Camptothecin-Carrying CuCo_2S_4 Nanoparticles: Sustained Drug Release And Anticancer Activity

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Research Article

Keywords: Sulfide nanoparticles, superparamagnetism, NIR absorption, nanocarrier, anticancer activity.

Posted Date: December 28th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1190820/v1>

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Abstract

Nanocarriers of anticancer drugs are delicately designed with precision addition at every attempt. In this paper, we report CuCo_2S_4 nanoparticles that show light-absorption in the NIR-II wavelength range and possess magnetic characteristics. The synthesized nanoparticles are characterized employing XRD, SEM, DLS, TGA, and XPS methods. The nanoparticles form a composite with biocompatible polymeric β -cyclodextrin. The nanoparticles possess a band gap energy of 2.25 eV. The magnetic property arises due to the cobalt-incorporation in the nanoparticles. The anticancer drug, camptothecin, is loaded in the nanocarrier with an 89% adsorption efficiency. The in vitro release of the drug occurs in a sustained fashion. Further, the in vitro anticancer potential of the nanocarrier is examined on breast cancer (MDA-MB-231) cell lines and the activities of the free- and the drug-loaded nanocarrier are compared. The cobalt-containing copper sulfide nanoparticle-poly- β -cyclodextrin composite works as a promising nanocarrier of camptothecin.

1 Introduction

Nanoparticles (NPs) have been investigated and utilized, with an increasing interest, as targeted drug delivery systems and for other biomedical applications.¹⁻³ The nanoparticles employed as drug carriers are designed as possessing properties that render them respond to stimuli like magnetic field, light, and pH.⁴⁻⁶ These properties enable them to find application in diagnosis and treatment of chronic diseases like cancer. For instance, magnetic resonance imaging (MRI), and magnetic hyperthermia, and magnetic field-assisted anticancer drug-delivery are possible when magnetic NPs are used.⁷⁻⁹ Some engineered NPs absorb near-infrared (NIR) radiation and heat up, thus acting as photothermal heating agents which allows the selective ablation of cancer cells.¹⁰ Moreover, magnetic-luminescent NPs also have been developed with a focus on multiple-modal imaging.¹¹ In a nutshell, delicate design and synthesis of NPs with tailor-made characteristics have occupied a place of paramount importance in the field of targeted drug delivery.

Besides the elemental composition of inorganic NPs used for biomedical applications, their size, shape, and surface modification have to be customized to make them effective therapeutic carriers.^{12,13} The size of the NPs are desired to be in the range of 50-150 nm to make them reside longer in the circulatory system.¹⁴ In addition, NPs with large aspect ratios undergo longer plasma residence.¹⁵ Another essential requirement is to modify the surface of the NPs to render them biocompatible, dispersible in aqueous medium, and to enable the loading of anticancer drugs.^{16,17} Biocompatible polymers serve the above-mentioned purposes. The loading of anticancer drug molecules in the polymer sheath that covers the inorganic NPs occurs mostly through non-covalent interactions.¹⁸ For instance, the drug molecule gets inserted between the coiled polymeric strands. Nevertheless, an intriguing method of loading the drug is through the formation of supramolecular host: guest complexes on the surfaces of NPs.¹⁹ It enables the loading of a larger amount of drugs. The inorganic NP-biocompatible polymer combination acts as a suitable choice of nanocarrier with precision-added properties.

Copper sulfide NPs have emerged as new generation materials for photothermal therapy (PTT) as they display a large NIR absorption.²⁰ Attempts have been made to dope elements into CuS NPs to further fine-tune their properties.^{21,22} We hypothesize that the substitution of a magnetic element like cobalt can make them magnetic while remaining NIR- absorbing materials. In addition, a nanocomposite (NC) of Co-doped copper sulfide with a carbohydrate polymer possessing cavity-containing molecular component can aid achieving an enhanced drug-loading and biocompatibility. For this purpose, we chose a β -cyclodextrin-containing polysaccharide as the coating polymer (poly-CD). Herein, we report the synthesis and characterization of Co-doped copper sulphide, preparation of its NC with the poly-CD, of the anticancer drug, camptothecin (CPT), and the anticancer potential of the drug loaded NC on breast cancer cell lines.

