

## PREPARATION AND APPLICATION OF NANOSCALE CELLULOSE BIOCARRIERS

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### ABSTRACT

In this paper, an improved technology for preparation of crystalline and amorphous cellulose nanocarriers, as well as conjugates of these biocarriers with therapeutically active substances has been proposed. The technology of the nanocarriers includes the following main steps: (1) controlled acidic treatment of initial cellulose for its structural and chemical modification, (2) high-power mechanical disintegration of the modified cellulose in an aqueous media to obtain dilute dispersion of reactive nanoparticles, and (3) concentration of the dispersion. Finally, an interaction of various therapeutically active substances (TAS) with reactive acidic and reducing groups of cellulose nanocarriers (NC) was performed in order to form nano-scale conjugates NC-TAS for various applications. Due to optimal combination of specific properties, the proposed conjugates can be employed at the development of advanced types of hygienic, cosmetic and medical remedies used for delicate care and effective treatment.

**Keywords:** *Nano-cellulose, Biocarriers, Therapeutic Active Substances, Conjugates, Application*

### INTRODUCTION

As is known, diverse peptides, aminoacids, proteins, enzymes and others therapeutically active substances (TAS) are widely used in cosmetic and medical remedies used for delicate care and effective treatment of the skin (Filatov *et al.*, 2013). However, there are some problems that hinder the application of some types of TAS.

The first problem is caused by inactivation of TAS in the liquid remedies. Numerous inhibiting ingredients, unfavorable pH and increased temperatures cause acceleration of the inactivation process. For example, due to fast denaturation the proteolytic enzymes cannot be directly used in liquid remedies, such as creams, lotions and other preparations. The second problem is caused by possible decomposition of other ingredients of the remedies under effect of TAS. For example, papain has both protease and esterase activity and therefore it can decompose ingredients containing of peptide- and ester links, e.g. surfactants, emollients, thickeners, etc. The third problem is that the solutions of TAS cannot provide slow-release effect in the liquid remedies.

The most promising way to improve the stability of the therapeutically active substance may be its joining to special carriers. Furthermore, to ensure delivery of TAS, these carriers should have nanoscale dimensions and meet the following additional requirements (Joelovich and Figovsky, 2008):

- To be natural
- Contain specific reactive groups capable to attach the TAS
- To be stable and do not decompose under effect of TAS
- Not inhibit the attached TAS
- To be insoluble in liquid remedies
- Not interact with other ingredients of remedies

Among various natural materials, cellulose is the most appropriate feedstock for preparation of nanocarriers, since this most abundant natural polymer has a nanostructured organization and unique properties such as low density, hardness and abrasivity; ability to structural and chemical modification; biocompatibility; biodegradability in the nature, etc (Joelovich, 2014). Moreover, cellulose nanoparticles fully satisfy the requirements of the optimal nano-scale biocarrier (Joelovich, 2013). The main purpose of this paper was to describe the preparation methods and characteristics of crystalline and amorphous nano-cellulose biocarriers, as well as conjugates of the nanocarriers with various therapeutically active

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substances. Besides, applications of these nanocarriers and conjugates in medicine, cosmetics and personal care have been discussed.

## **MATERIALS AND METHODS**

### ***Materials and Chemicals***

Initial cellulose material was MCC Avicel PH-301 made of FMC BioPolymer Co. This MCC type has average particles size of 50 $\mu$ m and DP=170. Chemical pure 95wt.% sulfuric acid was used; the concentrated acid was diluted with water to 80 wt.% (density 1.727 g/cm<sup>3</sup>). Some other chemicals were used. Therapeutically-active substances were the following: L-arginine, silver nitrate and trypsin from bovine pancreas (6000 BAEE units/mg). All chemicals and therapeutically-active substances were supplied from Sigma-Aldrich Co.

