

Rapid Communication

Infrared to Visible Up-conversion in Biocellulose–yttrium Vanadate Nanoparticle Composite Membranes. Demonstration of Chloroaluminum Phthalocyanine Light Emission Under Up-converted Light Excitation

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ABSTRACT

YVO₄:(Yb³⁺–Er³⁺/Ho³⁺) nanoparticles were incorporated in biocellulose membranes obtained from *Gluconacetobacter xylinus*. Materials present the property of converting near-infrared (NIR) into higher-energy visible light. Nanoparticles were prepared by optimizing towards higher emission intensity at the absorption wavelength range of chloroaluminum phthalocyanine (ClAlPc) used as a photosensitizer in the photodynamic therapy. The NIR excitation wavelength is advantageous for biological applications, as it allows deeper penetration into tissues than the UV–visible radiation commonly used for luminescence excitation. Up-conversion emission spectra obtained under excitation at 980 nm showed a preferential green emission for the Yb³⁺–Er³⁺ system and a red emission for the Yb³⁺–Ho³⁺ one. In the last case, by using mixtures of nanoparticles and ClAlPc the red emission (680 nm) of the phthalocyanine was observed through excitation by the up-converted emission of the nanoparticles (650 nm) which were excited in NIR (980 nm).

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Near infrared to visible up-conversion is a well known phenomenon presented by materials containing lanthanide ions [1]. The efficiency of the up-conversion process can be high enough to be observed with naked eyes under moderate excitation power for materials containing a pair of lanthanide ions as Yb³⁺–Ln³⁺ (Ln = Er, Tm, Ho) [1–3].

Up-conversion emission in lanthanide doped materials has attracted great interest in the last 50 years and is well known for its numerous applications in light emitting devices. Up-conversion nanoparticles (NPs) could also be employed in new applications, such as the use of nanomaterials in biomedicine, which offers excellent prospects for the development of new noninvasive strategies for the diagnosis and treatment of cancer [2,4].

Up-conversion NPs can be also used as fluorescent bioprobes due to their photo-stability and low toxicity [3]. The advantages for *in vitro* and *in vivo* optical bio-imaging applications rely on the absence of photodamage to living organisms, very low auto-fluorescence background, high signal-to-noise ratio, detection sensitivity and high light

penetration depth in biological tissues [3,5]. Moreover, multiplexed biological labeling can be achieved conveniently under a single wavelength excitation by changing activator ions and relative concentrations. Capabilities for the simultaneous imaging and tracking of multiple molecular targets are therefore extended, allowing the classification and differentiation of complex human diseases [6].

Up-conversion nanoparticles have attracted focus on the photodynamic therapy (PDT) as well [6,7]. PDT is a minimally invasive treatment of neoplastic and non-neoplastic diseases and is a well-established technology that relies on the absorption and retention of a photosensitizer molecule in tumor cells associated with further irradiation with an appropriate visible light. Upon irradiation, the photosensitizer is promoted from its ground singlet state to its triplet excited state leading to reactive oxygen species [8,5].

The development of drug delivery systems for PDT is an increasing demand due to the hydrophobicity presented by most of the photosensitizers. New drug delivery systems have improved the therapeutic and toxicological properties of existing chemotherapeutic agents and fostered the implementation of new agents. By combining drugs with different polymers, it is possible to modify the pharmacokinetics and biodistribution, improving the efficacy and safety of the therapy. A wide assortment of biomaterials with suitable biological properties is

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offered, but considerable attention has been drawn to green, natural polymers like bacterial cellulose (BC). BC is a highly pure form of cellulose produced in the form of a swollen membrane. BC or biocellulose is a polysaccharide of glucose produced by *Gluconacetobacter* sp. that is superior to plant cellulose due to its purity and nano-morphology. BC presents high water-holding capacity, large surface area, high crystallinity besides being renewable, biocompatible and biodegradable [9,10]. The incorporation of organic/inorganic compounds in its structure is possible due to the network of ribbon-shaped nanosized cellulose fibrils and water presence. A number of studies in the literature report the successful use of BC membranes in biomedical applications due to their unique physical and mechanical properties [11–13]. Such membranes are particularly advantageous in the topical drug delivery systems that have the ability to absorb exudates and adhere to irregular skin surfaces, such as the oral mucosa. As the majority of transdermal patches are manufactured by superimposing different materials, a system composed of fewer or even a single layer, such as a BC film, could simplify the preparation procedure and lower production costs [14]. Moreover, a previous study reported the good skin tolerance of BC membranes [15]. Chitosan, which is chemically related to cellulose and plant cellulose nanocrystals, has been studied by Schmitt et al. [16] and Drogat et al. [17] in the photodynamic therapy. In view of these facts, the development of new delivery systems that can efficiently deliver CIAIPc could enable its clinical use for topical PDT.

