Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

Natural Membranes for Application in Biomedical Devices

Gonçalo Ribeiro [a], Miguel Conde [a], Júlia Carvalho [a], Luísa C. Rodrigues [a], Raquel Alves [a], Rute A. S. Ferreira [b], Agnieszka Pawlicka [c], Luís D. Carlos [b] & M. Manuela Silva [a]

[a] Centro de Química, Universidade do Minho, Gualtar, Braga, Portugal

[b] Departamento de Física, CICECO, Universidade de Aveiro, Campus de Santiago, Aveiro, Portugal

[c] Instituto de Química de São Carlos, Universidade de São Paulo, São Carlos, SP, Brazil

Version of record first published: 30 Jul 2012.

To cite this article: Gonçalo Ribeiro , Miguel Conde , Júlia Carvalho , Luísa C. Rodrigues , Raquel Alves , Rute A. S. Ferreira , Agnieszka Pawlicka , Luís D. Carlos & M. Manuela Silva (2012): Natural Membranes for Application in Biomedical Devices, Molecular Crystals and Liquid Crystals, 562:1, 147-155

To link to this article: http://dx.doi.org/10.1080/15421406.2012.679172

Mol. Cryst. Liq. Cryst., Vol. 562: pp. 147–155, 2012

ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421406.2012.679172

Taylor & Francis
Taylor & Francis Group

Natural Membranes for Application in Biomedical Devices

GONÇALO RIBEIRO,[1] MIGUEL CONDE,[1] JÚLIA CARVALHO,[1] LUÍSA C. RODRIGUES,[1] RAQUEL ALVES,[1] RUTE A. S. FERREIRA,[2] AGNIESZKA PAWLICKA,[3] LUÍS D. CARLOS,[2] AND M. MANUELA SILVA[1,*]

[1]Centro de Química, Universidade do Minho, Gualtar, Braga, Portugal
[2]Departamento de Física, CICECO, Universidade de Aveiro, Campus de Santiago, Aveiro, Portugal
[3]Instituto de Química de São Carlos, Universidade de São Paulo, São Carlos, SP, Brazil

Polymers from natural sources are particularly useful as biomaterials for medical devices applications. In this study, the results of characterization of a gelatin network electrolyte doped with europium triflate ($Eu(CF_3SO_3)_3$) are described. The unusual electronic properties of the trivalent lanthanide ions make them well suited as luminescent reporter groups, with many applications in biotechnology. Samples of solvent-free electrolytes were prepared with a range of guest salt concentration. Materials based on $Eu(CF_3SO_3)_3$ were obtained as mechanically robust, flexible, transparent, and completely amorphous films. Samples were characterized by thermal analysis (thermogravimetry analysis (TGA) and differential scanning calorimetry (DSC), electrochemical stability, scanning electron microscopy (SEM), and photoluminescence spectroscopy.

Keywords Cyclic voltammetery; lanthanide ions; natural polymers; photoluminescence; thermal analysis

1. Introduction

During medicinal evolution, many synthetic compounds were invented, and some of them could represent some problems on direct human use. Therefore, the research for natural substitutes was mandatory. In fact, chitin, agar, starch, and gelatin are examples of an answer for this problem, because they are similar to macromolecular substances; therefore the biologic system is prepared for its recognition, or there are some enzymes capable of gradual degradation of the polymers. There are several studies reporting many uses for these natural materials for electrolyte use [1–5] and increasing interest for medical application [6–9]. For example, studies for new chitosan blends for bone and cartilage tissue regeneration have been made [10,11]. Chitosan has also been studied for reduce the rejection problems for organ transplantation [12]. Another example is the use of the same

*Address correspondence to M. Manuela Silva, Centro de Química, Universidade do Minho, Gualtar, 4710-057 Braga, Portugal. E-mail: nini@quimica.uminho.pt

compound for tissue engineering as a potential therapeutical tool through the development of substitutes that are able to restore proper function of damaged organs or tissues [10–12].

During last years, environmental considerations made the use of natural polymers very attractive because of their biodegradability, low toxicity, and low disposal costs. So, it is interesting and important to be able to produce polymeric membranes from naturally occurring or "green" polymers. Given the well-established relevance of gelatin, as one of the most abundant renewable and biodegradable resource, as well as an environment-friendly "green" polymer, this natural material, which has been intensively studied in biomedical areas, is thought to be a "green" polymer candidate for membrane materials [13]. In addition, the easy preparation and some qualities of the gelatin improve this choice [14,15].

Gelatin is a protein of animal origin, obtained by the thermal denaturation of collagen, which has a triple-helix structure stabilized mainly by the formation of interchain hydrogen bonds between carbonyl and amines groups. After the denaturation process, the triple helix is broken and polypeptide chains adopt a random configuration. Gelatin is successfully used as a gelling agent of food dispersion systems, drug encapsulation, pharmaceutical, and cosmetic products, mainly in the formulation of biodegradable packaging [16].