### ***Preparation of Crystalline Nanocarrier***

The starting cellulose material – MCC, was placed into lab Erlenmeyer flask, wetted with calculated volume of water; and then 80 wt. % sulfuric acid was slowly added with cooling to obtain an acid/cellulose ratio of 10 and final acid concentration of 60 wt.%. The flask was placed into a water bath having a temperature of 45°C, heated and stirred for 60 min. After acidic treatment, contents of the flask were poured out into a tenfold volume of cold water while stirring. Cellulose sediment was separated from the liquid phase by centrifugation, washed with water, 5 wt.% sodium bicarbonate, and finally with distilled water to a pH from 3 to 6 (depending on requirements), separating it from the water by centrifugation. Then the washed sediment of crystalline particles was diluted with distilled water to a solids concentration of 1 wt. % and disintegrated in an ultrasound disperser at 20 kHz for 15 min. The 1 wt. % water dispersion of crystalline nanocarrier (CNC) was evaporated under vacuum at 80°C in order to increase its consistency to 10-15 wt. %. To obtain the dry crystalline nanocarrier, the concentrated water dispersion of CNC was freeze-dried and comminuted by lab Waring knife mill.

### ***Preparation of Amorphous Nanocarrier***

The starting cellulose material – MCC, was placed into lab Erlenmeyer flask, wetted with calculated volume of water; and then 80 wt. % sulfuric acid was slowly added with cooling to obtain an acid/cellulose ratio of 10 and final acid concentration of 66 wt.%. The flask was placed into a water bath having a temperature of 25°C and stirred until complete dissolution of MCC. After acidic treatment, contents of the flask were poured out into a tenfold volume of cold water while stirring. Sediment of amorphous cellulose was separated from the liquid phase by centrifugation, washed with water, 5 wt.% sodium bicarbonate, and finally with distilled water to a pH from 3 to 6 (depending on requirements), separating it from the water by centrifugation. Then the washed sediment was diluted with distilled water to a solids concentration of 1 wt. % and disintegrated in an ultrasound disperser at 20 kHz for 15 min. The 1 wt. % water dispersion of amorphous nanocarrier (ANC) was evaporated under vacuum at 80°C in order to increase its consistency to 10-15 wt. %. To obtain the dry amorphous nanocarrier, the concentrated water dispersion of ANC was freeze-dried and comminuted by lab Waring knife mill.

### ***Characterization of Nanocarriers***

Sizes and shape of nano-particles of CNC and ANC were investigated by method of scanning electron microscopy (Joelovich and Leykin, 2006). Crystallinity degree of the nanocarriers was investigated by method of wide angle X-ray scattering (Joelovich *et al.*, 2010). The average degree of polymerization was measured by the viscosity method using diluted solutions of dry cellulose particles in Cadoxen (Joelovich and Leykin, 2004). Hydrophilicity of the nanocarriers was estimated by content of nonfreezing water (Nakamura *et al.*, 1981). Content of sulfonic groups in the nanocarriers was calculated from results of sulfur assay (Joelovich, 2013), while content of reducing groups was determinate by standard TAPPI method T430.

### ***Preparation of Conjugates Nanocarrier - TAS***

Concentrated (10-15 wt.%) dispersion containing 1 g of the nanocarrier having acidic pH 3-4 was introduced into 10 mL of 2% solutions of TAS (L-arginine or silver nitrate), The mixture was kept overnight at stirring at room temperature and then centrifuged to isolate the cellulose sediment containing

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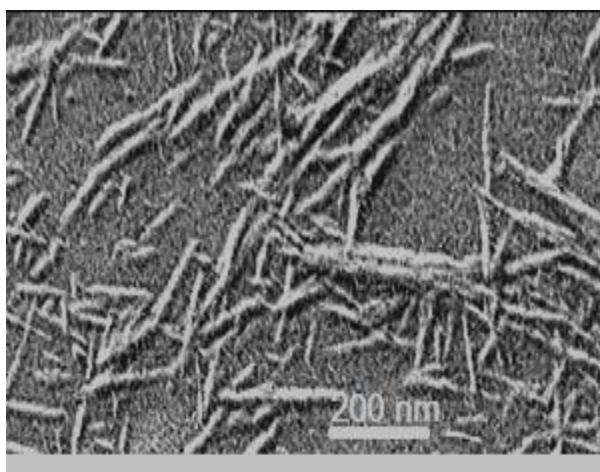
the attached TAS. To attach a proteolytic enzyme – trypsin, the 10-15 wt.% dispersion containing 1 g of the nanocarrier was introduced into 10 mL of 2% solutions of proteolytic enzyme in 0.1 M carbonate buffer. The mixture was kept overnight at stirring at room temperature and then centrifuged to isolate the cellulose sediment containing the attached TAS. Content of TAS in conjugate was calculated by the equation:

$$TAS = V(C_o - C)/W_s$$

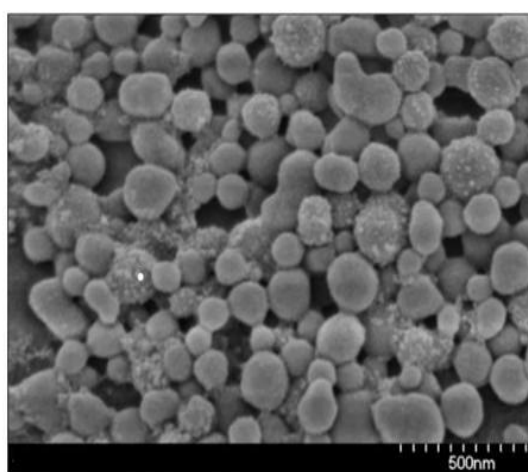
where  $W_s$  is solid content of the nanocarrier;  $V$  is volume of TAS-solution;  $C_o$  is initial concentration of the solution, and  $C$  is concentration of the solution after attachment of TAS by nanocarrier.

**RESULTS AND DISCUSSION**

The SEM investigations showed that nano-particles of CNC have rod-like shape with dimensions 100-200 x 10-20 nm (Figure 1). In contract to CNC, particles of ANC have spherical shape with average diameter of 80-150 nm (Figure 2).



**Figure 1: SEM image of nanoparticles of CNC**



**Figure 2: SEM image of nanoparticles of ANC**

The main characteristics of the nanocarriers were shown in Table 1.

**Table 1: Characteristics of the cellulose nanocarriers**

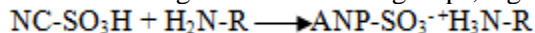
Characteristics	CNC	ANC
Average particle sizes, nm	150 x 15	100
Aspect ratio	10	1
Degree of polymerization	120-140	70-80
Degree of crystallinity, %	75-77	20-25
Allomorph type of crystallites	CI	CII
Specific gravity, g/cm <sup>3</sup>	1.57-1.58	1.50-1.51
Specific volume, cm <sup>3</sup> /g	0.63-0.64	0.66-0.67
Specific surface area, m <sup>2</sup> /g	120-200	40-50
Content of nonfreezing water, %	9-10	28-30
Content of SO <sub>3</sub> H-groups, meq/kg	48-56	200-220
Content of reducing groups, meq/kg	43-52	80-88

The rod-like nanoparticles of crystalline nanocarrier (CNC) have high degree of crystallinity, increased specific gravity, developed specific surface, relative low hydrophilicity and moderate content of reactive functional groups. As against, the spherical nanoparticles of amorphous nanocarrier (ANC) are characterized by low specific gravity, moderate specific surface, enhanced hydrophilicity and high content

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of reactive functional groups. Besides, due to expressed thickening properties the cellulose nanoparticles impart to dispersions an increased viscosity and phase stability (Ioelovich 2013, Ioelovich 2014) [3, 4].

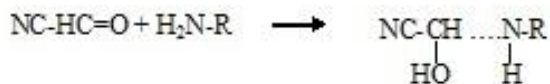
The presence of acidic -SO<sub>3</sub>H functional groups in the nanocarriers (NC) contributes to ion binding of TAS containing basic functional groups, e.g. L-arginine, by the following scheme:



Some others TAS can be attached to acidic functional groups of cellulose nanocarriers. So, after attachment of Ag-cations to NC a nano-antibacterial agent can be prepared:



On the other hand, the presence of reducing (aldehyde) groups in the nanocarriers provides additional opportunities for attachment of TAS. Consider, for example, the joining of proteolytic enzyme – trypsin to nanocarrier by labile chemical bonds with the formation of stabilized enzyme form:



To ensure a long-term storage, the pH of NC-Trypsin conjugate was adjusted to 3. Before usage, the pH of the conjugate should be increased to 8 in order to provide an optimal enzymatic activity. Along with trypsin, some other enzymes such as chymotrypsin, papain, collagenases, lysoamidases, lysozymes, etc can be attached to cellulose nanocarriers with the potential for controlled slow release.

As can be seen from Table 2, the content of TAS in conjugates with amorphous nanocarrier (ANC) was higher than in conjugates with crystalline nanocarrier (CNC). This result correlates with different content of specific functional groups in ANC and CNC.