2 Materials And Methods

2.1 Materials

The material needed for this work, namely copper chloride and cobalt nitrate, was obtained from Fischer Scientific. Thiourea was procured from Sigma Aldrich and ethyl acetate from NICE chemicals. Triethylamine and Cetyltrimethylammoniumbromide (CTAB) was obtained from Scientific chemical distributors. Analytical grade chemicals with purity above 98% was used.

2.2 Methods

2.2.1 Synthesis of CuCo_2S_4 nanoparticles

The β -CD polymer (poly-CD) was synthesized via a reported procedure.²³ A hydrothermal synthesis method was employed to synthesize poly-CD-coated CuCo_2S_4 NPs. Copper chloride and thiourea was mixed in the ratio 1:3 respectively in 20 ml deionized water and subjected to stirring for 15 minutes. To the above solution, 0.02 M cobalt nitrate dissolved in 10 ml of water was added followed by addition of 0.2 g of CTAB and 0.2 g poly-CD. The mixture was then added to 6 ml triethylamine dissolved in 10 ml of ethyl acetate. It was sonicated for 2 hours at room temperature and then transferred to a Teflon stainless steel autoclave. The temperature was maintained at 180 °C for 18 hours. The obtained poly-CD-coated- CuCo_2S_4 nanoparticles were alternatively washed with distilled water and ethanol to remove unreacted chemicals. The NPs were dried at room temperature.

2.2.2 Instrumentation

The synthesized NPs were characterised for their crystalline phase using X-ray powder diffraction (Bruker D8-Advance) using $\text{Cu K}\alpha$ ($\lambda = 0.154 \text{ \AA}$) radiation in the range of 10–80°. High-resolution transmission electron microscope (HR TEM–Jeol/JEM 2100) was used to determine the topography, morphology and size of the nanoparticles. The composition of the NPs was determined using Jeol/JEM 2100 Energy dispersive X-ray (EDX) instrument. ICP-MS-ThermoFisher CAP RQ ICP-MS device was used to measure elemental composition. The amount and the rate of weight changes of the nanoparticles with respect to

the temperature was determined using Thermogravimetric Analysis (TGA), TA instruments, USA Model SDT Q600. The elemental composition was measured using a Thermofisher Scientific Model Nexsa bas X-ray photoelectron spectroscopy (XPS). M-H curves to determine the magnetic property were obtained using a Lake-shore 7410 Vibrating Sample Magnetometer (VSM).

2.2.3 In vitro drug release

In vitro drug release was studied for NPs loaded with a chemotherapeutic drug camptothecin (CPT) and packed in a dialysis bag of MWCO 12-14 kDa. The bag was suspended in PBS solutions of pH 6.5 and 7.4. The bags were continuously stirred at room temperature. Samples were collected at regular time intervals. After sample collection, fresh PBS solution was added to maintain the pH throughout the release study. UV-vis absorption at 430 nm was recorded for each sample at each time point. The concentration of the released drug was determined by the standard calibration curve equation. The mean values for three independent time estimates were calculated. The percentage of drug loading and release was calculated using following equations:

$$\% \text{ Loading of CPT} = \frac{\text{Weight of CPT in the NPs}}{\text{Weight of NPs}} \times 100 \quad (1)$$

$$\% \text{ Release of CPT} = \frac{\text{Released CPT}}{\text{Total CPT}} \times 100 \quad (2)$$

2.2.4 Cell line culture and condition

Triple negative breast cancer cell line MDA-MB-231, was obtained from NCCS, Pune and cultured in liquid medium i.e., Dulbecco's Modified Eagle High Glucose (DMEM-HG) that was supplemented with 10% Fetal Bovine Serum Albumin (FBS), 1% sodium bicarbonate and 1% sodium pyruvate at 37 °C in a 5% CO₂ incubator. The cells were trypsinized using trypsin EDTA, and seeded in a 96 well plate for MTT and 6 well plate for flow cytometry analysis.

2.2.5 In-vitro MTT assay

MDA-MB-231 cells were trypsinized and grown in a 96-well plate at a density of 1 × 10⁶ cells/ml and incubated for 24 h. The cells were treated with free nanocarrier (poly-CD-coated NPs) and drug (CPT)-loaded nanocarriers. The concentrations of treatments were in the range of 0.25 µg/mL to 15 µg/mL. The sample was replicated three times It was then incubated for 24 h at 37°C. After incubation, the culture medium was removed from the treated plate, and 100 µL of MTT (0.5 mg/mL) was added to each well and incubated at 37 °C for 4 h. The resulting formazan crystals were dissolved in 100 µL of DMSO. The absorbance intensity was measured using a microplate reader (RT-2100 C) at 570 nm. The percentage of viable cells was plotted and the IC₅₀ value was calculated using GraphPad Prism 8.0 software.

