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Characterisation of Composites of Bacterial Cellulose and Poly(vinyl alcohol) Obtained by Different Methods

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Abstract
Bacterial cellulose (BNC)/poly(vinyl alcohol) (PVA) composites of various component ratios were obtained by three different methods: in situ synthesis of cellulose by Gluconacetobacter xylinus using the modified Schramm Hestrin culture medium mixed with PVA and two ex situ methods. One of them was the impregnation of synthesised BNC with PVA solution at 80 ± 5 °C, and the second one was involved the additional sterilisation of BNC/PVA mixture at 120 °C in an autoclave. It was found that the highest polymer content and basic weight was obtained using the in situ method. Composites obtained by these three methods were characterised by intermolecular interactions of BNC and PVA, which confirmed the FTIR spectroscopy. SEM revealed the internal structure of the composites, where the BNC fibres were partially coated by PVA.

Key words: *bacterial cellulose, poly(vinyl alcohol), polymer composites, FTIR, SEM.*

Introduction

Bacterial nanocellulose (BNC) is a natural polymer obtained during acetic acid bacteria fermentation. In the biotechnological process, the aerobic *Gluconacetobacter xylinus* strain synthesises cellulose membranes using glucose and other nutrients. This type of BNC is quite different from plant celluloses. Generally it is characterised by high purity (free of hemicelluloses, lignin, and other functionalities such as carbonyl or carboxyl groups), a high degree of polymerisation (up to 8,000) and a high crystallinity degree (80 – 90%) [1, 2]. Depending on the biosynthesis process, different weight ratios of the cellulose nanofibres and water can be obtained. Generally it is assumed that BNC membranes (hydrogels) contain from 1 to 5% of pure cellulose nanofibres and around 99 - 95% of water.

Owing to the high water content, bacterial nanocellulose gives a cooling sensation during application as a bandage. Moreover it does not cause any allergic reaction nor skin irritation, which makes it interesting for medical usage. Till now, BNC materials have found application in the medical treatment of burn wounds, diabetic foot and trophic wounds (hard to heal) [3 - 6]. BNC is also used in the food industry as a rich source of dietary fibre, which is common especially in the Asia as a sweet dessert called Nata de coco, where bacterial cellulose is properly modified [7, 8]. The particular internal structure of cellulose material allows to obtain speaker membranes, headphones and membranes used in filtration processes [9]. Modifications of cellulosic material give the possibility of using this type of composite in papermaking [10, 11].

Apart from the advantages due to the material structure and purity, the modification of BNC is necessary for new expected applications. This can be related to a change in the form and shape of the performed film and polymer structure. The shape of the BNC hydrogels can be designed effectively by choosing an appropriate reactor form. Usually fleeces of several centimeters high, films, spheres, and hollow bodies can be produced [12]. Nowadays material in the form of tubes is demanded for its application in the cardiovascular system as veins, nerve endings or supports for other organs. Moreover modified cellulose material is under investigation for cartilaginous material, as in [13, 14].

BNC composites can be divided according to the type of modifier added (organic, inorganic) or application (biomedical, industrial, optical or electronic material) [15]. Lately BNC based composite materials with improved mechanical and biological properties as well as electrical conductivity and magnetic properties have been produced.

Composites can be performed by *in situ* or *ex situ* modification of BNC material. Typical composite components are organic compounds such as bioactive agents, monomers, polymers (e.g. polyacrylates, resins, polysaccharides and proteins) as well as inorganic substances (metals and metal oxides). In the *in situ* method, the modifying material is added to the culture media which is built-in to the BNC structure, forming a composite during synthesis. In the *ex situ* technique, the modifying material is added to a previously synthesised BNC matrix, usually after the purification process.

BNC composites for medical application often contain chitosan (CS) or other polysaccharides as the second component. Chitosan, which is characterised by antimicrobial properties, has found application as an absorber of wound exudates, tissue scaffolds or drug delivery systems. Due to the similar structure and the presence of hydroxyl and amines groups in CS, strong matrix – filler interactions occur, which improves the physical, mechanical and biological properties of such biomaterials [13, 16].

