

TRANSMISSION ELECTRON MICROSCOPY: NOVEL APPLICATION OF ESTABLISHED TECHNIQUE IN CHARACTERIZATION OF NANOPARTICLES AS DRUG DELIVERY SYSTEMS

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ABSTRACT

Nanotechnology presents a modern field of science that in the last twenty-five years plays a dominant role in the biomedicine. Different analytical methods are used for evaluation of the physico-chemical properties of nanoparticles including chromatography, electrophoresis, X-ray scattering, spectroscopy, mass spectrometry, zeta potential measurement and microscopy on which this article will focus.

Herein, we present novel application of the long-established TEM technique that is focused on characterization and evaluation of various nanoparticles in development of drug delivery systems.

Transmission electron microscopy images were taken of samples from native nanoparticles, nanoparticles labeled using stannous chloride labeling procedure, inorganic silica nanoparticles loaded with budesonide and native micelles and micelles carrier of anticancer drug camptothecin. In the case of radiolabeled nanoparticles, beside for nanoparticle characterization, TEM technique was used to confirm the stability of the nanoparticles after radiolabeling. Furthermore, the porous structure of hybrid silica particles loaded with budesonide was examined under TEM.

Transmission electron microscopy technique offers exceptional benefits for nanoparticle characterization. Additionally, the necessity of ultrastructural analysis demonstrates the potential of TEM in the field of nanomedicine. Hence, the long-established and well-known TEM has been only partially exploited and offer researchers very detailed images of specimens at microscopic and nano scale.

Keywords: nanoparticle, transmission electron microscopy, drug delivery systems

INTRODUCTION

Nanotechnology presents a modern field of science that in the last twenty-five years plays a dominant role in the biomedicine e.g., development of new drug delivery systems, diagnostic tools, scaffolds and etc. [1-3]

Additionally, research in the field of biomedicine focused on nanoscience has been dramatically

increased which is proved with more than 60 000 published articles in qualified journals since 1995 and nowadays this discipline is known as nanomedicine [4].

Great variety of nanoparticles with special advantage of those in the range 1-100 nm have been used for research in different applications

namely, for drug delivery systems, contrast agents for *in vivo* imaging, sensor for detection of cells or organic components in living organisms and/or scaffolds in tissue engineering [4-6]. Nanoparticles can be made from different materials covering the space between organic and inorganic and various configurations between spheres, rods, cubes, etc. The world of nanoparticles gets more interesting since their surface can be functionalized which allows them to target specific cell types [4, 7].

Different analytical methods are used for evaluation of the physico-chemical properties of nanoparticles including chromatography, electrophoresis, X-ray scattering, spectroscopy, mass spectrometry, zeta potential measurement and microscopy on which this article will focus.

Namely, transmission electron microscopy represents one of the most valuable and powerful technique for characterization of nanoparticles. Its high resolution of structural details is considered essential for example to obtain information regarding the crystal structure and granularity of nanoparticles [8]. TEM is considered indispensable method for development of drug delivery systems since one can also explore the possible alterations in the morphology of the nanoparticle after incorporation of drugs at different concentrations [3, 7-10].

Besides its irreplaceable role in characterization of biological tissues, herein, we present novel application of the long-established TEM technique that is focused on characterization and evaluation of various nanoparticles in development of drug delivery systems.

MATERIALS AND METHODS

Materials

Uranyl acetate (Sigma Aldrich, Belgium), Ethanol (Merck, Germany), Poly(vinyl) formal (Sigma-Aldrich, Belgium), Chloroform (Merck, Germany) and MilliQ water was used. The Faculty of pharmacy in Skopje, Macedonia developed different nanoparticles covering different size range. Copper grid 100 mesh (Sigma Aldrich, Belgium) covered only with formvar or formvar/carbon was used for all TEM analysis.

Preparation of formvar film coated grids

25 mg polyvinyl formal was dissolved in 50 mL chloroform and was stirred for 24 hours on room

temperature. Before use, the solution was filtered and placed in a container where clean-glass slide was inserted and was let to sit at least a minute. Afterwards, the slide was removed from the container and filter paper was used for complete drying. Slide edges were scraped with a razor blade to loosen film. Grids were placed onto the film with shiny side down.