3 Results And Discussion

3.1 Characterization of the NPs

The Cu and Co-containing sulfide NPs were synthesized using a hydrothermal method. The synthesized NPs were coated with poly-CD. The polymer-coated NPs display an X-ray diffraction pattern as in Fig. 1A. As the poly-CD possesses a crystalline nature, broad peaks are not obvious in the XRD. The sharp peaks correspond to the sulfide NPs. The prominent peaks are observed at 2θ 27.12° (2 2 0), 31.3° (3 1 1), 32.4° (2 2 2), 47.3° (4 2 2), 52.3° (3 3 3), 58.8° (5 3 1), and 68.9° (4 4 4). These reflections correspond to the CuCo_2S_4 crystals of the face-centered cubic system. It is confirmed by their congruence with the JCPDS card number: 75-1570. The average grain size of the CuCo_2S_4 crystals was determined employing the Debye-Scherrer expression:

$$D = \frac{0.94\lambda}{\beta \cos \theta}$$

3

where λ , β , and θ represent the wavelength of Cu (K α), full-width-half-maximum in radians, and the angle of the most intense peak respectively. The crystalline size (D) is determined as 13.36 nm. Further, the dislocation density of the crystals was determined employing the following relation:

$$\text{Dislocation density, } \delta = \frac{1}{D^2}$$

4

where D represents the average crystallite size. The δ value is $6.5 \times 10^{-3} \text{ nm}^{-1}$. As the polymer contributes an extent of non-crystallinity to the sample of NPs, crystallinity index should be calculated. In a method of calculating the crystallinity, we utilized the following relation:

$$\text{Crystallinity Index, } CI = \frac{\text{Area of all the crystalline peaks}}{\text{Area of all the crystalline and amorphous peaks}}$$

5

The crystallinity thus determined is 46%.

Figure 1B shows the TEM images of the poly-CD-coated CuCo_2S_4 NPs. Nucleated nano-structures are observed in hexagonal shapes. Some of them appear sideways in orientation revealing that the formed NPs are hexagonal plate-shaped NPs. These nanostructures are consistent with the observation in crystal data revealed by the XRD pattern. The mean length and width of the NPs are 120.71 and 42.79 nm respectively. The poly-CD has coated the NPs as a thin layer. In addition, such polymers are transparent in TEM and do not appear vivid on the dark contrast-sulfide NPs.²⁴ The size of the polymer-coated NPs was further confirmed by carrying out a particle-size analysis by dynamic light scattering experiment. The DLS

shows a histogram with the mean size of the NPs as 122 nm. (Fig. 1C). The size range matches with that observed in the TEM images.

The elemental composition of the as-synthesized CuCo_2S_4 NPs was determined qualitatively, employing EDX spectroscopy. The EDX of the NPs is displayed in Fig. 2A. The peaks in the figure correspond to the elements viz., Cu, Co, and S. additional peaks pertaining to any other impurity is not observed. Thus, the EDX reveals the formation of pure CuCo_2S_4 NPs. To add further evidence for such composition of the NPs and to determine the presence of various elements in specific electronic states, X-ray photoelectron spectra were recorded. For XPS measurements, the poly-CD-coated NPs were used. Figs. 2B–F depict the Cu, Co, S, C, and O peaks of the XPS pattern. The $2p_{3/2}$ and $2p_{1/2}$ states of Cu appear at 932.25 and 952.20 eV respectively. (Fig. 2B). the deconvoluted XPS of Co shows peaks at 779.64 and 796.33 eV due to the $2p_{3/2}$ and $2p_{1/2}$ electronic states. (Fig. 2C). Fig. 2D depicts the $2p_{3/2}$ and $2p_{1/2}$ peaks at 161.68 and 162.81 eV respectively. These spectra arise from the cobalt-substituted copper sulfide. The spectra C and O are contributed by the polymer that resides on the surface of the CuCo_2S_4 NPs. The carbons of the C-O-C, C-C, and O-C=O functional groups display peaks at 284.04, 284.85, and 285.92 eV respectively, arising from the C 1s state. (Fig. 2E). the O 1s peaks of the C-O and C=O groups show peaks at the binding energy values viz., 532.38 and 531.63 eV. The XPS peaks and their positions reveal the composition of the poly-CD-coated CuCo_2S_4 NPs. Additional peaks due to any impurity is not observed in the XPS data.