Other types of BNC composites can be obtained after the immersion of BNC material into the gelatin solution, giving not only good mechanical properties but also high elasticity to the material. For example, a fracture strength and elastic modulus under the compressive stress of several orders of magnitude higher were obtained for BNC/gelatin in comparison to neat gelatin. These types of materials have found application in tissue engineering as scaf-

folds because they efficiently promote chondrocyte adhesion and proliferation cells [17]. More information on the various BNC composites with other natural fillers such as collagen, alginate, gellan gum andi-carragenan can be found in recent literature [18, 19].

BNC fibres are also used for the reinforcement of the synthetic polymers. An example of such a blend is BNC/poly(ethylene glycol) (PEG), which can be obtained during *in situ* or *ex situ* synthesis. Moreover the addition of hydroxyapatite leads to structure enhancement. BNC/PEG has found application as soft tissue replacement implants and scaffold for bone regeneration. Described in another work, BNC/poly(ethylene oxide) composites, characterised by high biocompatibility and elasticity, found application in medicine as drug delivery systems, surfactants and hydrogels.

The aim of our work was to obtain BCN composites with poly(vinyl alcohol), (PVA) for potential application as wound dressing and to select the optimal method of preparation. The properties and structure of materials obtained by three different methods were characterised by ATR-FTIR spectroscopy and scanning electron microscopy (SEM). The reaction efficiency and water content was determined by the gravimetric method.

PVA is a very popular, water-soluble polymer characterised by biocompatibility, nontoxicity and good mechanical properties. BNC/PVA composites described in previous works were designed for other biomedical applications such as materials for tissue reconstruction, drug delivery or cardiovascular stents [20 - 22]. However, there is no article describing blends obtained at higher temperatures necessary for sterilisation. The technological methods presented, used for composite preparation, are simple and possible to implement in industry. The effective combination of BNC fibres with poly(vinyl alcohol) allows to obtain a composite material that has good strength (feature of bionanocellulose) and flexibility (feature of PVA). Because BNC/PVA composites have similar mechanical properties to natural cardiovascular tissue it can be used as a material for aorta and heart valve leaflets. There is sparse information on cultivation conditions for the final efficiency and properties of BNC.

Materials and methods

Polymer

Poly(vinyl alcohol) PVA (M_w = 30,000 – 70,000) with a hydrolysis degree of 87 - 89% supplied by Aldrich was used for BNC composite preparation.

Microorganism and culture media

Gluconacetobacter xylinus E_{25} *strain,* supplied by Bowil Biotech Sp. z o.o. (Poland), was used to prepare cellulose membranes. A culture medium with the following volume content: 2.0% of glucose (as a carbon source), 0.5% of yeast extract, 0.115% of citric acid, 0.27% of disodium phosphate, and 0.05% of magnesium sulfate was used for BNC membrane preparation. This mixture was a modified Schramm Hestrin culture medium (SH). The pH was adjusted to 5.75 ± 0.03.

Preparation of bacterial cellulose (BNC)

Gluconacetobacter xylinus from the agar plate was inoculated in Schramm Hestrin medium (SH) placed in a conical flask. The culture medium was previously sterilised at 121 °C for 20 minutes. After the inoculation process, which took 2 days and was performed in an incubator at 30 ± 2 °C, the culture medium with bacteria in an amount of about 5% volume was transferred to the next culture medium.

The fermentation process was carried out in a container with a capacity of 0.05 m^2 filled with an SH medium containing 5 - 10% of inoculum up to 1/3 of the volume. The process took three days and was carried out in an incubator with gravity convection at 30 ± 2 °C). After this period, a thin homogenous BNC membrane was formed on the SH medium surface.

A purification process of the cellulose matrix obtained was carried out in a few stages. At the beginning the membrane was extensively washed by water in order to remove the residue of the culture medium. After that, the film was boiled for 1 hour in a 1% sodium base, neutralised by acetic acid and finally rinsed by deionised water till neutral pH. The final step was to remove excess water. In this way, each film had the same thickness of about 2 mm and a similar content of water [23].

Preparation of bacterial cellulose/poly(vinyl alcohol) composites by the *in-situ* method

A BNC/PVA composite was produced by the addition of PVA polymer to the culture media. The process was carried out in the same way as described before for BNC, with the only difference being the composition of the culture medium, which additionally contained PVA dissolved in water.

