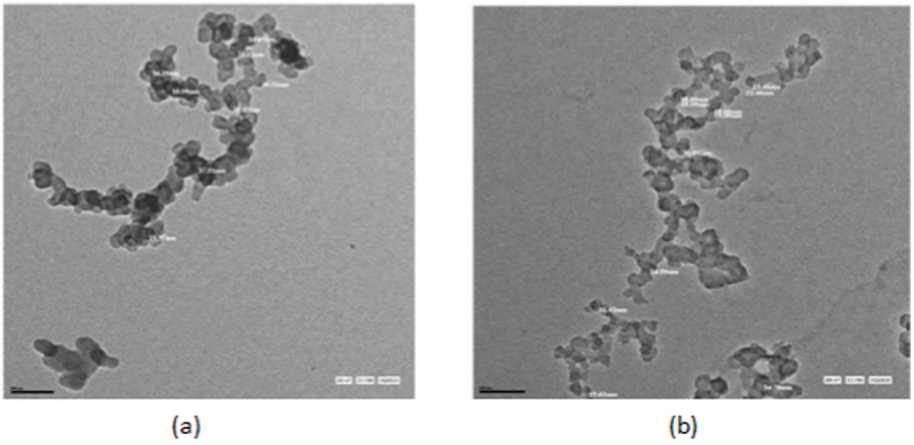

Fig. 5. TEM images of Chol-Dex nanostructures. Average particle size of Chol-Dex-5 and Chol-Dex-10 nanostructures was found to be ~31.7 and 23.2 nm, respectively. Scale bar: 100 nm.

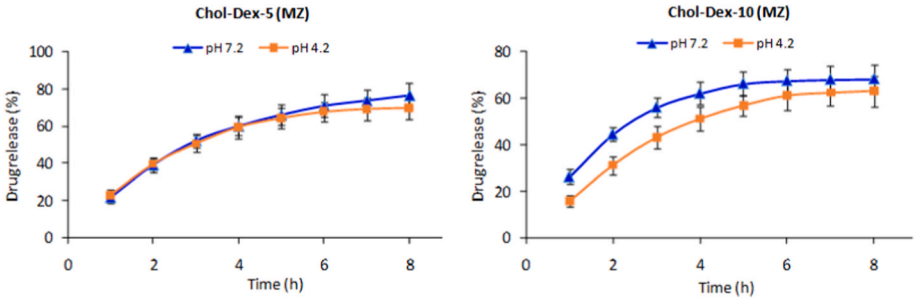

Fig. 6. Drug release profiles of metronidazole from Chol-Dex-5 (MZ) and Chol-Dex-10 (MZ) in 1x PBS of pH 4.2 and 7.2. Chol-Dex-5 (MZ): ~70 and 77% release at pH 4.2 and 7.2, respectively. Chol-Dex-10 (MZ): ~65 and 68% at pH 4.2 and 7.2, respectively.

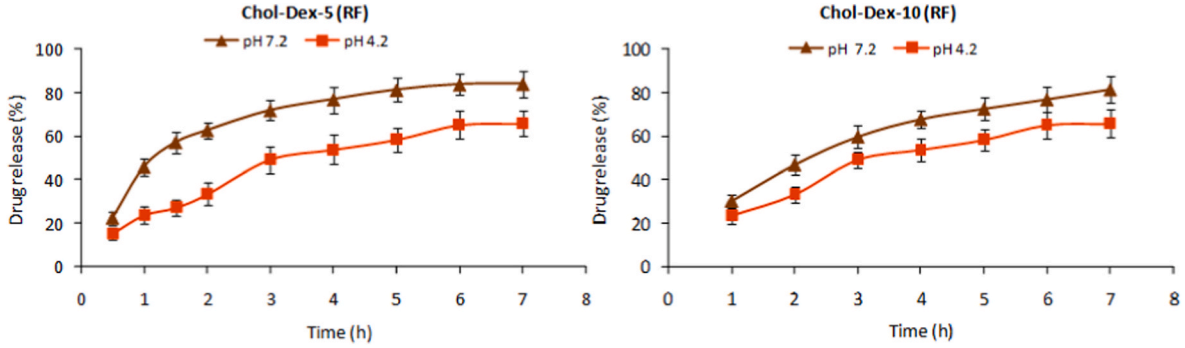

Fig. 7. Drug release profiles of rifampicin from Chol-Dex-5 (RF) and Chol-Dex-10 (RF) in 1x PBS of pH 4.2 and 7.2. Chol-Dex-5 (RF): ~66 and 84% release at pH 4.2 and 7.2, respectively. Chol-Dex-10 (RF): ~66 and 81% at pH 4.2 and 7.2, respectively.