BC membranes may be produced with different characteristics in order to select the most appropriate for the intended application. The structural properties of the membranes, e.g. interstitial spaces between the cellulose fibrils and thickness, are factors that influence loading efficiency into the polymeric matrix. The adequate interstitial spaces size must be high enough to ensure gaseous and fluid exchange and diffusion of drug to the external environment [18].

The aim of the present study is to describe the preparation of BC membranes containing YVO₄ NPs doped with the lanthanide ions Yb³⁺–Er³⁺ and Yb³⁺–Ho³⁺. The preparation of these nanoparticulate systems is well described in the literature [19,20] and the main goal was to obtain the property of energy upconversion from the infrared to the visible range, usually used to activate the photosensitizer molecules. The NIR excitation wavelength is advantageous for application in biological systems, as it allows deeper penetration into tissues than the UV or visible radiation commonly used for luminescence excitation. The emission properties were optimized in order to use the particles and membranes as a setup to activate photoprocesses useful for the treatment of neoplastic and non-neoplastic diseases susceptible to the photoactivation process. Chloroaluminum phthalocyanine (CIAIPc) [chloro(29H,31Hphthalocyaninato) aluminum] was used here. It belongs to the second generation of sensitizer molecules used in the photodynamic therapy (PDT) and other photodynamic processes (PDP). It is a tetrapyrrolic macrocycle that has nitrogen atoms linking the individual pyrrole units and a central metal ion (Al III) [21].

NPs and the composite biocellulose membranes (BC-NPs) were characterized in terms of morphological, structural and emission properties. YVO₄ NPs doped with the lanthanide ions Yb³⁺–Er³⁺ and Yb³⁺–Ho³⁺ were obtained as white powders. Fig. 1(A) displays a typical SEM image of YVO₄ NPs doped with the lanthanide ions Yb³⁺–Er³⁺. Particles with sizes below 100 nm can be observed together with larger agglomerates of particles. X-ray diffraction patterns (Fig. S2, Supplementary information) confirm the preparation of the tetragonal YVO₄ (JCPDS file 17-0341) [22]. The nanoparticles primary sizes could be estimated around 30 nm from the full width at the half-maximum of the diffraction peaks (200) at 25° by using the well-known Scherrer equation. Small quantity of cubic Y₂O₃ (JCPDS file 41-11-5) could also be detected. Fig. 1(B) shows the image of the BC membrane after incorporation of the particles (BC-NPs). Particles and agglomerates can be observed well wrapped by cellulose fibers. Interestingly enough, membranes are macroscopically homogenous, white in color and with no loose powder observed at the surface. One important characteristic of BC hydrated membranes concerns the property of incorporation of particles that end up incarcerated and attached to the cellulose structure by the nanometric cellulose fibers. Moreover, the 3D nanometric structure leads to a large surface area for particle stabilization [23].