Samples of different BNC : PVA ratio were obtained using the following formulations:

- 2% glucose in 100 ml of culture medium (SH) + 1% PVA. The ratio of glucose to poly(vinyl alcohol) was 2:1.
- 2% glucose in 100 ml of culture medium (SH) + 2% PVA. The ratio of glucose to poly(vinyl alcohol) was 1:1.
- 2% glucose in 100 ml of culture medium (SH) + 4% PVA. The ratio of glucose to poly(vinyl alcohol) was 1:2.

Preparation of bacterial cellulose/poly(vinyl alcohol) composites by *ex-situ* methods

The same type of PVA in the form of powder was added to deionised water at a 2.0 w/v% concentration, and then the mixture was heated in an autoclave at 121 °C for 20 minutes till the dissolution was completed. The PVA solution obtained was used for the preparation of composites with BNC by impregnation or repeated sterilisation.

Impregnation - The purified BNC pellicles were immersed in the PVA solution and mixed by a magnetic stirrer at 80 ± 5 °C for 2 h. Then the BNC membrane in the polymer solution was stored at room temperature for 12 h.

Sterilisation - The purified BNC pellicles were immersed in the PVA solution, heated in an autoclave at 121 °C for 20 minutes and kept at room temperature for 12 h.

The BNC/PVA pellicles obtained were transferred into deionised water for 30 min to remove excess PVA from the sample surface. Afterwards the BNC/PVA composites were dried in an oven at 45 ± 2 °C for 12 h to a constant weight.

In the *ex situ* method aqueous solutions of poly(vinyl alcohol) were used in appropriate concentrations: 1, 2 and 4%.

Attenuated total reflectance fourier transform infrared (ATR-FTIR) spectroscopy

ATR-FTIR spectra of dried films of PVA, BNC and composites were recorded using a Genesis II spectrophotometer (Mattson, USA) equipped with an ATR device – MiracleTM Pike Technologies containing zinc-selenide crystal. The spectra resolution was 4 cm^{-1} and 64 scans were accumulated.

Scanning electron microscopy (SEM)

The samples were gold sputter coated and their surfaces were observed with scanning electron microscopy LEO 1430 at an accelerating voltage of 10 kV and at various magnifications. A secondary electron (SE) detector, enabling very fine detail to be resolved, was used. The most representative pictures were taken and reported in the article.

Results

General remarks and visual observation of the samples

All of the BNC samples used for both *ex situ* modifications: impregnation and sterilisation were obtained from the same production process. The bionanocellulose membrane formed was cut to the proper dimensions. A composite material BNC/PVA obtained by in the situ method was produced under standard culture conditions. The process was conducted in an incubator for three days at 30 ± 2 °C, in which the Schramm-Hestrin culture medium used for the process additionally contained a suitable concentration of PVA: 1, 2 and 4%.

After drying, BNC and its composites had a different appearance. The neat BNC membrane was turbid, with an evenly milky - white colour and smooth structure. The PVA addition caused an increase in the transparency - the sample with 4% of PVA solution was clear but rather rough. Moreover samples with a PVA addition were less fragile, while the neat BNC was brittle and resembled a piece of paper. These kinds of differences were observed for materials obtained by *in situ* and *ex situ* methods with sterilisation. In the case of samples attained by the *ex situ* method with the impregnation all the samples: neat BNC and BNC/PVA composites, they had a similar appearance without any visible differences.

No phase separation was visible with the naked eye, the internal structure only being observed with the aid of a microscope.

Table 1. Effect of poly(vinyl alcohol) addition by various methods on the BNC/PVA's basic weight and polymer content.

Addition PVA, g/100 ml	Method of composite preparation					
	in situ		*ex situ* (sterilization)		*ex situ* (impregnation)	
	Basic weight, g/m²	Polymer content, %	Basic weight, g/m²	Polymer content, %	Basic weight, g/m²	Polymer content, %
0	25.56	2.38	24.41	2.26	20.83	1.45
1	38.48	3.39	33.15	2.69	24.91	1.46
2	48.08	4.55	37.24	3.20	24.60	1.63
4	71.60	6.92	44.81	4.28	24.80	1.73

Effect of PVA addition on the total polymer content in composites and their basic weight

The basic weight and the total polymer content in the neat BNC membrane and its composites with PVA obtained by different methods were compared and the results are summarised in ***Table 1***.