**Table 2: Content of various TAS in conjugates, mg/g**

TAS	CNC	ANC
L-arginine	5	21
Silver nitrate	2	6
Trypsin	12	23

The nano-scale conjugates can find wide application in personal care, cosmetics, pharmaceuticals and medicine. The CNC-TAS conjugates based on crystalline nanocarriers paves a new path in development of advanced remedies of cosmetics and personal care. These nanocrystalline conjugates are characterized by nano-size, high crystallinity, developed surface, increased thickening ability and some other specific properties imparting to cosmetic and hygienic products the following effects:

- Crystalline nanoparticles of the conjugates of peeling creams cause selective exfoliation of the dead skin tissues and not damage the healthy skin during the peeling procedure
- Due to high specific surface the nano-conjugates of liquid remedies provide an effective cleaning of the skin
- Nanoscale conjugates have a gentle sensation
- Crystalline nanoparticles do not swell and do not interact with inactive ingredients of the liquid remedies
- Nanocrystalline conjugates do not precipitate in liquid media
- Organic cellulose nanoparticles are compatible with organic ingredients and impart to liquid remedies a nice texture, increased viscosity, water retention ability and phase stability
- Due to pronounced thickening ability of natural-based nano-conjugates, their use in cosmetic and hygiene compositions permits reduce an amount of synthetic thickeners, detergents and other harmful ingredients
- Other specific effects



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In particular, due to unique combination of hard nanocrystalline structure, high specific surface and pronounced antibacterial activity, the conjugate CNC-Ag can be used as promising multifunctional agent, which provides an effective peeling, cleaning, thickening and disinfection of the skin.

As is known, the dentifrices contain micron-size abrasive inorganic particles (e.g. silica dioxide), which provide the cleaning of teeth by means of abrasive detrition. Unfortunately, the cleaning process with abrasives is accompanied by gradual damage to the tooth enamel. The use of nanocrystalline cellulose conjugate, e.g. CNC-Ag, can create opportunities for the development of fundamentally new types of dentifrices, which do not damage the teeth enamel and have a nice texture, excellent compatibility, fast cleaning ability along with bactericide and fungicide activity.

The conjugates ANC-TAS based on amorphous nanocarriers can be used for delivery of slow-release therapeutically active substances to cure of deep skin diseases, injuries, wounds and burns. In particular, ANC-Trypsin conjugate removes selectively a dead skin cells and cleans wounds without damage of healthy tissues (Joelovich, 2014). Main application area of the conjugate ANC-Ag is skin disinfection. The conjugate ANC-Arginine can be used for effective treatment and regeneration of damaged skin tissues; cure of herpes simplex virus and in other arginine-therapy areas (Stechmiller, 2005; Witte and Barbul, 2003). Moreover, due to nano-scale size the ANC-TAS conjugates are able to penetrate through the lipid layer and epidermis within the skin strata to cure deep skin diseases and injuries (Filatov *et al.*, 2013; Joelovich and Figovsky, 2008). The high thickening ability of the ANC-TAS conjugates allows reducing the amount of synthetic thickeners and imparts the phase stability to various liquid drug remedies.

### **Conclusion**

The improved technology for preparation of crystalline and amorphous cellulose nanocarriers, as well as conjugates of these biocarriers with therapeutically active substances has been proposed. The technology of the nanocarriers includes the following main steps: (1) controlled acidic treatment of initial cellulose for its structural and chemical modification, (2) high-power mechanical disintegration of the modified cellulose in an aqueous media to obtain dilute dispersion of reactive nanoparticles, and (3) concentration of the dispersion. It was found that the crystalline nanocarrier consists of rod-like nanoparticles, while the amorphous nanocarrier contains spherical nanoparticles. Besides, the obtained nanocarriers are characterized by low degree of polymerization, developed surface and presence of reactive acidic and reducing functional groups.

To attach therapeutically active substances (TAS) an interaction of these substances with reactive groups of the nanocarriers was performed. Due to optimal combination of specific properties, the proposed conjugates can be employed at the development of advanced types of hygienic, cosmetic and medical remedies. The CNC-TAS conjugates based on crystalline nanocarriers paves a new path in development of advanced hygienic, cosmetic preparations and dentifrices. The conjugates ANC-TAS based on amorphous nanocarriers can be used for delivery of slow-release therapeutically active substances to cure of deep skin diseases, injuries, wounds and burns.

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