Fig. 2 shows the up-conversion emission properties of the NPs of YVO₄:Yb³⁺:Ho³⁺ and YVO₄:Yb³⁺:Er³⁺ as a function of laser power at 980 nm. In Fig. 2(A) bands are observed in the spectral regions of 520–560 nm and 620–680 nm, corresponding to the Ho³⁺ transitions ⁵S₂ → ⁵I₈ and ⁵F₅ → ⁵I₈ [22]. In Fig. 2(B) bands are observed in the spectral regions of 515–565 nm and 640–680 nm corresponding to Er³⁺ transitions: ²H_{11/2} → ⁴I_{15/2}, ²S_{3/2} → ⁴I_{15/2}, and ⁴F_{9/2} → ⁴I_{15/2} respectively [20]. In the process of up-conversion emission, the intensity of emission generated (I_E), increases non-linearly, with the pumping intensity (I_{ex}), i.e., I_{CAE} ∝ (I_{ex})ⁿ, where *n* is the number of photons in the infrared that are sequentially absorbed by Yb³⁺ and transferred to Ho³⁺ and Er³⁺ ions leading to emission of one visible photon. The value of *n* is necessarily an integer, however, non-radiative processes such as thermal effects and size effects may influence and contribute to decrease the slope to lower values. The density of the excitation energy is high enough to promote a high absorption of the sample and raising the temperature in the irradiated region, inducing thermal effects that can result in lower emission intensity. The effect is more important for smaller crystal sizes [24]. Concerning Ho³⁺ emission bands at ~540 nm (⁵S₂ → ⁵I₈) and ~650 nm (⁵F₅ → ⁵I₈), the values of *n* were determined to be 1.69 and 1.74, respectively, which suggests two-photon processes for excitation of green and red emissions. These results are coherent with similar work reported in the literature [22]. For Er³⁺ bands at ~525 nm (²H_{11/2} → ⁴I_{15/2}) and ~550 nm (⁴S_{3/2} → ⁴I_{15/2}) values of *n* equal to 2.93 and 2.59, respectively, are obtained indicating that three photons are absorbed. Three photons are indeed observed for the green emission in Yb³⁺:Er³⁺ [23–25]. Higher concentrations of Yb³⁺ (20% molar ratio in our case) tend to activate the three-photon process

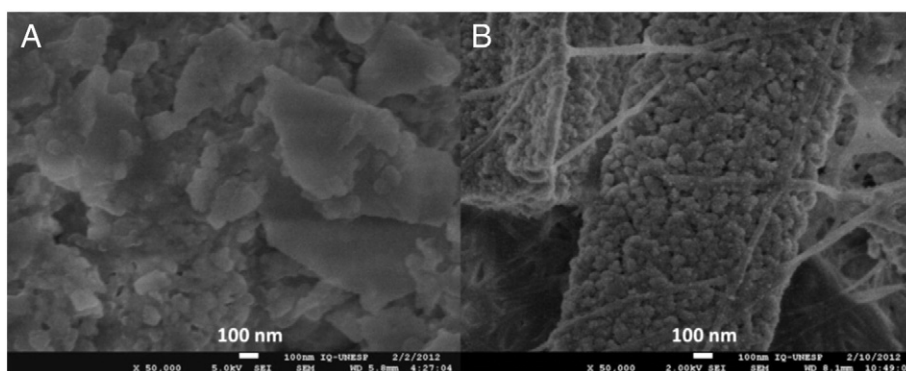


Fig. 1. SEM images of YVO₄ NPs doped with the lanthanide ions Yb³⁺–Er³⁺ (NPs) (A) and dried biocellulose membrane containing these NPs (BC-NPs) (B).