The basic weight (i.e. grammage) of the samples increased with the concentration of PVA for the methods applied. The same tendency was observed also in the case of the percentage of polymer content in the composite. Exceptions are the values obtained by *ex situ* – impregnation, where no such relation between the PVA concentration and grammage exists.

Comparing these data, one can state that the *in situ* method, where the PVA solution was added directly to the culture medium, and the *ex situ* method combined with sterilisation in an autoclave

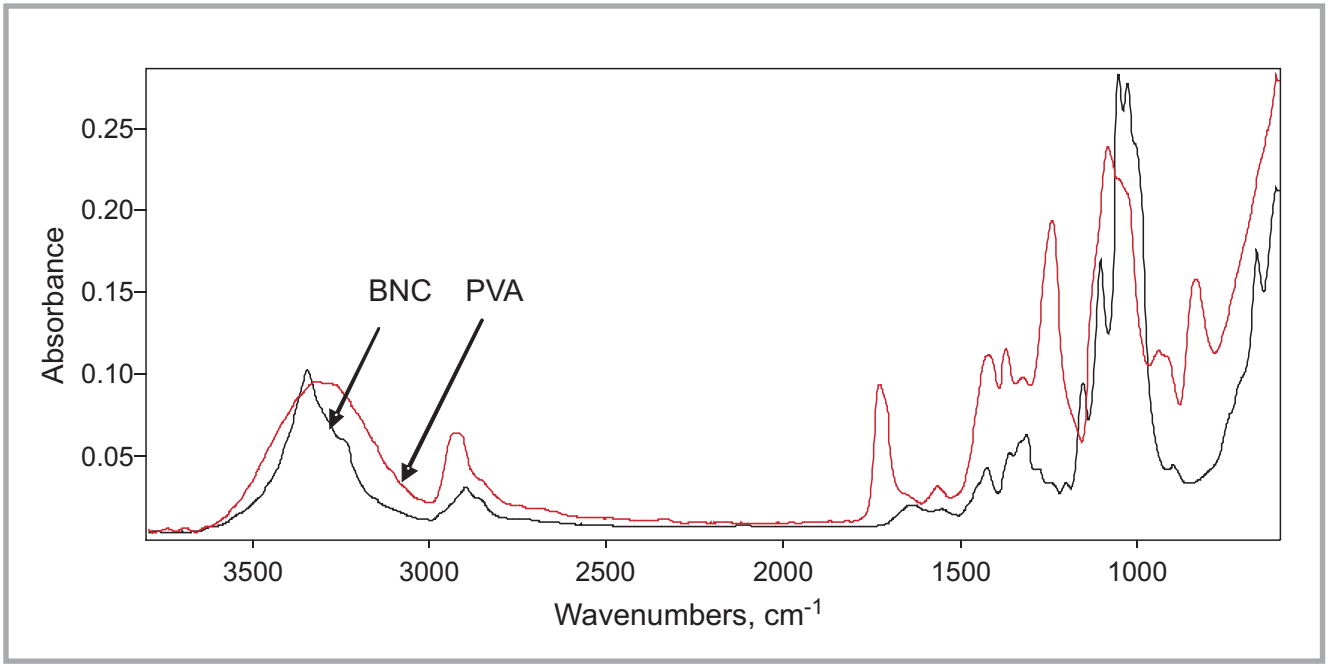

Figure 1. *Comparison of ATR-FTIR spectra of neat PVA and BNC.*

Table 2. *Characteristic absorption bands in FTIR spectra of PVA, BNC and their composites (wave numbers taken from spectra obtained in this work, assignment based on references).*

Sample	Chemical group	Wavenumber, cm^{-1}		References
		Range	Maximum	
PVA	O–H (stretching) involved in intermolecular and intramolecular hydrogen bonds	3500 - 3000	3320	[24, 26-28]
	C–H (stretching) from alkyl groups	3000 - 2800	2930	
	C=O (stretching) residues of acetate groups	1775 - 1660	1731	
	C–O–C (stretching)	1150 - 1035	1084 1049	
	CH_2 (deformation)	1500 - 1158	1420 1370 1325 1245	
BNC	O-H (stretching)	3400 - 3300	3345	[22, 24]
	C-O (stretching)	1080 - 1015	1052 1030	
BNC/PVA	O-H (stretching)	3500 - 3240	3340 3305	[22]
	C-O (stretching)	900 – 1100	1055 1030	

were more effective than impregnation at 80 ± 5 °C.