Grid carbon coating

Formvar film coated copper grids were additionally stabilized with carbon film that was applied automatically under vacuum from 10 Pa to 0 Pa with Carbon coater (Jeol JEC-530, AutoCarbon Coater, Japan).

Native and radiolabeled nanoparticle samples

Poly (lactic-co-glycolic acid): Poloxamer blend amphiphilic nanoparticles were prepared using modified nanoprecipitation technique and afterwards were radiolabelled using stannous chloride and sodium borohydride. Details of the preparation procedure is described in details elsewhere [6].

Samples were stained with 2% uranyl acetate, and then small volume (2 μ l) was placed on 100-mesh formvar coated copper grid with additional carbon film. The grid was set up on filter paper in order to absorb excess solvent and dried under vacuum.

Inorganic silica nanoparticle loaded with budesonide samples

Budesonide loaded inorganic silica particles were prepared by acetic acid catalyzed hydrolysis and polycondensation of TEOS in ethanol/ water mixture at room temperature, followed by spray drying. Details of the preparation procedure is described elsewhere [10]. The porous structure of the samples was characterized using TEM. The samples were prepared by dispensing the particles in MilliQ water followed by vigorous mixing. Then, a small quantity of the particles dispersion (2 μ L) was placed on coated copper grid (100 mesh) with additional carbon film. The grid was placed on filter paper (WhatmanTM 42, Austria) in order to absorb excess solvent and air dried at ambient temperature.

Block copolymer carrier for 7-ethyl-10-hydroxy camptothecin samples

Amphiphilic poly(acrylic acid)-poly(ϵ -caprolactone)-poly(acrylic acid) block copolymer carrier for 7-ethyl-10-hydroxy camptothecin (micelles) were prepared by nanoprecipitation method which in details

is described elsewhere [11]. Samples of native micelle solution were stained with 2% aqueous solution of uranyl acetate and small amount (2 μ L) of each sample was placed on formvar/carbon coated copper TEM grid (100 mesh). Excess solvent was absorbed using filter paper and the grid was allowed to dry in a vacuum oven.

Transmission electron microscopy characterization

Transmission electron microscopy images were taken of samples from native nanoparticles, nanoparticles labeled using Stannous chloride labeling procedure (SCLP), inorganic silica nanoparticles loaded with budesonide and native micelles and micelles carrier of anticancer drug camptothecin. After drying, each sample was loaded into TEM (JEM-1400, Jeol, Japan) attached to digital camera (Veleta TEM Camera, Olympus, Germany) and controlled by iTem software v.5.

RESULTS AND DISCUSSION

Transmission electron microscopy is considered to be among the most valuable tools and techniques for detailed physicochemical characterization of nanoparticles [1-11]. This is supported by published literature, since during the last 20 years more than 15 000 articles that deal with this subject practice TEM among other methods for characterization [4]. Moreover, to add to the fact that TEM is invaluable tool in nanomedicine, is that more than 7000 scientific articles in the last 15 years use this technique to explore the effects of nanostructures on biological systems. Moreover, 2400 scientific articles were published in the period of 2014 to 2016 [4].

Herein, we present TEM analysis on nanoparticles that have been performed in our laboratory focused on drug delivery systems.

In the case of radiolabeled nanoparticles, beside for nanoparticle characterization, TEM technique was used to confirm the stability of the nanoparticles after radiolabeling. Hence, TEM imaging

of the sample was performed at the beginning and at the end of SCLP.

TEM images clearly present that there were not any changes in the spherical morphology, smooth surface, and size of the nanoparticles during the labeling procedure (Figure 1). On the other hand, presence of distinctive large irregularly shaped particles of stannic oxide were identified in the sample at the end of SCLP (Figure 2) [6].

