The effect of cyclodextrin on both the agglomeration and the in vitro characteristics of drug loaded and targeted silica nanoparticles
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Abstract One of the problems in the use of nanoparticles (NPs) as carriers in drug delivery systems is their agglomeration which mainly appears due to their high surface energy. This results in formation of NPs with different sizes leading to differences in their distribution and bioavailability. The surface coating of NPs with certain compounds can be used to prevent or minimize this problem. In this study, the effect of cyclodextrin (CD) on the agglomeration state and hence on the in vitro characteristics of drug loaded and targeted silica NPs was investigated. A sample of NPs was loaded with anticancer agents, then modified with a long polymer, carboxymethyl-β-cyclodextrin (CM-β-CD) and folic acid (FA), respectively. Another sample was modified similarly but without CD. The surface modification was characterized using fourier transform infrared spectroscopy (FT-IR). The polydispersity (PD) was measured using dynamic light scattering (DLS) and was found to be smaller for CD modified NPs. The results of the in vitro drug release showed that the release rate from both samples exhibited similar pattern for the first 5 hours, however the rate was faster from CD modified NPs after 24 hours. The in vitro cell viability assay confirmed that CD modified NPs were about 30% more toxic to HeLa cells. These findings suggest that CD has a clear effect in minimizing the agglomeration of such modified silica NPs, accelerating their drug release rate and enhancing their targeting effect.

Key words: Cyclodextrin; agglomeration; targeting; in vitro

1. Introduction

Nanoparticles are particulate dispersions or solid particles with a size in the range of 10-1000 nm [1]. Due to their unique properties, they have gained a great research interest in the area of drug delivery systems. Their ultra small size makes their taking up by cells much easier than larger molecules [2], [3]. They have a large surface area to volume ratio and their surface can be easily modified with different functional groups [4], [5]. More importantly, they can be used to improve the pharmacokinetic and pharmacodynamic properties of different types of drug molecules that are encapsulated into NPs and forming targeted delivery systems of both imaging and anticancer agents [6]. Furthermore, They have been used in vivo to protect the drug molecules in the systemic circulation, ensure delivery of the drug to the site of action at a controlled and sustained manner and to reduce the undesirable side effects of the drugs [6].

In spite of these advantages, using NPs as carriers in drug delivery systems have certain limitations. For instance, their small size and large surface area can lead to their agglomeration, making their physical handling , processing, synthesis, storage and transportation difficult in both the liquid and dry forms [1].

One of the most important characteristics of NP systems is their particle size and size distribution [7]. It has been found that these parameters determine the in vivo distribution,
biological fate, toxicity, targeting ability, drug loading, drug release and stability of the NPs [1], [8]. Therefore, the problem of agglomeration and non uniform size distribution have to be overcome in order to prepare efficient drug delivery systems based on NPs.

The agglomeration state of the NPs and their size can be determined by their surface coatings [5]. Several coatings have been used to prevent or minimize the agglomeration of the NPs and keep them in colloidal state [9]. Such coatings include polymers like polyethylene glycol (PEG), poly(vinylpyrrolidone) (PVP), natural polymers like dextran, chitosan, pullulan, and surfactants like sodium oleate and dodecylamine [10].

Cyclodextrins (CDs) have also been used for surface coatings of different types of NPs [11] in order to enhance their steric hindrance and minimize their agglomeration [11], [12]. CDs are (1,4) linked macrocyclic oligosaccharides with cage-like structure having a hydrophobic interior and a hydrophilic exterior [13]. Due to these properties, they have been widely used to enhance the aqueous solubility of hydrophobic drugs [14]. In the present work, however, we intend to study the effect of CD, which conjugated to our previously investigated targeted drug delivery system [15], on the agglomeration of silica NPs and hence on their in vitro characteristics such as drug release rate and targeting ability.

2. Materials and methods

2.1. Materials

FITC-labeled propylcarboxylic acid functionalized silica NPs (particle size 200 nm, pore diameter 4 nm), Poly(propyleneglycol)bis(2-aminopropylether diamine, D4000) and carboxymethyl-B-cyclodextrin sodium salts (CM-β-CD) were purchased from Sigma Aldrich. Folic acid (purity > 98%) and Phosphate Buffered Saline (PBS, PH = 7.4) were obtained from bioworld. All other reagents and materials used for cell culture were used as received.