ATR FTIR spectroscopy

Figure 1 presents FTIR spectra of the composite components: PVA alone and BNC films. It is clearly seen that the main differences appear in the hydroxyl and carbonyl regions. Spectra of PVA and BNC exhibit an intensive band at 3000 - 3500 cm^{-1} due to the presence of OH groups. However, the band at 3320 cm^{-1} observed in the PVA spectrum is much broader, which indicates the hydrogen bond's formation.

The narrower OH band (with the maximum at 3345 cm^{-1}) in the BNC spectrum proves the dominance of the free hydroxyl groups, which is in agreement with the literature data [22, 24]. The carbonyl band is present only in the PVA spectrum, which is due to the incomplete hydrolysis of this polymer, derived from poly(vinyl acetate). The second band existent in the PVA (but not in BNC) spectrum is observed at 836 cm^{-1} , which can be attributed to CH_2 deformation vibrations, typical for vinyl polymers.

The other absorption bands appear in similar ranges, notwithstanding evident marked shifts. For instance, the maximum position of the C-O double band in BNC appears at 1052 and 1030 cm^{-1} (typical for the presence of aliphatic primary and secondary alcohols from cellulose), while the main peak in this range for PVA exists at 1084 cm^{-1}. The low intensive band at 1637 cm^{-1} occurs in all samples containing BNC (but does not exist in PVA alone), which is due to water absorbed by cellulose [25].

The assignment of the most important absorption bands observed in the spectra of the samples studied is shown in ***Table 2***.

FTIR spectra of BNC/PVA composites contain bands characterising both individual polymers. There were no distinct differences for samples obtained by different methods, thus exemplary spectra for the BNC/PVA composites prepared *in situ* are presented in ***Figure 2***.

The shape of the hydroxyl band in the spectra of BNC/PVA is similar to that observed in BNC. However, it is broader, which confirms the intermolecular interaction between hydroxyl groups of PVA and BNC. The band between 2800 and 3000 cm^{-1} is related to the stretching of the aliphatic C-H bond [22]. The band at 1731 cm^{-1} is assigned to carbonyl groups, as a residue of non-hydrolyzed acetate groups of PVA. The intensive absorption at 900 – 1100 cm^{-1}, appearing in the spectra of all samples studied, is due to the presence of C-O stretching vibrations. The intensity of bands characteristic for BNC decreases regularly with increases in the PVA content in composites in all cases. Although it is difficult to draw quantitive conclusions on the base of ATR-FTIR spectra, this observation indicates the effectiveness of all obtaining methods.

It should be pointed out that in all the spectra of the composites (irrespective of the obtaining methods), carbonyl (stretching) and methyl (deformation) bands were found, which clearly indicates the presence of PVA mixed with BNC in spite of washing the composite samples with water.

Crystallinity index (CI)

The crystallinity index for the BNC material tested was evaluated on the basis of spectroscopy analysis. Absorbance bands at 1430 cm^{-1} are related to the crystallinity content of cellulose and is called the 'crystallinity absorption band'. The crystallinity content was calculated as the absorbance ratio for the bands at 1372 cm^{-1} (C–H deformation in CH_3 or OH in plane bending) to 2900 cm^{-1} (CH stretching of CH_2 and CH_3 groups) and an absorbance ratio of 1430 cm^{-1} (CH_2 bending or OH in plane bending) to the band at 890 cm^{-1} (glucosidic linkages between the sugar units) [29].

The following average values calculated for several spectra were found :

$$A_{1430}/A_{890} = 1.45 \text{ and}$$

$$A_{1372}/A_{2900} = 0.78$$

These values obtained for pure BNC are very similar to those for commercially available microcrystalline cellulose (respectively, 1.40 & 0.73) [25].

It should be added that CI cannot be calculated for the composites because the crystallinity bands of cellulose and CH_2 deformation bands of PVA overlap.