entrapped nanocomposites (Fig. 7), drug release profiles revealed that in case of Chol-Dex-5 (RF), ~84% drug was released in 7 h at pH 7.2 while ~66% released at pH 4.2. Likewise, from Chol-Dex-10 (RF), ~81% of drug was released in 7 h at pH 7.2 and in acidic pH (4.2), drug release was found to be ~66%. **Here, the effect of pH was not observed**. In Chol-Dex-5 (RF), the difference in the release of the drug in the first 2.5 h was more pronounced, however, after that, the release was almost similar. In both the cases, the higher release of drugs was found from Chol-Dex-5 which might be attributed to greater size of the micellar nanostructures and lower degree of interactions with the drug molecules. Drug release from the micellar nanostructures occurs via different mechanisms. However, in degradable polymeric micelles, release of therapeutics takes place by a two-step process, viz., in the first step, drug release happens via diffusion and in the later step, degradation of polymer followed by slow rupturing of the micellar structures effects the release process. Diffusion of the solvent into the micellar nanostructures forces the drug to come out of the micellar core. Subsequent degradation of the amphiphilic polymeric units via cleavage of ester linkages causes drug to release from the core of the micelles into the surrounding medium. The step occurs slowly thus sustained release is maintained over a longer duration. As the release of both the drugs was higher at pH 7.2, these results confirmed that these nanocomposites could be used to deliver drugs in colonic area to treat diseases bypassing the acidic environment of the stomach.

3.5. Cell viability assay

In order to assess the toxicity of void Chol-Dex nanostructures and

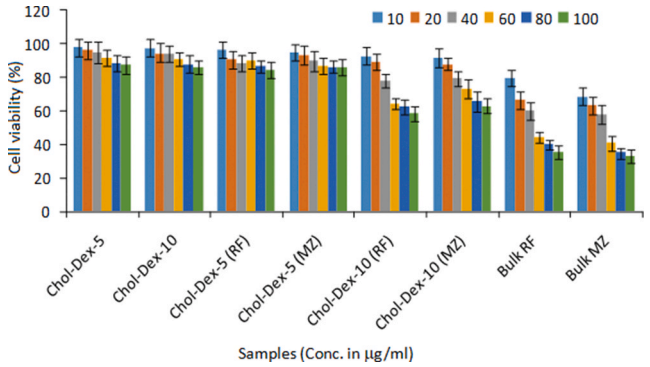

Fig. 8. Cell viability assay of void nanostructures (Chol-Dex-5 and 10), two amphiphiles encapsulated with rifampicin and metronidazole [Chol-Dex-5 (MZ/RF) and Chol-Dex-10 (MZ/RF)], bulk drugs (RF and MZ) at concentration of 10, 20, 40, 60, 80, 100 µg/ml on HEK 293 cells.

drug entrapped nanostructures in vitro, cell viability assay was performed using MTT. Here, HEK 293 cells were exposed to bulk drugs (MZ and RF), void Chol-Dex nanocomposites and drug loaded Chol-Dex nanostructures at various concentrations (10, 20, 40, 60, 80 and 100 µg/ml). The results are depicted in Fig. 8, which exhibited that void Chol-Dex nanostructures were almost non-toxic. Drug-loaded nanostructures showed variable viability depending on the concentration. Metronidazole- and rifampicin-loaded Chol-Dex-5 nanocomposites were found to be non-toxic at all the concentrations. However, in case of metronidazole- and rifampicin-loaded Chol-Dex-10 nanocomposites, these have displayed some toxicity at high concentration (~100 µg/ml). The drug molecules exhibited high toxicity in the range of ~60–100 µg/ml concentration. These observations showed a considerable decrease in the toxicity of the drugs entrapped in the nanocomposites suggesting that these carriers could be used as drug delivery systems for other hydrophobic drugs also. These results are in complete agreement with the previous reports [35–38]. In these reports, amphiphilic cholesterol conjugates, capable of forming self-assembled or aggregate structures, have been prepared using various hydrophilic molecules such as polyethylene glycols, monosaccharides, polysaccharides, synthetic polymers, etc. and evaluated for drug delivery applications. All these micellar nanostructures were found to be almost non-toxic and could significantly reduce the toxic effects of the bulk drug by releasing them in a controlled manner that too onto the specific sites without harming healthy cells.

4. Conclusion

In the projected work, we have attempted to develop non-toxic drug delivery systems useful for colon targeting employing a natural polysaccharide and colon-targeting ability, dextran, and a biocompatible molecule, cholesterol. Post-conjugation, the self-assembly of these amphiphilic Chol-Dex conjugates resulted in the micelles with hydrophobic core and hydrophilic shell capable of entrapping hydrophobic drugs. Two hydrophobic drugs, metronidazole and rifampicin, were entrapped in their nanopockets which then showed sustained drug release over a period of time. Cytocompatibility of the entrapped drugs also improved. Higher percentage of drug release at pH 7.2 showed the potential of these carriers to deliver drugs in the colonic areas. Hence, the designed nanostructures have more versatility and non-toxic in nature. Therefore, such type of nanomicelles can be successfully used to target the colon owing to their biodegradable and biocompatible properties.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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