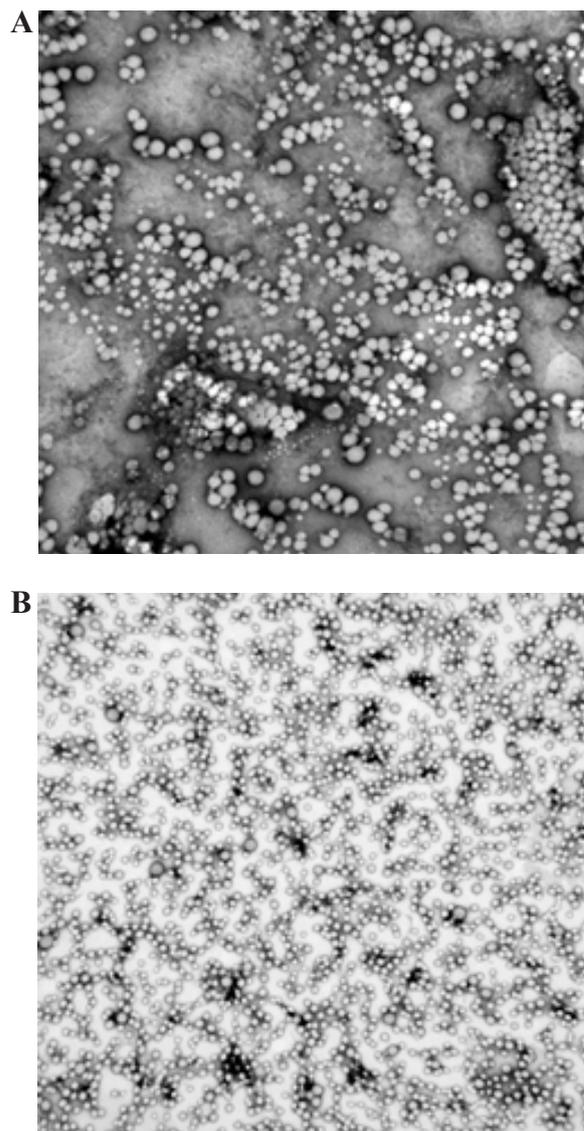


Figure 1. Transmission electron microscopy images of native sample of the prepared amphiphilic nanoparticles (a) $\times 60k$ magnification, (b) $\times 40k$ magnification

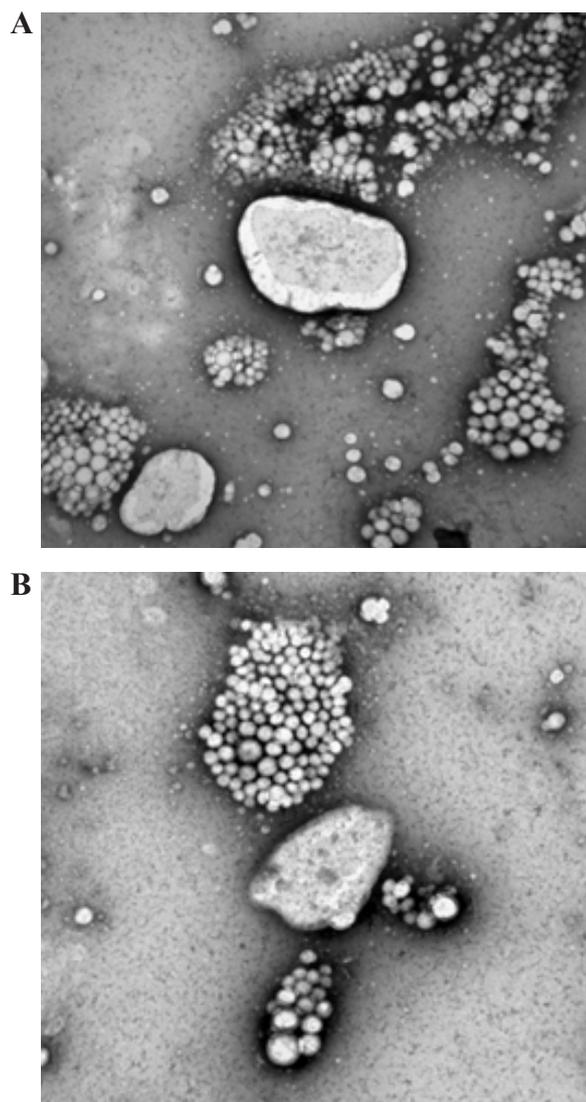


Figure 2. Transmission electron microscopy images (a) and (b) of sample after the SnCl_2 radiolabeling procedure ($\times 60k$ magnification)

Other application of a TEM technique that we are reporting herein is in a study of hybrid-silica-xerogel particles that were developed as carriers of budesonide (BDS) for efficient local treatment of inflammatory bowel diseases [10]. In this study, TEM was utilized to analyze the shape and morphology of the prepared inorganic silica and hybrid silica particles. Figure 3a is evidence for the uniform size of the well-formed spherical shape and smooth surface of the inorganic silica particles, whereas Figure 3b and 3c demonstrates the hybrid silica particles that are slightly larger porous spheres.

