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# Beta-glucan complexes with selected nutraceuticals: Synthesis, characterization, and stability

Miroslav Veverka<sup>a</sup>, Tibor Dubaj<sup>b</sup>, Ján Gallovič<sup>a</sup>, Vladimír Jorík<sup>b</sup>,  
Eva Veverková<sup>c</sup>, Matej Mičušík<sup>d</sup>, Peter Šimon<sup>b,\*</sup>

<sup>a</sup> EUROFINS BEL/NOVAMANN Ltd., 940 02 Nové Zámky, Slovakia

<sup>b</sup> Faculty of Chemical and Food Technology, Slovak University of Technology, 812 37 Bratislava, Slovakia

<sup>c</sup> Faculty of Natural Sciences, Comenius University, 842 15 Bratislava, Slovakia

<sup>d</sup> Polymer Institute, Slovak Academy of Sciences, 845 41 Bratislava, Slovakia

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## ABSTRACT

The preparation of β-glucan complexes with representative FDA-approved nutraceuticals was investigated. The complex-forming nutraceuticals used were phosphatidylcholine, folic acid, boswellic acids, ascorbic acid, coenzyme Q10, quercetin, and curcumin. The complexes were characterized by infrared spectroscopy, differential scanning calorimetry (DSC), NMR spectroscopy, X-ray photoelectron spectroscopy (XPS), and X-ray powder diffraction (XRPD). The absence of crystalline form of the nutraceuticals in the complexes was confirmed by XRPD and DSC; absence of complex formers on β-glucan surface was confirmed by XPS. The results suggest deep penetration of complex formers into helices forming the β-glucan matrix. The stability tests have proven the stabilizing effect of β-glucan matrix on the complex formers towards light, oxidation, and higher temperature. This paper also introduces a new assessment method and the characterization of complexes using the line profile analysis of XRPD.

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## 1. Introduction

β-Glucans are polysaccharides encountered as constituents in various plants including oat, barley, seaweed, and also in some bacteria, yeast, and mushrooms. β-(1,3)-D-Glucans are composed of β-(1,3)-linked D-glucopyranosyl units forming the main chain, i.e. the spatial organization of glucan chains is governed by the glycosidic linkage pattern and the conforma-

tion. β-Glucans from various sources differ in their structure, solubility, conformation, and thus biological activity (Descroix, Ferrières, Jamois, Yvin, & Plusquellec, 2006; Gawronski, Park, Magee, & Conrad, 1999). Accordingly, the structural differences could affect both the β-glucan extraction and the biological activity (Yan, Allendorf, & Brandley, 2005). β-Glucan possesses several beneficial properties, including the ability to eliminate free radicals in a way similar to antioxidants (Kofuji et al., 2012). *In vivo* studies on diabetic rats found that β-glucan

\* Corresponding author. Tel.: +421 2 59325530; fax: +421 2 52926032.

E-mail address: [peter.simon@stuba.sk](mailto:peter.simon@stuba.sk) (P. Šimon).

Abbreviations: AA, ascorbic acid; BA, boswellic acids; BG, β-glucan; CF, complex former; CU, curcumin; DSC, differential scanning calorimetry; FA, folic acid; FDA, Food and Drug Administration; FTIR, Fourier transform infrared spectroscopy; PC, phosphatidylcholine; Q10, coenzyme Q10; QU, quercetin; XPS, X-ray photoelectron spectroscopy; XRPD, X-ray powder diffraction

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has an anti-inflammatory effect on the liver (Uskoković et al., 2013) and alleviates the oxidative stress (Mihailović et al., 2013). Peng et al. (2013) have shown that  $\beta$ -glucan-rich oat improves obesity, body fat, and serum parameters in rats. A review by Roupas, Keogh, Noakes, Margetts, and Taylor (2012) suggests that  $\beta$ -glucan is responsible for most of the beneficial health effects of edible mushrooms. Liu, Gunn, Hansen, and Yan (2009) revealed that the molecular size and complexity of  $\beta$ -glucan, more than the enrichment or the presence of only the  $\beta$ -(1,3)- or  $\beta$ -(1,6)-linkage, affect its interaction with human monocytes. As a result of broad studies, FDA (Food and Drug Administration) approved the usage of claims regarding the benefits of soluble fibre on health for foods containing oats.

