Are Cellulose nanocrystals 'alien particles' to human experience?

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Abstract

A wide family of cellulose-based additives are authorized worldwide as fillers and thickening agents in foods, pills and tablets, and microcrystalline cellulose (MCC) is, among these, the most important one. Since MCC manufacturing is similar to the main production route of cellulose nanocrystals (CNCs), it is reasonable to wonder whether the MCC would contain CNCs as minor components. In this Short Communications we provide first results about the occurrence of CNCs in MCC, observed by dynamic light scattering and transmission electron microscopy after serial filtrations of MCC suspensions. The incidence of cellulose nanoparticles has been proved in several different trials in our ongoing works on diverse MCC samples and the nanoparticles isolated showed shape and dimensions similar to those commonly produced by acidic hydrolysis at laboratory level. Therefore, the presence of CNCs in many products is considered as a certainty. The foods and the pharmaceuticals we have been consuming so far, do indeed contain traces of CNCs to such an extent that this wide presence in consumed products should be taken into account when considering possible limitations of the use of these nanoparticles in food contact materials manufacture.

Introduction

A general, strong prejudice on the use of nanomaterials in food contact materials (FCM) persists all around the world and the European legislation, since 2011, established that in the manufacture of FCM "substances in nano-form should be used only if explicitly authorized", even ignoring for these applications the functional barrier concept [1]. It is worth reminding that according to EU Recommendation 2011/696, nanomaterial means a *natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm*.

Even though this precautionary policy may be understandable when considering novel substances or inorganic/metallic species, these limitations definitely affect the development of innovative, more sustainable, biodegradable and high performance packaging materials. On the other side, fundamental and applied research has already demonstrated the great potential of cellulose nanoparticles, both cellulose nanocrystals (CNCs) and microfibrillated cellulose (MFC), in the improvement of fundamental properties of FCM [2-4]. In particular, CNCs have been shown to be very interesting barrier coatings, capable of further reducing the gas permeability than synthetic polymers (e.g. EVOH) to a much thinner thickness [5,6]. In addition, no studies to date have demonstrated any dangerousness of the CNCs [3,7] and recent results [8] suggested that cellulose nanoparticles might potentially be used as regulators of lipid absorption; used as food additives or supplements they might provide a safe and non-chemical means of reducing fat absorption, thus allowing weight loss.

CNCs are nanoparticles whose shape and dimensions are largely influenced by the type of cellulosic sources and processes used for their fragmentation. However, they are generally reported as rod-like particles, with length of 100-200 nm and width of 5-10 nm. Such features are practically excluded from many diffusional migration phenomena. It has been demonstrated, in fact, that measurable migration may occur only for nanoparticles up to approximately 3.5 nm in diameter. For 10 nm diameter particles, an apparent diffusion coefficient (*D*) of 1.1E^{-35} cm^2 s^{-1} was theoretically calculated in a LDPE host matrix. Such extremely low *D* results in almost null mobility of the migrants and undeterminable risk of migration [9]. In this context, the only real risk is that cutting, breaking, or similar mechanical stresses of the packaging materials containing CNCs, can lead to a release of nanocellulose in the food.

In foods and in pharmaceutical products (pills and tablets), the presence of cellulose is very common because a wide group of cellulose-based additives is authorized worldwide as thickening, filler and functional agents. Recently, European Food Safety Authority (EFSA) has re-evaluated 10 different chemically modified and unmodified celluloses as food additives, concluding that there was no need for a numerical Admitted Daily Intake (ADI) and that there would be no safety concern about the reported uses [10]. Among all these additives, microcrystalline cellulose (MCC) is certainly the most important. MCC is a cellulose-based, powder-like product, known since the '50s, whose global annual production is currently around 120,000 tonnes [11,12]. In general, wood and cotton powder are common sources for the production of MCC, although other biomasses have been proposed for its production [13]. In any cases, MCC manufacturing is quite similar to the main route for CNCs production and generally consists of a chemical acidic hydrolysis, possibly followed by ultra-sonication.

Therefore, it is reasonable to wonder whether the MCC would contain CNCs as minor components. The aim of this short communication is reporting first results obtained seeking for the presence of CNCs in different types of MCC, focusing also on the needs for more extended and deeper investigation in this field.

Materials and Methods

Two different types of cellulose microcrystalline were used: MCC for column chromatography, Merck KGaA, Darmstad Germany and MCC, USP (United State Pharmacopeia) approved, Blackburn Distribution, Nelson UK. Ultrapure Milli-Q® water, 0,22 µm filtered, 18.2 MΩcm, 3ppb TOC (MilliporeMerckKGaA, Darmstad Germany), was used in all the steps of suspensions preparation and filtration.