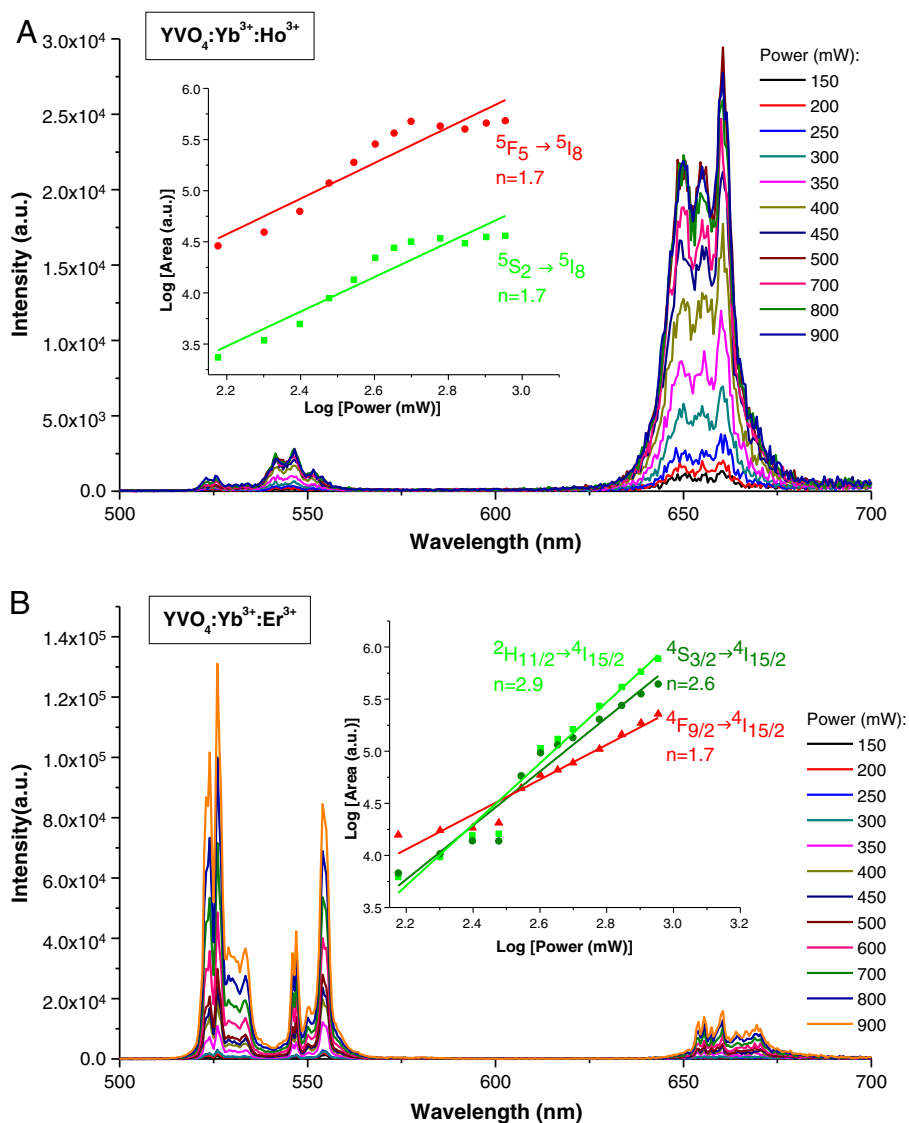


Fig. 2. Up-conversion emission spectra obtained by pumping at 980 nm at different powers. NPs of $\text{YVO}_4:\text{Yb}^{3+}:\text{Ho}^{3+}$ (A) and $\text{YVO}_4:\text{Yb}^{3+}:\text{Er}^{3+}$ (B).

to populate levels $2H_{11/2}$ and $4S_{3/2}$. The absorption of a third photon excites the Er^{3+} from the $4F_{9/2}$ excited level to the $2H_{9/2}$ upper level and nonradiative relaxation populates $2H_{11/2}$ and $4S_{3/2}$ levels from which the green emission is observed. The n value obtained for the Er^{3+} emission at 660 nm ($4F_{9/2} \rightarrow 4I_{15/2}$) ($n = 1.67$) corroborates the results obtained in [20] for a two photons process. BC-NP membranes exhibit the very same up-conversion emission properties. As already mentioned, up-conversion membranes could find several different applications including the one discussed hereafter.

The up-conversion emission intensity from the different samples containing different relative quantities of Er^{3+} and Ho^{3+} (not shown here) were compared with respect to the emission band in the red region. That is the region of absorption of the photosensitizer of interest. In this sense the sample $79\text{YVO}_4:20\text{Yb}^{3+}:1\text{Ho}^{3+}$ was observed to exhibit the best red light output under 980 nm excitation.

Fig. 3 displays the absorption spectrum of CIAIPc in ethanol solution. Main bands are observed at 351 nm and 671 nm, attributed to the well known Soret band and Q-band respectively, and normally observed for phthalocyanines [21]. The up-conversion emission spectrum of the Yb-Ho containing nanoparticles is also shown. The overlap between the red up-conversion emission and the red absorption band of the photosensitizer is clearly observed.