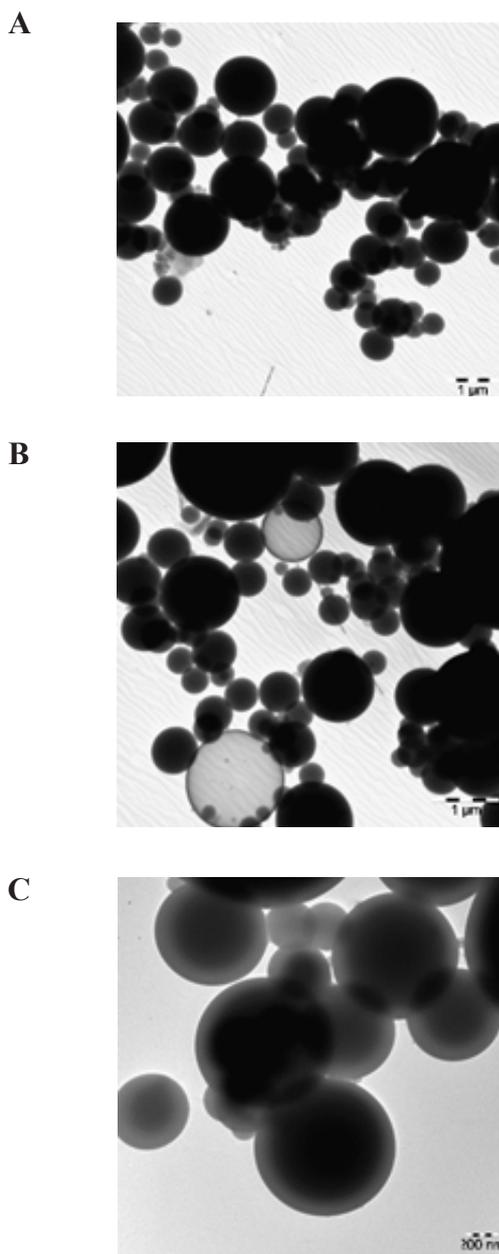


Figure 3. a) Inorganic silica particles; b and c) hybrid silica particles

Additionally, TEM technique was used to confirm the surface, the shape and the unimodal distribution of the particles in the study that aimed to develop self-assembled polymeric micelles for targeted delivery in tumor cells [11]. The particles are shown on Figure 4. Authors used TEM as confirmatory and added value technique to the particle size analysis of the sample in the reported research [11].