Scanning electron microscopy

SEM images of BNC reveal a typical fibrous structure (***Figure 3.A***, see page 74), while PVA alone exibits a flat homogeneous surface, without any defects (invisible). The average diameter of cellulose fibres determined from SEM images ranged from 50 to 100 nm.

The morphology of BNC/PVA composites depends on the synthesis method, which exhibits the structural patterns presented in ***Figures 3.B, 3.C*** and ***3.D***. *In situ* synthesis leads to very good mixing of both components, resulting in less visible fibres and a completely flat surface area (***Figure 3.B***). This effect is related due to the strong interactions and formation of the hydrogen bond between PVA and BNC. In ***Figures 3.C*** and ***3.D***, illustrating the morphology of composites prepared by the *ex situ* method, a mi-

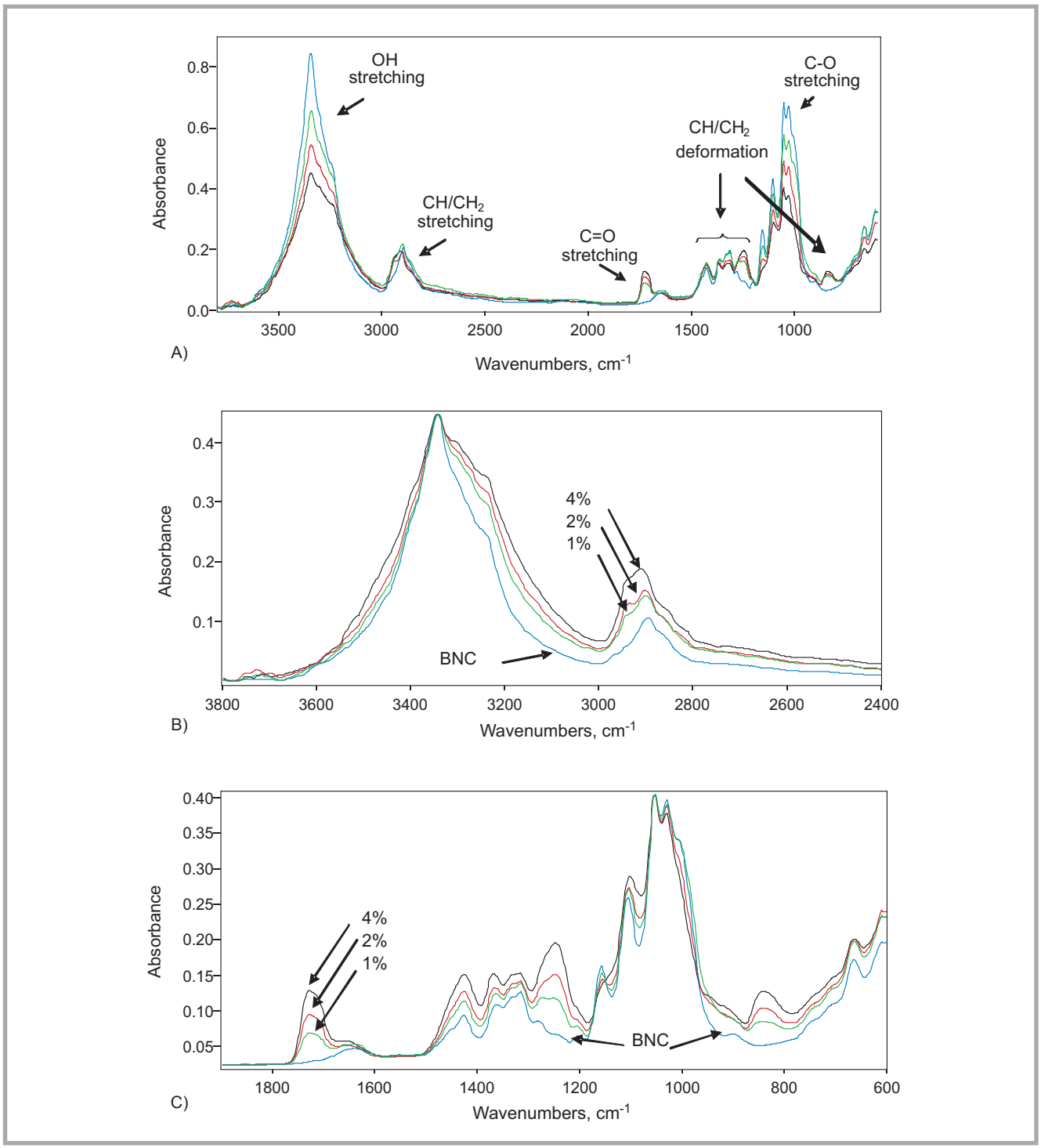

Figure 2. *FTIR spectra of BNC and BNC/PVA blends (obtained by addition of 1%, 2% and 4% PVA solution); A –non-normalized spectra, B and C – normalised spectra in the range of 2400-3800, and 600-1900 cm^{-1}, respectively.*

crofibrilal structure is also present, but fibres are partially coated or even "stuck" by PVA. Such connections between BNC fibrils and PVA macrochains prevent phase separation, typical for other immiscible polmeric systems.