2.2. Preparation of drug loaded FA-CM-β-CD aminated silica NPs and FA-aminated silica NPs.

The procedures for drug loading and synthesis of these two types of modified silica NPs were presented in our previous work [15].

2.3. FITR and DLS analysis of the modified silica NPs

The FTIR spectra of the silica NPs were obtained using WQF-521 Fourier transform infrared spectrophotometer within the mid-IR range (500-4000 cm⁻¹). The mean particle size (Z-average) and PD of the NPs were measured by DLS technique using (Zetasizernano series, Malvern U.K). The NPs were diluted as suspensions (4.5 x10⁻3 mg/ml) in ethanol with refractive index (RI) = 1.362 for ethanol and 1.48 for silica NPs and viscosity= 1.20 cp. The suspensions were first sonicated for 5 minutes to separate the bigger aggregates. The measurements were performed in triplicates for each sample with 6 runs each time and the average was then taken.

2.4. In vitro drug release study

Certain amounts of each of drug loaded FA-CM-β-CD aminated silica NPs and FA-aminated silica NPs were mixed with 2 ml PBS (pH = 7.4) in a dialysis bag (SnakeSkin Dialysis Tubing, 22 mm x 35 feet dry diameter). Each sample was then added to 23 ml of PBS in a beaker and shaken at 37° C in water path. At different time intervals (1, 2, 4, 5, 24, 28, 30 and
48 hours), aliquots of 1.5 ml were removed, replaced with 1.5 ml of fresh PBS, to maintain the same sink conditions. The amount of released drug in the supernatant was measured via UV-Vis spectroscopy using (Uv/vis spectroscopy spuv-19). Absorbance was measured at λ_max specific for the anticancer agents used. The corresponding drug concentration was calculated based on the calibration curves obtained. All experiments were performed in triplicate. The percentage of cumulative drug release was then calculated and the values represent the average of three experiments for each sample.

2.5. Antiproliferative (cell viability) Assay

HeLa Cells were dispensed (100 μl/well) into 96-well tissue culture plates (flat bottom) at an optimized concentration of 15000 cells/well in complete tissue culture medium. After 24 h, the media in each well were completely removed and the attached cells were treated in triplicates with 200 μl of 0.05 mg/ml of two different suspensions of modified silica NPs, suspended in complete tissue culture medium. Plates were incubated for 48 h, and then cell viability was measured by using MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenytetrazolium bromide) assay. From each well, 100 μl of culture media was removed and 10 μl of thiazolyl blue tetrazolium solution was added and placed in CO2 incubator for 3 hours. Then the reaction was stopped by adding MTT solubilization solution (100 μl/well), mixed well and incubated for another one hour. Absorbance was measured at 550 nm by microplate reader. Cell viability (% survival) was calculated and then used to get percentage of cell toxicity.

3. Results and Discussions:
3.1. Characterization of the conjugated NPs
3.1.1. FTIR Analysis

To study the effect of CD on the agglomeration and hence on the other in vitro characteristics of the silica NPs, two different modifications were prepared in this study. A sample of the commercially available propylcarboxylic acid functionalized silica NPs was first loaded with anticancer agents then modified with a long polymer (Poly(propyleneglycol)bis(2-aminopropylether diamine, D4000), CM-β-CD and FA, respectively. The other sample was modified similarly but without CD. Thus, we have FA-CM-β-CD aminated and FA-aminated silica NPs, respectively. The FTIR spectra of the two modifications are shown in Figure 1. The first modification includes the formation of amide bonds after the addition of both the diamine and the CD, while the folic acid is embedded into CD cavity via host–guest interaction and thus the peaks of folic acid will be mainly hidden, as previously reported [12]. On the other hand, the second modification, in which the CD is absent, includes two amide bonds after both the diamine and the folic acid. In this case, the peaks specific for FA should be more clear than the first modification.

As shown in Figure 1c, the FTIR spectrum of FA-CM-β-CD aminated silica NPs has new small peaks at about 2800 – 2900 cm⁻¹ compared to unmodified NPs. On the other hand, the spectrum of FA-aminated silica NPs (Figure 1d) has more and broader peaks related to FA. As shown in Figure 1b, unconjugated FA has few peaks above 3100 cm⁻¹ which appeared as a broad peak in Figure 1d and one at about 2800 cm⁻¹ which existed in Figure 1d at the same position. This indicated that a covalent bond was involved in FA attachment in the second modification while it was via host guest interaction in the first modification.
Figure 1: FTIR spectra of (a) pure (propylcarboxylic acid functionalize silica NPs, (b) unconjugated FA, (c) FA-CM-β-CD aminated silica NPs and (d) FA- aminated silica NPs.