To check the possible CNCs presence in MCC, 7 water suspensions of the two MCC, in the concentrations ranging from 0 to 9% (m/v), were submitted to a serial filtrations protocol. Paper filters with nominal cut-off 8-12, 5-8 and 1 µm (Sartorius Stedim, Varedo Italy) and Polyvinylidene Fluoride (PVDF) hydrophilic membranes filters (Durapore® Millipore Merck KGaA, Darmstad Germany) with nominal cut-off 0.22 µm were used in the serial filtrations. The last filtered supernatants were analysed by dynamic light scattering (DLS) for equivalent hydrodynamic diameters, polydispersity and light scattering intensities using a Litesizer500, Anton Paar, Graz, Austria; the DLS measurements were performed at 25.0 ± 0.1 °C, with a 35 mW laser diode light (λ = 658 nm) and collecting the scattered light at 90° (side scattering angle). The last supernatants, possibly containing particles with expected dimensions lower than 0.22 µm, were freeze-dried for transmission electron microscopy (TEM) observations (LEO 912AB, Zeiss, Oberkochen, Germany) at an accelerating voltage of 80 kV, in order to characterize the morphology and the dimensions of the isolated particles.

Results and Discussion

Whatever the MCC concentrations in the different water suspensions filtered, it was always detected, by DLS measurements, equivalent hydrodynamic diameters around 100-150 nm in the supernatants obtained after the last filtration under the 0.22 µm cut-off, as it is shown in Figure 1, with a relatively low level of polydispersity around 20%. In order to confirm the presence of nanoparticles in MCC only, i.e. excluding the presence in the water or due to the procedure used, the filtered Milli-Q® water (MCC concentration 0%) was also tested. The diameters recorded in this case are inconsistent and not reliable values because of the cumulant fit error very high (poor fitting of the correlation function), the high number of runs needed to get a result and the very low mean intensity was recorded (Figure 2). Moreover, the presence of nanoparticles appeared roughly proportional to the initial MCC concentration as it is shown by the increasing scattering intensity (DLS, kcounts/s), at least in the range shown in Figure 2.

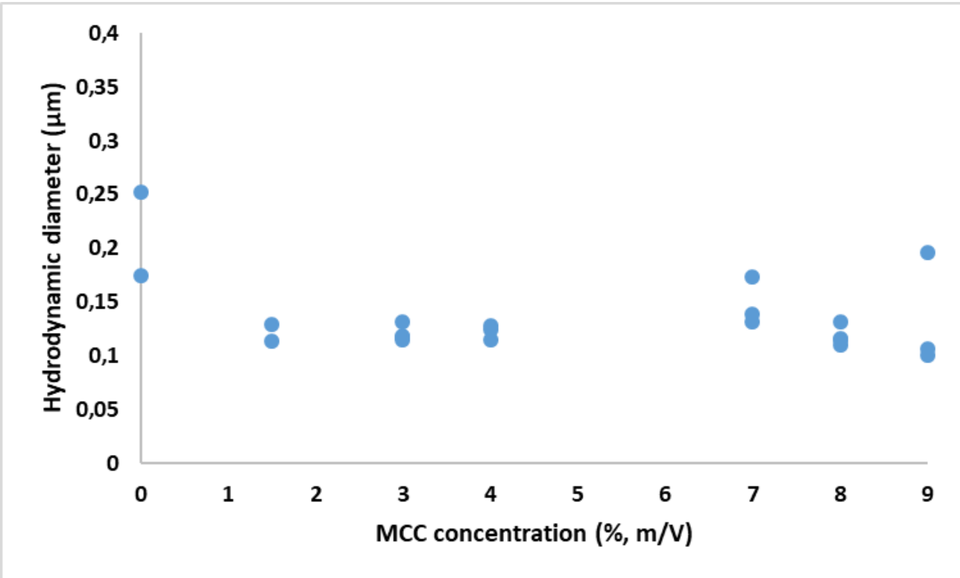

Figure 1 – Particle size, equivalent hydrodynamic diameters, measured by DLS for different MCC concentrations, after the serial filtration protocol (n=3).

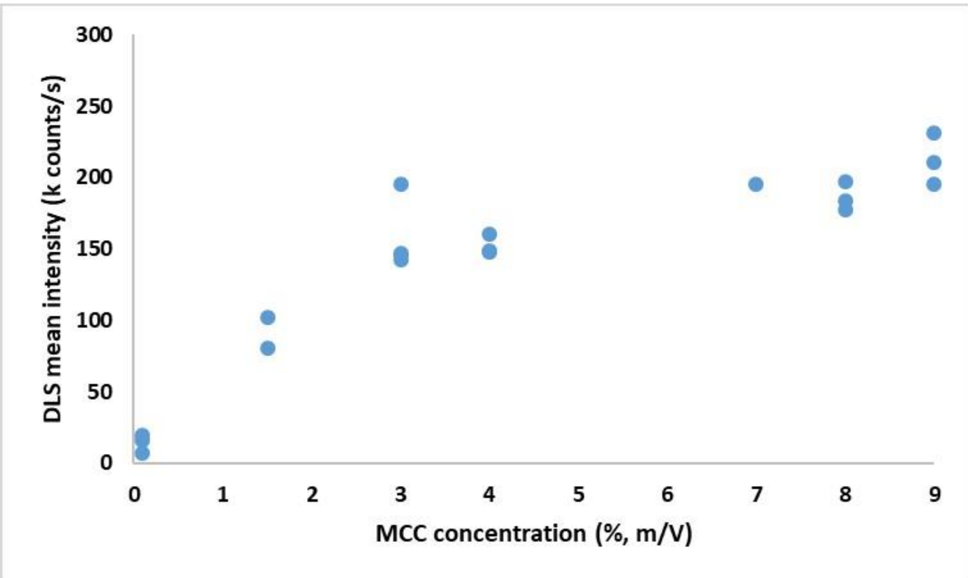

Figure 2 – Scattering intensity from DLS measurements for different MCC concentrations, after the serial filtration protocol (n=3).