Summary and conclusions

Three different methods were applied for preparation of BNC/PVA composites, all of which led to well mixed polymeric systems. However, taking into account the basic weight and total polymer content, it can be concluded that the most efficient is the *in situ* method, in which PVA is a component of the culture medium. ATR-FTIR spectroscopy confirms the presence of PVA in composites obtained also by *ex situ*. Good results were also obtained by the *ex situ* method combined with sterilisation.

The advantage of BNC/PVA blends is higher transparency comparing to BNC alone. BNC forms a network of entangled fibres, which exists in all composites. SEM images reveal the coating of BNC fibrils by PVA due to the strong intermolecular interactions between both polymers. Moreover the samples were not brittle, thus BNC/PVA composites can be recommended as a good material for dressing production.

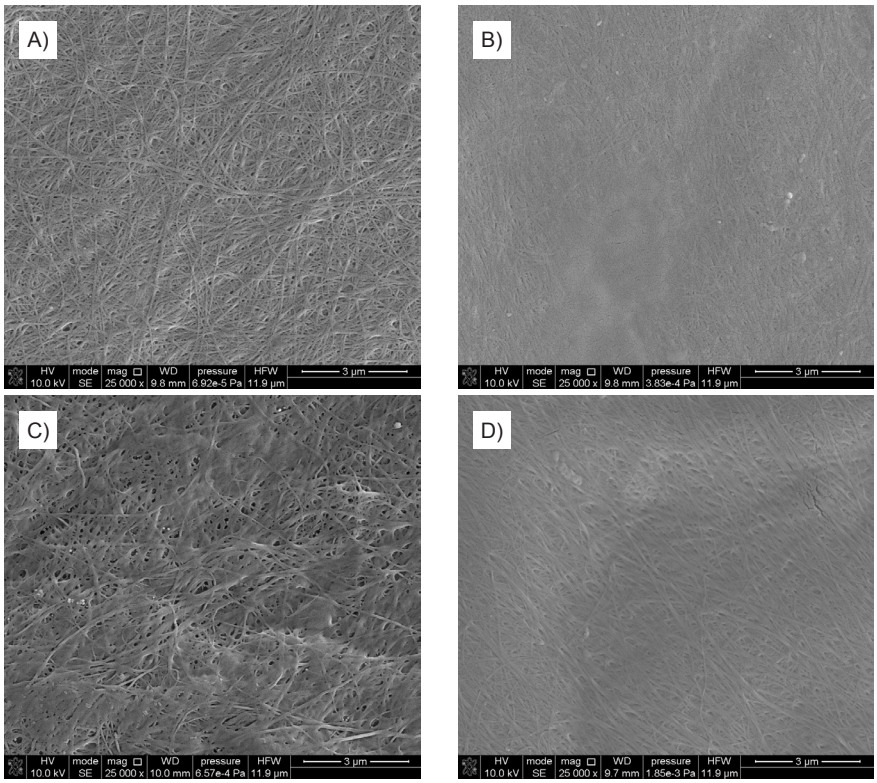

Figure 3. *SEM images of BNC (A) and BNC/PVA composites obtained by different methods: in situ (B), impregnation (C), sterilisation (D), magnification - 25,000×.*

The future application of materials based on this type of cellulose and their further development can be predicted.

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