The addition of a luminescent molecule, which acts as a marker in the medical procedure, is of paramount importance. Through the last decade, considerable research effort has been made on the study of luminescent lanthanide complexes [17,18] due largely to the applications of such materials in biomedical and optical technologies [19]. Since the discovery of their industrial uses, the relationship between research and these elements has been kept alive until today when many high-technology applications of lanthanide-containing materials such as energy-saving lighting devices and responsive luminescent stains heavily rely on the brilliant and pure-color emission of lanthanide ions [19–23]. In fact, the complexes formed with these ions have considerable advantages: easily recognizable, line-like emission spectra, long lifetimes, and relative insensitivity to photo-bleaching [22].

Most of the trivalent lanthanide ions are luminescent, either fluorescent or phosphorescent, and have the ability to interact with biological tissues or organs [22–24]. There are some reports of the temperature dependence of the europium luminescence [25].

The present study aims to use the gelatin attached with Eu(III) and evaluate the dependence of temperature of the matrix as well as the luminescent characteristics of the produced samples.

2. Experimental Section

2.1. Materials

2.2. Sample Preparation

Samples were prepared according to an optimized procedure described elsewhere [2]. Commercial colourless gelatin (2.0 g) was dispersed in 15 mL of Milli-Q water and heated under magnetic stirring for a few minutes up to 50°C for complete dissolution. Then, 0.5 g of glycerol (Himedia, 99.5%) as plasticizer and different quantities of $Eu(CF_3SO_3)_3$ (Aldrich, 98%) were added to this solution under stirring. This solution was then poured on Petri plates to form transparent films. Next, some pieces were cut for some lamellas and put on a Buchi TO51 tube oven for a complete drying.

2.3. Measurements

2.3.1. Thermal Analysis. Samples of dry films were subjected to thermal analysis under a flowing argon atmosphere between −60°C and 120°C and at a heating rate of 5°C.min^{-1} using a Mettler DSC 821e. Samples were transferred to 40 μL aluminium cans with perforated lids within a dry argon-filled glovebox. Samples for thermogravimetric studies were prepared in a similar manner, transferred to open crucibles, and analyzed using a Rheometric Scientific TG1000 thermobalance operating under flowing argon between 30°C and 700°C and at a heating rate of 10°C.min^{-1}.

2.3.2. Electrochemical Stability. Evaluation of the electrochemical stability window of electrolyte compositions was carried out under an argon atmosphere using a two-electrode cell configuration. The preparation of a 25 μm diameter gold microelectrode surface, by polishing with a moist cloth and 0.05 μm alumina powder (Buehler), was completed outside the drybox. The cell was assembled by locating a clean lithium disk counter electrode (cut from Aldrich, 99.9%, 19 mm diameter, 0.75 mm thick) on a stainless steel current collector and centering a sample of electrolyte on the electrode surface. A small volume (2 μL) of THF was placed on the microelectrode surface. The microelectrode was then located on the electrolyte surface and supported firmly by means of a clamp. The use of THF to soften the electrolyte was necessary to achieve a reproducible microelectrode/electrolyte interfacial contact. An Autolab PGSTAT-12 (Eco Chemie) was used to record voltammograms at a scan rate of 200 mV/s. Measurements were performed at room temperature, within a Faraday cage.

2.3.3. SEM. To evaluate the morphology of the as-prepared samples, Scanning Electron Microscopy (SEM) micrographs were obtained using LEO 440 equipment.

2.3.4. Photoluminescence Spectroscopy. Emission spectra in steady-state mode were recorded at room-temperature on a Fluorolog–3 2-Triax, Horiba Scientific, with a modular double grating excitation spectrometer (fitted with a 1200 grooves.mm^{-1} grating blazed at 330 nm) and a TRIAX 320 single-emission monochromator (fitted with a 1200 grooves.mm^{-1} grating blazed at 500 nm, reciprocal linear density of 2.6 nm.mm^{-1}), coupled to a R928 Hamamatsu photomultiplier, using the front face acquisition mode. The excitation source was a 450W Xe arc lamp. The emission spectra were corrected for detection and optical spectral response of the spectrofluorimeter, and the excitation spectra were corrected for the spectral distribution of the lamp intensity using a photodiode reference detector.

3. Results and Discussion

3.1. Thermal Behavior of Electrolytes

The onset of thermal decomposition was estimated from thermogravimetric analysis. The thermograms (Fig. 1) show the relation between weight and temperature (dot-dashed line) and between weight percentage and temperature (straight line). TGA analysis is consistent with a minimum thermal stability of 200°C, a value considered acceptable for most applications under normal operating conditions.

