International Journal of Pharmaceutics 535 (2018) 95–105

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Research paper

DNA interaction, anti-proliferative effect of copper oxide nanocolloids prepared from metallosurfactant based microemulsions acting as precursor, template and reducing agent

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ARTICLE INFO

Keywords:
Metallosurfactant
Microemulsions
Copper nanoparticles
CT-DNA
Circular dichroism
Gel electrophoresis

ABSTRACT

In the present study, we have synthesized mixed cuprous/copper oxide nanosuspensions by metallosurfactant based microemulsion technique. Three metallosurfactants were synthesized which includes two non-ionic double chained metallosurfactants with C_{12}, C_{16} chains with coordinated copper i.e. Cudda and Cuhexa, respectively. Another cationic double chained metallosurfactant with loosely bound metal (Cuctac) was also prepared. The prepared metallocomplexes were characterized using FTIR, elemental analysis, and NMR. The effect of the position of metallosurfactant in microemulsion on the fabrication and properties of nanosuspensions was elucidated. In this method, no external reducing agent and capping agent were added and tween 80 acted both as reducing and stabilizing agent for the nanoparticles. The synthesized nanoparticles were characterized and it was observed that mixed copper and cuprous oxide particles are present in colloidal suspension for all the three studied metallosurfactants. The kinetics of formation of mixed copper/cuprous oxide nanosuspensions (Ns) and their stability was estimated using Uv-visible spectroscopy. Further, the binding and interactions of copper nanosuspensions with calf Thymus DNA (CT-DNA) were assessed using Uv–vis spectroscopy, circular dichroism and gel electrophoresis. Additionally, the antioxidant activity of the Cu Ns was checked using DPPH assay. The role of positive charge on nanoparticles as evaluated from Zeta potential was responsible for DNA affinity. The DNA conformational changes in the presence of nanosuspensions and relevant scavengers were investigated. Further, the anti-proliferative activity of copper Ns was assessed using HeLa cells and Cuhexa derived Ns were proved to be active with highest activity at a low concentration and were nontoxic towards normal cell lines. In summary, this work demonstrates a softer approach for the synthesis of copper nanosuspensions with a size range of 2–5 nm and evaluated the role of type and structure of metallosurfactant on size, stability of particles and anti-proliferative activity.

1. Introduction

In the last few decades, the emphasis has been laid on the use of non-viral vectors in gene therapy because it evades many secondary effects. Different formulations are being explored for this functionality which includes surfactant self-assemblies such as micelles (Mazzoli et al., 2015), liposomes, catanionic vesicles (Dias et al., 2002) and/or nanoparticles (Rahban et al., 2010). Along with, intensive investigations have been made by studying the interaction of various transition metal complexes with DNA, in order to develop newer material for gene therapy (Mal et al., 2014; Mandegani et al., 2016; Manikandamathavan et al., 2012).

In recent times, researchers have used metal functionalized surfactants, commonly known as metallosurfactants, for analyzing the interactions with DNA (Veeralakshmi et al., 2015; Nagaraj et al., 2014). Metallosurfactants serve the advantages of both surfactant and metal complex, as a metal ion is present in the structure of an amphiphilic molecule, may be as a head group, tail or as a counter ion. Recently, Lebrón et al have explored the interaction behavior of Ruthenium metallosurfactant with DNA. The effect of hydrophobic tail and

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http://dx.doi.org/10.1016/j.ijpharm.2017.10.059
Received 17 May 2017; Received in revised form 23 September 2017; Accepted 31 October 2017
Available online 02 November 2017

presence of cyclodextrin was studied using various analytical techniques. (Lebróna et al., 2015) Similarly, Kumar et al have investigated cobalt based metallosurfactant in terms of DNA binding affinity and found that binding to DNA has occurred via groove binding, van der Waals interactions and/or electrostatic interactions. (Kumar et al., 2009) Metallosurfactants have shown the perspective of the development of newer materials for utility in various fields like drug delivery (Lavasanifar et al., 2000), heterogeneous catalysis (Li et al., 2008), a template for nanoparticle synthesis (Chaudhary et al., 2015), anticancer agents (Kaur et al., 2016), and antimicrobial agents (Veeralakshmi et al., 2014).

These metallosurfactants have been widely used to synthesize nanoparticles using micelles (Kaur et al., 2017) and microemulsions (Maillard et al., 2003; Petit et al., 1993) as templates to generate nanoparticles. Pileni et al have fabricated copper (Pileni et al., 1997), silver (Maillard et al., 2002), cobalt (Lisiecki and Pileni, 2003) nanoparticles using metal surfactant prepared from anionic surfactant sodium (II)bis(2-ethylhexyl)sulfosuccinate (AOT). The particles were synthesized using microemulsion technique, where the metal surfactant is present in one microemulsion and a reducing agent such as $NaBH_4$ is present in second and the addition of two resulted in the controlled formation of particles. The microemulsion technique is the bendsome preparation method which enables to control the particle properties such as the mechanism of particle size control, morphology, geometry, surface area, and homogeneity. Along with other advantages, the method in the present case is more advantageous because no external stabilizing or reducing agent has been used for the fabrication of nano colloidal solutions.