Fig. 4 shows the up-conversion emission spectrum obtained under 980 nm excitation for the nanoparticle ethanol suspension. Assignments of the observed bands have been already done in the discussion

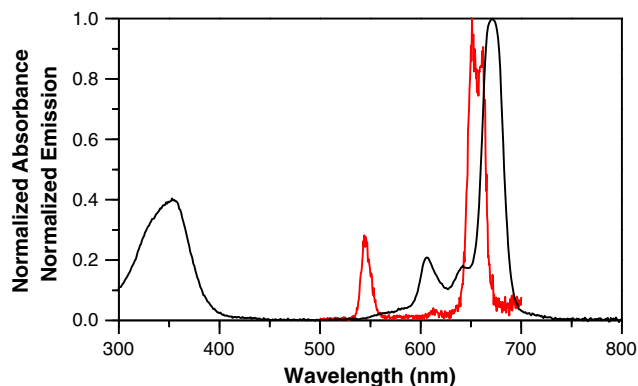


Fig. 3. Absorption spectrum of ethanolic solution of CIAIPc (black) and up-conversion emission spectrum of the BC- $\text{YVO}_4:\text{Yb}^{3+}:\text{Ho}^{3+}$ membranes (red).

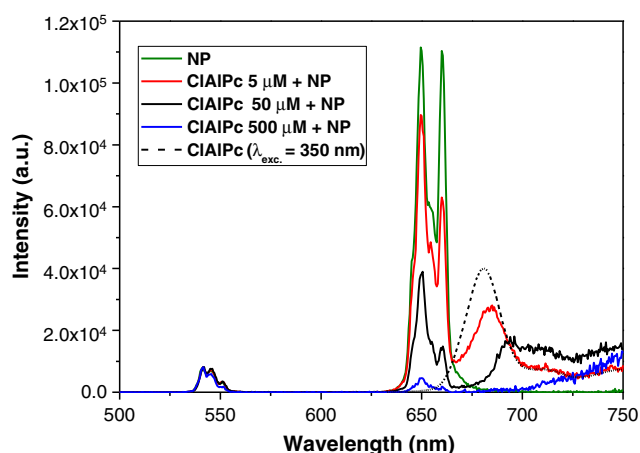


Fig. 4. Up-conversion emission spectra (EXC-980 nm) normalized by the green band in the 550 nm region: ethanolic suspension of $\text{YVO}_4:\text{Yb}^{3+}:\text{Ho}^{3+}$ nanoparticles (NP); ethanolic suspension of $\text{YVO}_4:\text{Yb}^{3+}:\text{Ho}^{3+}$ nanoparticles mixed with an ethanolic solution of CIAIPc with concentrations: 5, 50 and 500 $\mu\text{mol} \cdot \text{L}^{-1}$. The dashed spectrum shows CIAIPc not normalized emission under 350 nm excitation.

of Fig. 2(A) above. Fig. 4 also shows the up-conversion emission spectra obtained for suspensions containing the nanoparticles, to which CIAIPc was added in different concentrations. The red to green band intensity ratios amount to ~ 18.5 for the nanoparticle suspension. When CIAIPc was present, that ratio was observed to decrease to ~ 13.6 ; 4.7; and 0.6 for the CIAIPc concentrations of 5, 50 and 500 $\mu\text{mol} \cdot \text{L}^{-1}$, respectively. This fact suggests that the red emission band was attenuated with respect to the green one with the increase in the CIAIPc relative content.

In fact, the red up-converted emission from the nanoparticles was absorbed by the CIAIPc molecules (Q-band) leading to the well known triplet state emission at 680 nm, that is observed more clearly at the spectrum of the sample containing 5 $\mu\text{mol} \cdot \text{L}^{-1}$ of CIAIPc (red spectrum in Fig. 4). For higher concentrations of CIAIPc this emission is attenuated together with the red up-conversion band, due to the increasing absorption at that wavelength region, leading to reabsorption of the emitted light. The CIAIPc triplet emission obtained under 350 nm is also shown in the figure (dashed) corroborating the assignment of the emission band. The same spectroscopic characteristics were observed for the composite membranes. Therefore, in order to observe the CIAIPc emission excited by the up-converted light, a compromise exists between the relative quantities of NPs and CIAIPc and also the pumping intensity.

Considering possible biological targets the observation of the phthalocyanine emission at 680 nm under 980 nm excitation is very interesting suggesting the application of these composite systems in PDT processes activated by IR light.