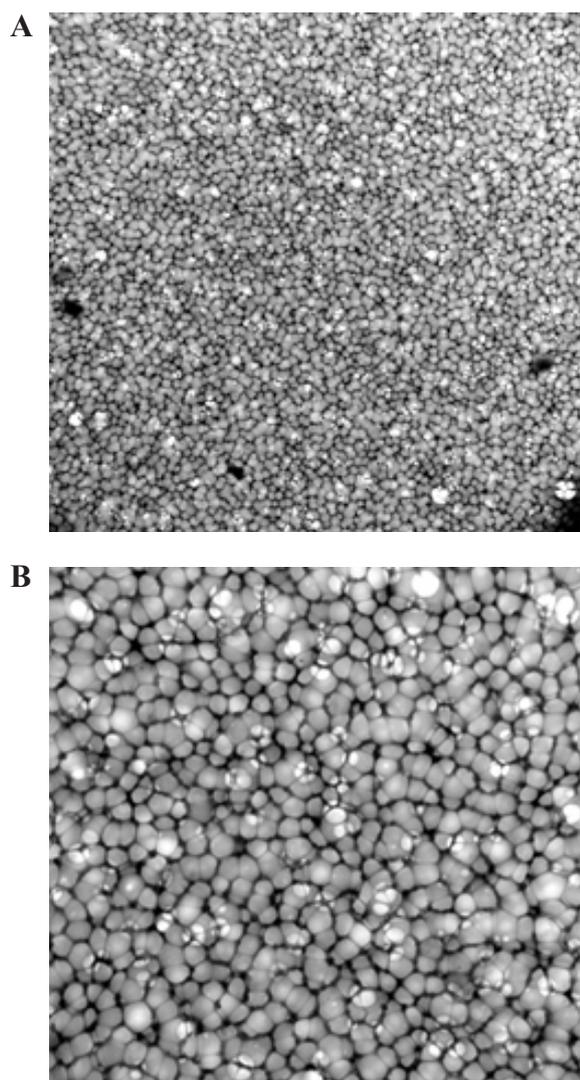


Figure 4. TEM images of loaded nanoparticles a) x8000 magnification b) x 20000 magnification

Nanotechnology is at the forefront of development of novel materials from gold nanoparticles for targeted delivery in cancer cells through carbon nanotubes in renewable energy [3, 4].

Despite nanoparticles' diverse application, the performance of all nanoparticles is defined by the same physical properties, including particle size, size distribution, shape, and surface feature.

Nanoparticle size characterization therefore presents key step in nanotechnology research & development and quality control [12]. During the development phase especially in pharma industry, electron microscopy is one of the most powerful tools for determining these critical performance-defining attributes. This is in detail reflected by the US Food and Drug Administration's (FDA) recommendation of the technique in identifying and

demonstrating the efficacy and safety of innovator and generic drug submissions [12].

CONCLUSION

Transmission electron microscopy technique offers exceptional benefits for nanoparticle characterization. Additionally, the necessity of ultrastructural analysis demonstrates the potential of TEM in the field of nanomedicine. Hence, the long-established and well-known TEM has been only partially exploited and offer researchers very detailed images of specimens at microscopic and nano scale.

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Резиме

ТРАНСМИСИСКА ЕЛЕКТРОНСКА МИКРОСКОПИЈА: НОВА ПРИМЕНА НА ВОСПОСТАВЕНАТА ТЕХНИКА ВО КАРАКТЕРИЗАЦИЈА НА НАНОЧЕСТИЧКИ КАКО СИСТЕМИ ЗА ИСПОРАКА НА ЛЕК

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Нанотехнологија претставува ново модерно научно поле, кое во последните 25 години доминира во биомедицината. Различни аналитички методи се користат при евалуација на физичкохемиските својства на наночестиците, вклучувајќи ги тука: хроматографијата, електрофорезата, распрснување на рендген-зраци, спектроскопија, масена спектрометрија, мерење на зета-потенцијал и микроскопија.

Во ова истражување ја прикажуваме новата апликација на добро етаблираната трансмисиска електронска микроскопија, фокусирајќи се на карактеризација и евалуација на различни наночестици во развојот на фармацевтски дозирани форми.

Слики од трансмисиска електронска микроскопија беа земени од следните примероци: нативни наночестици, наночестици радиоактивно означени со SnCl₂, неоргански силика наночестици полнети со будезонид, нативни мицели и мицели носачи на антиканцер супстанција – камптотекин. Покрај карактеризацијата на наночестиците, ТЕМ беше користен за потврда на стабилноста на радиоактивно означените наночестици во споредба со нативните наночестици. Дополнително, техниката ТЕМ беше користена за проучување на порозната структура на хибридните силика наночестици, носачи на будезонид, во споредба со структурата на нативните неоргански силика наночестици.

Трансмисиската електронска микроскопија претставува техника од исклучително значење при карактеризација на наночестиците, особено во фармацевтската индустрија и во наномедицината. Со овој труд сакаме да ја потенцираме идејата дека техниката ТЕМ е само парцијално експлоатирана и таа им нуди на истражувачите детална слика на ултраструктурата на примероците во микроскопско и нанониво.

Клучни зборови: наночестици, трансмисиска електронска микроскопија, фармацевтски дозирани форми