In the present case, we have synthesized copper based metallosurfactants, which was further used for the fabrication of nanoparticles using tween mixed microemulsions. In this methodology, no external reducing agent was added (Andersson et al., 2005) and the particles were fabricated using single microemulsion. To carry out the investigations, three double tailed surfactants were synthesized. Firstly, metallosurfactant containing two C_{16} chains was prepared with coordinated copper (bishexadecylamine copper dichloride; Cuhexa). The second surfactant was prepared to have two 12 carbon chains, in order to compare the varying chain lengths (bisdodecylamine copper dichloride; Cudda). Since the above two surfactants were nonionic, therefore for another comparison one cationic metallosurfactant was prepared containing loosely bound metal (C_{16}; bis hexadecyl trimethyl ammonium copper tetrachloride, Cuctac). The prepared complexes were characterized using FTIR, elemental analysis, and NMR. Thereafter, the copper nanoparticles were prepared using microemulsion formed from tween 80, oleic acid and butanol as a template. After the characterization of nanoparticles using HRTEM, FESEM, EDX, XRD, AFM, and Zeta potential, the kinetics of formation of mixed copper/cuprous oxide nanosuspension and their stability was estimated using Uv-visible spectroscopy. Also, the role of microemulsion as a template, as a reducing agent and as a capping agent was evaluated using three synthesized copper-based double tailed metallosurfactants. Further, the interactions of as-synthesized copper nanostructures with CT-DNA were assessed using Uv-visible spectroscopy, CD and gel electrophoresis. Additionally, the antioxidant activity of the Cu Ns was evaluated using DPPH assay.

2. Materials and methods

2.1. Materials

Agarose, ethidium bromide (95% purity), Hexadecyl Amine (98% purity), bromophenol blue dye, D-Mannitol (98% purity), Tween 80, Calf Thymus DNA and Trizma base (99.9% purity) were purchased from Sigma-Aldrich. Oleic acid (85-93% purity) was provided by CDH (central drug house). Ethanol (99.9% purity) was provided by Changshu Yangyuan Chemical China. DPPH (2, 2-dipheny-1-picrylhydrazyl, 95% purity) was provided by Sisco Research Laboratories. Glacial Acetic Acid (99-100% purity) was purchased from Merck. Copper chloride was provided by Himedia. Butanol (99% purity) was purchased from qualigens. DMF (N, *N*-Dimethyl formamide, 99% purity) was purchased from Fisher Scientific. All the chemicals were used as purchased without any further purification.

2.2. Synthesis of metallo-surfactant

The mixing of $CuCl_2.H_2O$ and hexadecyl amine (hexa) was done in 1:2 M ratio in DMF. This homogeneous mixture is then refluxed for 4 h under constant stirring at 74°C. Refluxed product is dried under vacuum. The color change from blue to green indicated the formation of the bishexadecylamine copper dichloride (Cuhexa) at the first sight and refluxing was done to complete the reaction.

Similarly, Bisdodecylamine copper dichloride (Cudda) was prepared by refluxing mixture of $CuCl_2.H_2O$ and dodecyl amine (dda) in 1:2 in DMF for 4 h as per our previously reported method (Kaur et al., 2017).

The hexadecyl trimethyl ammonium copper tetrachloride (Cuctac) was prepared according to methodology reported previously by refluxing $CuCl_2.H_2O$ and hexadecyl trimethyl ammonium chloride (ctac) in 1:2 M ratio in ethanol for 2 hs (Kaur et al., 2016).

2.3. Synthesis of copper and cuprous oxide nanoparticles

Copper nanoparticles were prepared using tween-based microemulsion. The microemulsion was composed of oleic acid (0.082 g), tween (as a nonionic surfactant, 0.328 g), CuX, X = dda, hexa, ctac (as metallosurfactant, 0.0925 g), butanol (as cosurfactant, 0.579 g) and double distilled water (0.0678 g) for 1 ml. Firstly, metallosurfactant was dissolved in the oil phase and on the other hand butanol, water and tween were weighed and mixed together. The two fractions were mixed while heating using a hot plate stirrer (at 80°C). The mixing resulted in the formation of a clear brown color microemulsion for cuctac, green colour for cudda and light green for cuhexa, which was quenched immediately using ice-cold double-distilled water in 1:10 (v/v) for the fabrication of nanoparticles. The nanosuspensions were then separated by centrifuging at 10,000 rpm for 1 h. Nanosuspensions (Ns) were filtered using 0.2 µm millipore filter before characterization. The concentration of nanoparticles in suspension was estimated using the following equation (Chaudhary et al., 2017)

$$c = \frac{C_M M_{cuo/cu_2o}}{\frac{4}{3} r^3 \pi \rho N_o} \quad (1)$$

where C_M represents the molar concentration of metallosurfactant i.e. Cuhexa, Cudda, and Cuctac, $M_{CuO/Cu2O}$ is the molar mass of CuO and Cu_2O, r is the average radius of nanoparticles as obtained from HRTEM, ρ is the density of nanoparticles present in the suspension (0.983 g cm^{-3}, 0.987 g cm^{-3}, 0.979 g cm^{-3} for Cuhexa, Cudda and Cuctac, respectively), and N_0 is Avogadro's number. The concentration obtained for Cuhexa, Cudda and Cuctac based nanoparticles are 0.170 mM, 0.119 mM, and 0.042 mM, respectively.

2.4. Experimental technique

C, H, N analyses of the synthesized metallosurfactants were performed by PerkinElmer 2400 CHN/analytischer Vario EL II Fab. Nr. 11,975,059. All the CHN experiments were performed under nitrogen. Infrared spectroscopic studies were performed using PerkinElmer- FTIR Spectrum-100 operated in the spectral domain of 400 − 4000 cm^{-1} at a resolution of 4 cm^{-1}. KBr pellets were used to hold the sample, and 32 scans were taken for statistical averaging. For ^{1}H NMR studies, BRUKER AVANCE II was employed with 400 MHz radio frequency. Tetramethylsilane was used as internal standard and deuterated chloroform was used as a solvent.