In conclusion, [biocellulose–yttrium vanadate nanoparticle–CIAIPc phthalocyanine] (BC–NP–CIAIPc) composite membranes have been prepared. They present the property of converting infrared energy into visible light. By using nanoparticles containing the pair $\text{Yb}^{3+}\text{--}\text{Ho}^{3+}$ the visible emission could be adjusted to the wavelength of excitation of the CIAIPc phthalocyanine, a well known photosensitizer used in photodynamic therapy. CIAIPc emission at 680 nm was therefore observed upon excitation at 980 nm. IR excitation excited the up-conversion of the nanoparticles and the up-converted light was then absorbed by CIAIPc leading to the triplet emission. The wavelength used for primary excitation (980 nm) is advantageous for application in biological systems, as it allows deeper penetration into tissues than the UV or visible radiation commonly used for luminescence excitation. Therefore, BC–NP–CIAIPc membranes proved effective as a new biomaterial for a setup to sensitizer-active photoprocesses useful for the treatment of neoplastic and non-neoplastic diseases susceptible to the photoactivation process.

Moreover, this system could simplify the preparation procedure and lower production costs in topical or transdermal drug delivery systems.

Supplementary information (SI) for this paper also includes: detailed experimental procedures for the synthesis and characterizations of nanoparticles and composite membranes. Additional characterizations of nanoparticles: Fourier transform infrared (FTIR) spectra, thermogravimetric (TG) curves and X-ray diffraction (XRD) profiles are also presented as SI.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.colcom.2014.08.001>.

References

- [1] F. Auzel, Upconversion and anti-stokes processes with f and d ions in solids, *Chem. Rev.* 104 (2004) 139–173.
- [2] J.A. Barreto, W. O'Malley, M. Kubeil, B. Graham, H. Stephan, L. Spiccia, Nanomaterials: applications in cancer imaging and therapy, *Adv. Mater.* 23 (2011) H18–H40.
- [3] J. Chen, J.X. Zhao, Upconversion nanomaterials: synthesis, mechanism, and applications in sensing, *Sensors* 12 (2012) 2414–2435.
- [4] M. Wang, G. Abbineni, A. Clevenger, C. Mao, S. Xu, Upconversion nanoparticles: synthesis, surface modification and biological applications, *Nanomedicine* 7 (2011) 710–729.
- [5] P. Zhang, W. Steelant, M. Kumar, M. Scholfield, Versatile photosensitizers for photodynamic therapy at infrared excitation, *J. Am. Chem. Soc.* 129 (2007) 4526–4527.
- [6] T.L. Doane, C. Burda, The unique role of nanoparticles in nanomedicine: imaging, drug delivery and therapy, *Chem. Soc. Rev.* 41 (2012) 2885–2911.
- [7] D. Bechet, P. Couleaud, C. Frochet, M. Viriot, F. Guillemain, F. Barberi-Heyob, Nanoparticles as vehicles for delivery of photodynamic therapy agents, *Trends Biotechnol.* 26 (2008) 612–621.
- [8] M.K.K. Oo, Y. Yang, Y. Hu, M. Gomez, H. Du, H. Wang, Gold nanoparticle-enhanced and size-dependent generation of reactive oxygen species from protoporphyrin IX, *ACS Nano* 6 (2012) 1939–1947.
- [9] D. Klemm, F. Kramer, S. Moritz, T. Lindström, M. Ankerfors, D. Gray, A. Doris, Nanocelluloses: a new family of nature-based materials, *Angew. Chem. Int. Ed.* 50 (2011) 5438–5466.
- [10] S. Kalia, A. Dufresne, B.M. Cherian, B.S. Kaith, L. Avérous, J. Njuguna, E. Nassiopoulou, Cellulose-based bio- and nanocomposites: a review, *Int. J. Polym. Sci.* 2011 (2011) 1–35.
- [11] S.A. Guzun, M. Stroescu, F. Tache, T. Zaharescu, E. Grosu, Effect of electron beam irradiation on bacterial cellulose membranes used as transdermal drug delivery systems, *Nucl. Inst. Methods Phys. Res. B* 265 (2007) 434–438.
- [12] C. Bodhibukkana, T. Srichana, S. Kaewnopparat, N. Tangthong, P. Bouking, G.P. Martin, R. Suedee, Composite membrane of bacterially-derived cellulose and molecularly imprinted polymer for use as a transdermal enantioselective controlled-release system of racemic propranolol, *J. Control. Release* 113 (2006) 43–56.
- [13] E. Trovatti, C.S.R. Freire, P.C. Pinto, I.F. Almeida, P. Costa, A.J.D. Silvestre, C.P. Neto, C. Rosado, Bacterial cellulose membranes applied in topical and transdermal delivery of lidocaine hydrochloride and ibuprofen: in vitro diffusion studies, *Int. J. Pharm.* 435 (2012) 83–87.
- [14] C. Padula, G. Colombo, S. Nicoli, P.L. Catellani, G. Massimo, P. Santi, Bioadhesive film for the transdermal delivery of lidocaine: in vitro and in vivo behavior, *J. Control. Release* 88 (2003) 277–285.
- [15] I.F. Almeida, T. Pereira, N.H.C.S. Silva, F.P. Gomes, A.J.D. Silvestre, C.S.R. Freire, et al., Bacterial cellulose membranes as drug delivery systems: an in vivo skin compatibility study, *Eur. J. Pharm. Biopharm.* 86 (2014) 332–336.
- [16] F. Schmitt, L. Lagopoulos, P. Kauper, N. Rossi, N. Busso, J. Barge, et al., Chitosan-based nanogels for selective delivery of photosensitizers to macrophages and improved retention in and therapy of articular joints, *J. Control. Release* 144 (2010) 242–250.
- [17] N. Drogat, R. Granet, C. Le Morvan, G. Bégaud-Grimaud, P. Krausz, V. Sol, Chlorin-PEI-labeled cellulose nanocrystals: synthesis, characterization and potential application in PDT, *Bioorg. Med. Chem. Lett.* 22 (2012) 3648–3652.
- [18] W.K. Czaja, D.J. Young, M. Kaweck, R.M. Brown, The future prospects of microbial cellulose in biomedical applications, *Biomacromolecules* 8 (2007) 1–12.
- [19] D. Giamme, V. Buisette, K. Lahlil, T. Gacoin, J.-P. Boilot, D. Casanova, E. Beaupaire, M.-P. Sauvat, A. Alexandrou, Emission properties and applications of nanostructured luminescent oxide nanoparticles, *Prog. Sol. State Chem.* 33 (2005) 99–106.