The results presented in Fig. 1 show an increase in thermal stability with increasing salt concentration, confirming that the salt has a stabilizing influence on the host matrix. This observation has been previously reported for other polymer electrolytes [2,3]. From the

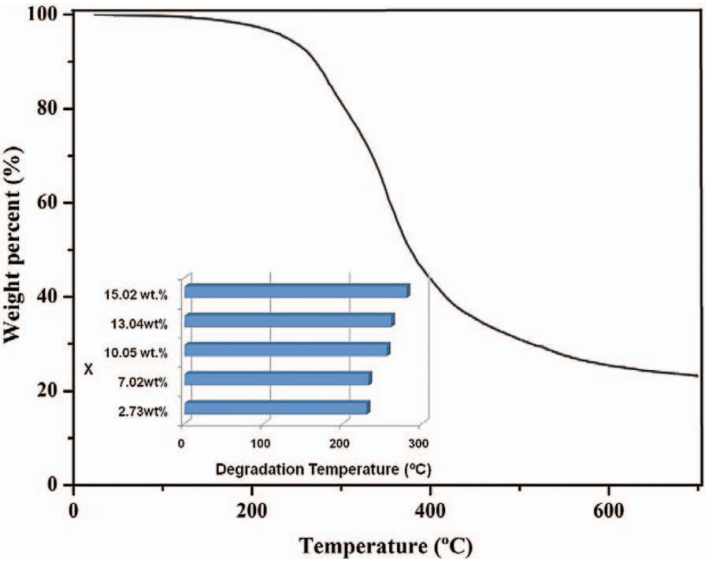

Figure 1. TGA curve of gelatin $Eu(CF_3SO_3)_3$. The inset shows a degradation temperature for samples with different quantities of $Eu(CF_3SO_3)_3$.

DSC curves (Fig. 2) of the gelatine-based electrolyte system, it was possible to conclude that this morphology is an entirely amorphous structure over the range of temperatures studied.

3.2. Electrochemical Stability of Electrolytes

The electrochemical stability of the $Eu(CF_3SO_3)_3$ electrolyte was determined by micro-electrode cyclic voltammetry over the potential range −1.0 to 9.0 V (Fig. 3). The potential limit for the electrolyte composition was determined as the potential at which a rapid rise

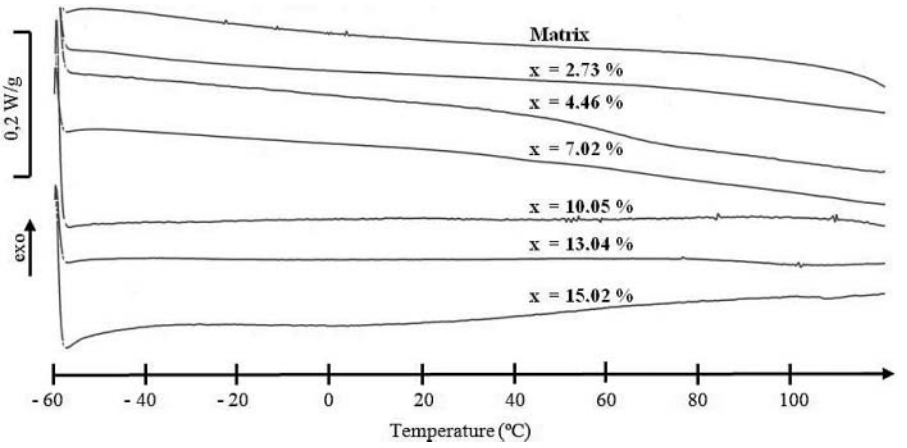

Figure 2. DSC thermograms of selected samples.

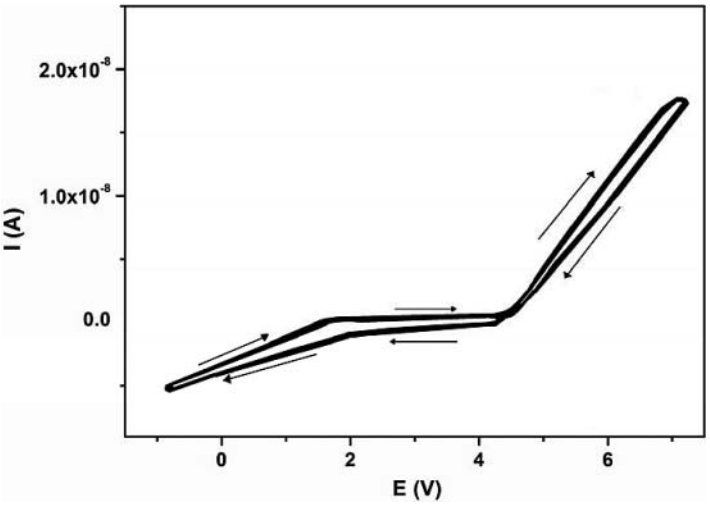

Figure 3. Room temperature cyclic voltamogram of the gelatin $Eu(CF_3SO_3)_3$ obtained with a 25 μm gold microelectrode as working electrode and lithium counter and reference electrodes (sweep rate = 100 mVs^{-1}).

in current was observed and where the current continued to increase as the potential was swept in the same direction. On the cathodic sweep, a low current peak at approximately 5.0 V vs. Li/Li^+ was observed and attributed to the reduction of decomposition products that were formed at the anodic limit. The overall stability of the electrolyte is good with no electrochemical oxidation occurring at potentials less than 5.0 V.