3.1.2. DLS Analysis

The mean particle size of the two modified NPs as well as their polydispersity were measured using DLS. As shown in Table 1, the mean particle size is bigger for FA-CM-β-CD aminated silica NPs compared to FA-aminated silica NPs due to the presence of CD. However, the PD was smaller for FA-CM-β-CD aminated NPs. Since the PD measures the heterogeneity of sizes of the particles, the smaller value indicates a narrower size distribution which is most likely related to a lesser tendency of the NPs to form agglomerates [16]. These results confirmed that the presence of CD in this targeted system improves the NPs distribution and minimizes the chance of their agglomeration.

| Sample                          | Mean particle size (nm) | Polydispersity (PD) |
|---------------------------------|-------------------------|---------------------|
| FA-CM-β-CD aminated silica NPs | ~ 615 nm                | ~ 0.4               |
| FA-aminated silica NPs         | ~ 505 nm                | ~ 0.6               |

3.2. In vitro drug release study
The in vitro drug release profiles for both FA-CM-β-CD aminated and FA-aminated silica NPs were measured and the percentage of cumulative drug release was calculated. In general, as shown in Figure 2, both modifications showed an initial burst release of the drug followed by a controlled release. More importantly, they exhibited similar pattern for the first 5 hours, but a faster rate was observed for CD modified NPs after that. This is due to the fact that the particle size exert a clear effect on the various properties of nano drug delivery systems such as their release rate [17]. The absence of CD in FA-aminated silica NPs resulted in combining the NPs to create product with particle distribution shifted towards larger sizes and smaller surface area. Therefore, the actual number of NPs exposed to media will be less and the release rate of drug from such modified NPs will be slower. It also seems that the agglomeration increased with time hence the difference between both modifications was obvious after a certain length of time as shown in Figure 2.

Figure 2: In vitro drug release of FA-CM-β-CD aminated silica NPs and FA-aminated silica NPs in PBS (pH 7.4).

3.3. In vitro cell viability assay

NPs (0.05 mg/ml) were for cell viability assay, two different suspensions of the two modified silica encapsulated with the same amount of drug and incubated with HeLa cells for 48 hours under the same conditions. As shown in Figure 3, FA-CM-β-CD aminated silica NPs (sample 1) were about 30% more toxic toward HeLa cells compared to FA-aminated silica NPs (sample 2).

It is well known that the size is one of the major factors determining cellular uptake efficiency of NPs and several internalization pathways depend on the size [7]. As a general rule, smaller NPs are internalized into cells easier than larger ones [7] and our results were in agreement with this fact. Since FA-aminated NPs were more susceptible to agglomeration and their particle size distribution was shifted toward larger sizes, their internalization via receptor
mediated endocytosis was slower than FA-CM-β-CD aminated NPs. Thus, these two FA targeted systems have different targeting ability which was observed from the differences in their toxicity effects toward HeLa cells (Figure 3). From these findings, it was concluded that the presence of CD, in such targeted NPs, minimizes their agglomeration, accelerates their drug release rates and enhances their internalization and targeting efficiency.

![Figure 3](image)

**Figure 3**: The percentage of cell toxicity of (1) FA-CM-β-CD aminated silica NPs and (2) FA-aminated silica NPs toward HeLa cells after incubation for 48 hours.

4. Conclusions

In this study, we investigated the effect of CD on the agglomeration and hence on the in vitro characteristics of our previously prepared TDDS which is based on silica NPs. The NPs were first loaded with anticancer agents then modified, as we previously described, with a long polymer, CM-β-CD and FA, respectively. For comparison purposes, another set of NPs were treated similarly but with the absence of CD. The results obtained showed that the polydispersity for CD modified NPs was smaller indicating a lower tendency of these NPs to form agglomerates. The results of the in vitro drug release showed that the release rate from both samples was similar for the first 5 hours, however the rate was faster from CD modified NPs after 24 hours. The in vitro cell viability assay demonstrated that CD modified NPs were more toxic to HeLa cells indicating their easier internalization. These findings suggest that CD has a clear effect in minimizing the agglomeration of such modified silica NPs, accelerating their drug release rate and enhancing their targeting effect.