- [20] G. Mialon, S. Türkcan, G. Dantelle, D.P. Collins, M. Hadjipanayi, R.A. Taylor, T. Gacoin, A. Alexandron, J.-P. Boilot, High up-conversion efficiency of YVO₄:Yb, Er nanoparticles in water down to the single-particle level, *J. Phys. Chem. C* 114 (2010) 22449–22454.
- [21] A.C. Tedesco, J.C.G. Rotta, C.N. Lunardi, Synthesis, photophysical and photochemical aspects of phtalocyanines for photodynamic therapy, *Curr. Org. Chem.* 7 (2003) 187–196.
- [22] W. Ryba-Romanowski, S. Golab, G. Dominiak-Dzik, P. Solarz, T. Lukasiewicz, Conversion of infrared radiation into red emission in YVO₄:Yb, Ho, *Appl. Phys. Lett.* 79 (2001) 3026–3028.
- [23] H.S. Barud, C. Barrios, T. Regiani, R.F.C. Marques, M. Verelst, J. Dexpert-Ghys, Y. Messaddeq, S.J.L. Ribeiro, Self-supported silver nanoparticles containing bacterial cellulose membranes, *Mater. Sci. Eng. C* 28 (2008) 515–518.
- [24] X. Bai, H. Song, G. Pan, Y. Lei, T. Wang, X. Ren, S. Lu, B. Dong, Q. Dai, L. Fan, Size-dependent upconversion luminescence in Er³⁺/Yb³⁺-codoped nanocrystalline yttria: saturation and thermal effects, *J. Phys. Chem. C* 111 (2007) 13611–13617.
- [25] F. Song, G. Zhang, M. Shang, H. Tan, J. Yang, F. Meng, Three-photon phenomena in the upconversion luminescence of erbium–ytterbium-codoped phosphate glass, *Appl. Phys. Lett.* 79 (2001) 1748–1750.