TEM observations, carried out on the freeze dried supernatants from the last filtration (0.22 μm cut-off), confirmed both the presence of CNCs in MCC, and the dimensions estimated by DLS. Also the typical spindle shape (rod-like)? of the cellulose nanocrystals was revealed through TEM observations; the dimensions estimated from Figure 3 are approximately 150-250 nm in length and 20-50 nm in width; dimensions and aspect ratio are consistent with those, commonly measured on CNCs obtained by acidic hydrolysis.

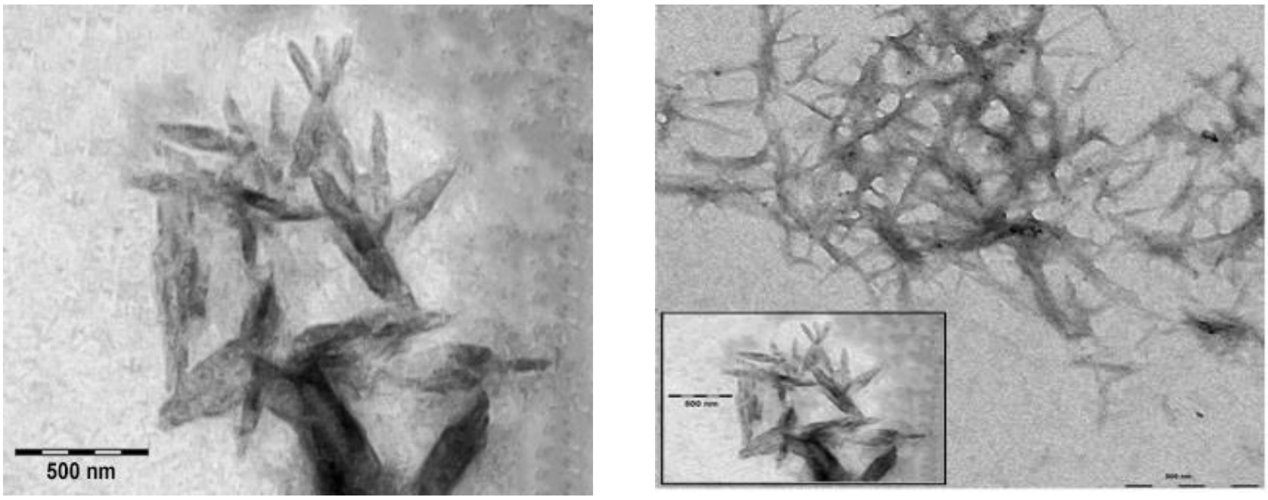

Figure 3–Representative 500 nm scale TEM images of primary size and morphology of CNCs revealed after serial filtration of MCC suspension.

The freeze-dried material obtained through the serial filtration has been also analysed by Fourier-transform infrared (FTIR) spectroscopy with a Perkin Elmer instrument (Spectrum 100), equipped with attenuated total reflectance (ATR) accessory, and the results (data not shown) confirmed the cellulosic nature of the isolated. First results, to be confirmed, revealed a concentration in the order of parts per million (ppm) of cellulose nanocrystals in the MCC samples tested.

Works are currently in progress in order to verify the possibility that additional amounts of nanocrystals might be produced from MCC by pH, time and temperature values, typical of gastric digestion. Moreover, a further fundamental undergoing research program is to find out an accurate procedure to estimate the CNCs amount in different media. In fact, it is worth reminding that a reliable procedure to assess quantitatively the CNCs, is an essential pre-requisite for planning migration tests of possible FCM which contain, as fillers or coatings, cellulose nanocrystals.

CONCLUSION

In conclusion, it should be considered the presence of CNCs in many foods and pharmaceutical products as a certainty; the foods we have been consuming so far contain traces of CNCs, to such an extent that this wide presence in consumed products should be taken into account when considering possible limitations of the use of these nanoparticles in FCM manufacture.

A thorough investigation is in progress in order to set up a reliable procedure able to quantify the concentration of the cellulose nanoparticles by means of a combination of electron microscopy, imaging techniques and other appropriate methodologies based on dynamic light scattering. The physicochemical characterization of such organic nanocrystals in terms of shape, dimensions and especially concentration and stability in different media represents a fundamental and challenging stage of the scientific assessment of the risk for the application of nanotechnologies in food and feed chain.

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