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**Conflicts of Interest**: The authors declare no conflict of interest.

**References**
[1] V. J. Mohanraj and Y. Chen, “Nanoparticles – A Review,” *Trop J Pharm Res*, vol. 5, no. June, pp. 561–573, 2006.

[2] S. S. Suri, H. Fenniri, and B. Singh, “Nanotechnology-based drug delivery systems,” *J Occup Med Toxicol.*, vol. 2, p. 16, 2007.

[3] L. B. Peppas, “Recent advances on the use of biodegradable microparticles and nanoparticles in controlled drug-delivery,” *Int. J. Pharm.*, vol. 116, no. 1, pp. 1–9, 1995.

[4] I. Slowing, B. Trewyn, and V. Lin, “Effect of surface functionalization of MCM-41-type mesoporous silica nanoparticles on the endocytosis by human cancer cells,” *J. Am. Chem.Soc.*, vol. 128, no. 46, pp. 14792–14793, 2006.

[5] R. H. S. and M. M. Christoph Bantz, Olga Koshkina, Thomas Lang, Hans-Joachim Galla, C. James Kirkpatrick, “The surface properties of nanoparticles determine the agglomeration state and the size of the particles under physiological conditions,” *Beilstein J Nanotechnol*, vol. 15, no. 5, pp. 1774–86, 2014.

[6] S. Singh, V. K. Pandey, R. P. Tewari, and V. Agarwal, “Nanoparticle based drug delivery system : Advantages and applications,” *Indian J Sci Technol.*, vol. 4, no. 3, pp. 177–180, 2011.

[7] L. Shang, K. Nienhaus, and G. U. Nienhaus, “Engineered nanoparticles interacting with cells : size matters,” *J Nanobiotechnology*, pp. 1–11, 2014.

[8] B. G. Hoshyar N, Gray S, Han H, “The effect of nanoparticle size on in vivo pharmacokinetics and cellular interaction.,” *Nanomedicine*, vol. 11, no. 6, pp. 673–92, 2016.

[9] W. H. De Jong, “Drug delivery and nanoparticles : Applications and hazards,” *Int J Nanomedicine*, vol. 3, no. 2, pp. 133–149, 2008.

[10] G. M. Gupta AK, “Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications,” *Biomaterials.*, vol. 26, no. 18, pp. 3995–4021, 2005.

[11] B. Gidwani and A. Vy as, “A Comprehensive Review on Cyclodextrin-Based Carriers for Delivery of Chemotherapeutic Cytotoxic Anticancer Drugs,” *Biomed Res. Int.*, vol. 2015, 2015.

[12] C. Su et al., “Carboxymethyl-β-cyclodextrin conjugated nanoparticles facilitate therapy for folate receptor-positive tumor with the mediation of folic acid,” *Int. J Pharm.*, vol. 474, no. 1–2, pp. 202–211, 2014.

[13] S. K. Das, R. Rajabalaya, S. David, N. Gani, J. Khanam, and A. Nanda, “Cyclodextrins-the molecular container,” *Res. J. Pharm. Biol. Chem. Sci.*, vol. 4, no. 2, pp. 1694–1720, 2013.

[14] G. Tiwari, R. Tiwari, and A. K. Rai, “Cyclodextrins in delivery systems: Applications.,” *J. Pharm. Bioallied Sci.*, vol. 2, no. 2, pp. 72–9, 2010.

[15] A. M. Khattabi, W. H. Talib and D. A. Alqdeimat, "A Targeted Drug Delivery System of Anti-Cancer Agents Based on Folic Acid-Cyclodextrin-Long Polymer Functionalized Silica Nanoparticles," *J Drug Deliv Sci Technol, (in press).*

[16] M. D. N, R. Eskandari, H. Zolfagharian, and M. Mohammad, “Preparation and in vitro characterization of chitosan nanoparticles containing Mesobuthus eupeus scorpion venom as an antigen delivery system,” *J. Venom. Anim. Toxins incl. Trop. Dis.*, vol. 18, no. 1, pp. 44–52, 2012.

[17] S. Honary and F. Zahir, “Effect of Zeta Potential on the Properties of Nano-Drug Delivery Systems - A Review (Part 2 ),” *Trop J Pharm Res.*, vol. 12, no. April, pp. 265–273, 2013.