It is apparent from the results discussed above that the synthesized material carries CuCo_2S_4 NPs with the poly-CD immobilized on their surface. To further evaluate the amount of poly-CD on the surface of the NPs, thermogravimetric profiles of the coated CuCo_2S_4 NPs were measured. Fig. 3 shows an initial weight loss of 4.42% and then plummeting down of the weight up to 12.46%. these decay profiles imply that the polymeric structure on the surface changes conformation and then starts melting. The melting is sharp at about 440°C. above this temperature, there is complete degradation of the coated polymer and it extends up to above 750°C. the residue remains with a weight percentage of 79.86 with an implication that the coated polymer offers about 20% of weight to the composite nanostructure.

3.2 Optical and magnetic characteristics of the NPs

The UV-Vis absorption spectrum of the poly-CD-coated CuCo_2S_4 NPs is shown in Fig. 4A. the band at the visible spectral region is shorter (lower absorbance) than the NIR band, which is vividly due to the 3d electrons of the transition metals in the NPs.²⁵ The stronger absorption is further contributed by the Co incorporated into the crystals, which makes the normally observed CuS absorption spectrum enhance in absorbance at the NIR wavelength.²⁶ The aqueous dispersion of the poly-CD coated NPs, displaying such an NIR absorption, presents them suitable for photothermal heating. Further, the absorption wavelength was determined by an extrapolation of the line to the linear portion of the absorption spectrum. The optical band gap was determined employing the relation:

$$Eg = \frac{hc}{\lambda}$$

where h represents the Planck's constant, c , the velocity of light, and λ is the wavelength derived from the tail of absorption. In the present work, the energy band gap calculated is $E_g = 2.25$ eV (Fig. 4B). this value is slightly different from the reported secondary band gap of 2.12 eV for a smaller size CuCo_2S_4 NPs.²⁷ Furthermore, it is larger than the band gap reported for CuS NPs (1.80 eV).²⁸ The photoluminescence spectrum of the poly-CD-coated CuCo_2S_4 NPs is shown in Fig. 4C. The luminescence spectral band is broad and intense, showing wavelength of emission at 510 nm. This property presents the NPs to be followed employing luminescence imaging.

The magnetic properties of the poly-CD-coated NPs were investigated by employing VSM. The room-temperature magnetization property, with an applied sweeping magnetic field, of the NPs is displayed in Fig. 4D. the magnetization curve shows a small coercivity value of 78.09 Oe which is close to superparamagnetic limit.²⁹ The saturation magnetization (M_s) value is 0.024 emu g^{-1} . Such magnetic NPs attain saturation quickly at the application of an external magnetic field. In addition, they relax quickly due to the absence of broad hysteresis loops. Therefore, the synthesized NPs are suitable for application in bio-imaging using MRI.³⁰

3.3 Drug-loading and release

The polymer-coating on the NPs poses the advantages viz., (i) it makes the NPs dispersible in aqueous medium, (ii) renders them biocompatible, and (iii) enables the loading of organic therapeutic molecules. A drug carrier should accommodate an appreciable amount of the therapeutics. In addition, the release of the drug is preferred to be sustained and slow.³¹ Therefore, the drug loading and the drug-release kinetics were investigated in vitro. The experimental procedures have been elaborated in the methods section. The percentage of the model drug CPT, loaded in the poly-CD-coated CuCo_2S_4 NPs (nanocarrier) is estimated employing the equation (1). The percentage of adsorbed CPT on the nanocarrier is 89.04%. It implies a large amount of drug is loaded on the nanocarrier. CPT forms an inclusion complex with the β -CD molecule.³² Therefore, the poly-CD is a suitable choice to be employed as the polymeric host structure that is immobilized on the surface of the NPs. The CPT release profile from the nanocarrier (Fig. 5) reveals that it is disseminated in a sustained fashion, well over a period of 360 hours at the physiological pH of 7.4. The cumulative release of CPT at 24 hours is 25%. In addition, the release rate is accelerated when the pH is made slightly acidic (6.5). In this lower pH, release is enhanced to 40% at 24 hours. Therefore, it is obviously seen that the drug release is pH dependent and it is tunable with the application of a stimulus. It is worth mentioning that the tumor microenvironment is acidic in nature.³³ Therefore, the designed nanocarrier is suitable for selective release of CPT at the tumor site.