The studies on  $\beta$ -glucan complexes are quite scarce. It was observed that  $\beta$ -glucan in dilute sodium hydroxide solution formed a complex with congo red where the presence of a hydrophobic interaction was suggested (Ogawa, Dohomaru, & Yui, 1994). Sakurai et al. (2005) suggested that  $\beta$ -(1,3)-glucans interact with some polynucleotides to form triple-stranded and helical macromolecular complexes consisting of two polysaccharide-strands and one polynucleotide-strand.  $\beta$ -Glucans from the cell wall of *Saccharomyces cerevisiae* have shown in vitro affinity to zearalenone. Both hydrogen bonds and van der Waals interactions were identified in the complex (Jouany, Yiannikouris, & Bertin, 2005; Yiannikouris et al., 2004). The complexes of tea polyphenols with oat  $\beta$ -glucan exhibited much higher activities of superoxide dismutase and glutathione peroxidase in liver than the corresponding physical mixture (Wu et al., 2011a, 2011b). Another study showed use of highly-porous  $\beta$ -glucan microparticles encapsulated with an antigen for drug delivery (Jamás, Ostroff, & Easson, 1990). The patent application (Kral et al., 2011) describes water-soluble  $\beta$ -glucan complexes with active pharmaceutical substances. The formation of water-soluble complexes is achieved by addition of an active pharmaceutical ingredient in an organic solvent to the solution ( $\approx 2\%$ ) of water-soluble glucan. The complexation of complex former (CF) on polysaccharides results in a change of its stability and release (Oidtmann et al., 2012; Tong & Wen, 2008; Xiong, Melton, Eastel, & Siew, 2006). Significantly enhanced bioavailability of curcumin-impregnated soluble dietary fibre dispersions based on galactomannan was reported by Krishnakumar et al. (2012).

The literature review indicates that  $\beta$ -(1,3)-glucans are very attractive materials not only as a food additive and a preventive therapeutic but also as a homopolymer for drug delivery (Ranade & Cannon, 2011). The unique properties of  $\beta$ -(1,3)-glucans undoubtedly originate from their inherent, very strong helix-forming character which has not been observed for other polysaccharides (Numata & Shinkai, 2011; Sletmoen & Stokke, 2008). On the other hand, it was reported that  $\beta$ -glucan is absorbed poorly, conjugated extensively, and its bioavailability is low under oral administration (Kishida, Sone, & Misaki, 1992; Sandula, Kogan, Kacurakova, & Machova, 1999). Phospholipids (e.g., phosphatidylcholine, PC) play a major role in drug delivery that can modify the solubility behaviour and rate of drug release for the enhancement of absorption across the biological barriers (Ting, Jiang, Ho, & Huang, 2014). The phospholipid complexes improve the water solubility when applied e.g. with flavonoids or curcumin (Giori & Franceschi, 2009; Semalty, Semalty, Singh, & Rawat, 2010; Singh, Rawat, Semalty,

& Semalty, 2012a, 2012b; Yaniu, Yunmei, Zhipeng, & Qineng, 2006).

The present study deals with the development of a BG:PC complex with the aim of improving the undesirable physico-chemical characteristics of  $\beta$ -glucan, e.g. poor absorption. Further complex formers applied were the representative GRAS (Generally Recognized As Safe, FDA) nutraceuticals, i.e., folic acid (FA), boswellic acids (BA), ascorbic acid (AA), coenzyme Q10 (Q10), quercetin (QU), and curcumin (CU). The  $\beta$ -glucan complexes were characterized by infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), NMR, X-ray powder diffraction (XRPD), and X-ray photoelectron spectroscopy (XPS). The protective effect of  $\beta$ -glucan matrix on thermal, light, and oxidation stability of complexed nutraceuticals was also investigated.

## 2. Materials and methods

### 2.1. Materials

$\beta$ -Glucan (93% purity) in micronized form was obtained as a gift sample from Natures Ltd. (Trnava, Slovakia). Phosphatidylcholine (97% purity) was obtained as a gift sample from Phospholipid GmbH (Cologne, Germany). The complex formers and all the solvents (analytical grade; methanol, ethanol, acetone, dimethyl sulphoxide, and dimethylformamide) were purchased from Sigma-Aldrich (Bratislava, Slovakia); all the chemicals were used as received.

### 2.2. Preparation of BG:PC complex

The BG:PC complex was prepared by stirring  $\beta$ -glucan (100 mg) and phosphatidylcholine in the weight ratio from 1:1 to 4:1. Both reactants were placed in a 10-ml round bottom flask containing 3 ml of the solvent (Method A: dimethylformamide, Method B: dimethyl sulphoxide, and Method C: water). The mixtures were vigorously stirred at 22 °C for 3 days, and then allowed at 5 °C for 12 days. Thereafter, the solvent (Method A) was removed by evaporation under reduced pressure. To the reaction mixture (Method B) water (4 ml) was added and then stirred for 6 h. To the reaction mixture (Method C) ethanol (3 ml) was added and then stirred for 6 h. All the complexes were isolated by centrifugation (6000 g, 20 min) and washed with  $3 \times 1$  ml of chilled ethanol (60%, v/v). The materials were dried under vacuum at 45 °C and stored in a desiccator. The IR spectra, XRPD, NMR, and DSC records confirmed identity of products obtained by the Methods A–C. For testing, the materials were scaled up following the same procedures.

### 2.3. Preparation of BG:CF complexes

To  $\beta$ -glucan (350 mg) in dimethyl sulphoxide (Method A, 8 ml) or water (Method B, 8 ml) a few drops of NaOH solution ( $0.2 \text{ mol dm}^{-3}$ ) were added to pH value 11.3–12.5. The mixture was sonicated for 60 min at 25–30 °C in an ultrasonic bath and then complex former was added (5:1 or 10:1, BG:CF, w/w). The reaction mixture was stirred for 20 min and then pH was adapted to 5.6–2.1 by an addition of HCl (3%, w/w) and the

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