3.4 In vitro anticancer activity

The in vitro anticancer activities of the free- and the nanocarrier were carried out on human breast cancer cell lines (MDA-MB-231). After an incubation period of 24 hours as described in the experimental section,

the cytotoxicity was analyzed employing an MTT assay. Figs. 6A and B represent the percentage cell viability as a function of concentration of the free- and CPT-loaded nanocarriers respectively. The cytotoxicity is observed to be dose-dependent in both the cases. Nevertheless, it is more in the case of CPT-loaded nanocarrier than the free-nanocarrier. The half-maximal inhibitory concentration (IC_{50}) of the free nanocarrier is $161.5 \mu\text{g mL}^{-1}$, indicating that the cells are considerably viable at the addition of the nanocarrier alone. Contrary to that, the loading of CPT in the nanocarrier leads to an enhancement of the anticancer activity. The IC_{50} value of the CPT-loaded nanocarrier is $68.93 \mu\text{g mL}^{-1}$. Therefore, it can be concluded that the loaded drug gets slowly released from the nanocarrier and destroys the cancer cells. Further, all of the amount of loaded CPT is not released at the time period of 24 hours. The cumulative release as discussed in the previous section suggests a 40% of CPT disseminated over a period of 24 hours. Therefore, it follows that the anticancer activity occurs due to the portion of the CPT that is released from the nanocarrier. The reported IC_{50} of the free-CPT is indeed more than the CPT-loaded nanocarrier reported herein. Nevertheless, it should be noted that such direct application of CPT to the culture of cells renders the former rapidly and unrestrictedly diffusing into mammalian cells and hence make them more cytotoxic.³⁴ This is contrary to the slow internalization of the drug-loaded nanocarrier through the endocytosis pathway. Therefore, the poly-CD-coated CuCo_2S_4 nanocarrier is a promising nanocarrier of CPT, that possesses additional features of magnetic, NIR absorbing, and photoluminescence characteristics.

4 Conclusions

We synthesized CuCo_2S_4 nanoparticles and coated them with poly-CD. The nanoparticles show a hexagonal crystal structure with a mean size of 122 nm which is a size suitable for employing as a nanocarrier. The NIR-II absorption of the NPs is broad and more intense than the visible spectral band. The optical band gap is 2.25 eV. In addition, the nanocomposite reveals a superparamagnetic behavior due to the presence of incorporated cobalt. It makes them suitable for control by external magnetic field. The poly-CD coating renders the material biocompatible and enables the loading of CPT by means of a non-covalent host : guest association. The drug-release from the nanocarrier takes place over a period of about 360 hours. Further, the drug-release is pH-dependent and it is accelerated at pH 6.0 compared to the normal physiological pH (7.4). the drug-loaded nanocarrier reveals an anticancer efficacy ($IC_{50} = 68.93 \mu\text{g mL}^{-1}$) that presents it as a suitable choice as a CPT-transporter. The cobalt-addition and the poly-CD coating thus add precision to the promising sulfide NPs.

Declarations

Acknowledgement

The research work was carried out without any funding by external agencies. The authors thank the management of KITS for providing necessary facilities.

Compliance with ethical standards

Conflict of interest None declared.

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Figures

Figure 1

(a) XRD, (B) TEM, and (C) DLS of CuCO_2S_4 -poly-CD nanocomposites.

Figure 2

(a) EDX spectrum of CuCo₂S₄-poly-CD, X-ray photoelectron spectrum of elements present in CuCo₂S₄-poly-CD NPs (Cu, Co, S, C, O shown from B to F respectively).

Figure 3

TGA profile of CuCo₂S₄-poly-CD NPs.

Figure 4

A. Absorbance graph of CuCo₂S₄-poly-CD NPs, B shows the band gap energy of CuCo₂S₄-poly-CD NPs calculated using Tauc's plot, C and D show the magnetization profile and photoluminescence spectra of CuCo₂S₄-poly-CD NPs respectively.

Figure 5

Drug release profile of CuCo_2S_4 -poly-CD NPs at different pH's.

Figure 6

A and B show the in-vitro cytotoxicity assay on MDA-MB-231 cell lines of CuCo_2S_4 and CPT loaded CuCo_2S_4 -poly-CD respectively.

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