3.3. SEM

In order to evaluate the material morphology SEM as turned into one of the most important tools. In fact, with this technique it is possible to acquire high resolution tridimensional images for the material surface, allowing a more detailed study.

The results showed in Figs. 4(a) and (b) reproduce system based on gelatin doped with europium(III) trifluoromethanesulfonate.

The homogeneity without any phase separation and good surface uniformity of the samples can be observed in the SEM analysis (Figs. 4(a) and (b)). Gelatin-europium-based SPE samples were translucent and showed very good adhesion properties.

3.4. Photoluminescence Measurements

Ligand-sensitized luminescence of lanthanide has a relevant interest to analytical chemists due to its potential applications in biochemistry and luminescent analysis of biochemical drugs as well as for the trace determination of lanthanide ions and some organic analytes [26]. Therefore, some optical methods for the measurement of absorption, reflectance or luminescence of certain materials have been improved [25].

Figure 5 displays the room temperature emission spectra of gelatin $Eu(CF_3SO_3)_3$ (4.76 wt.%) material acquired under different excitation wavelengths. All the spectra are composed of a large broad band between 360 and 520 nm, whose emission energy deviates toward the red region as the excitation wavelength increases from 330 to 395 nm, ascribed

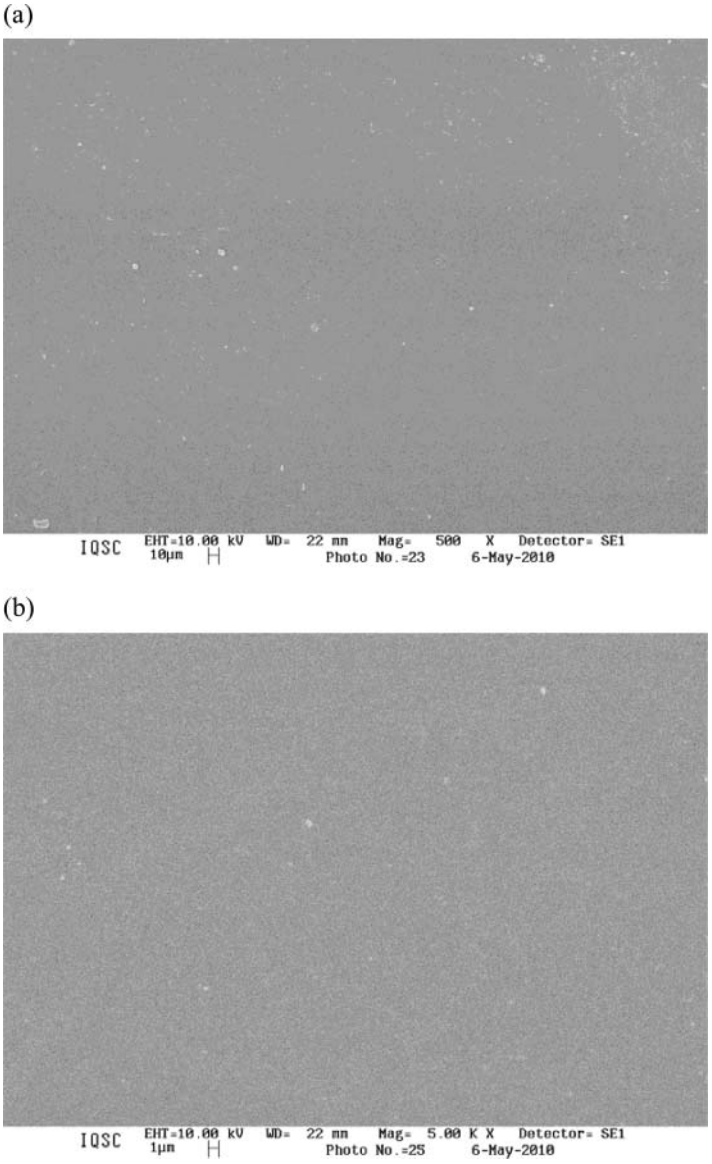

Figure 4. SEM pictures of the (a) $Eu(CF_3SO_3)_3$ (4.76 wt.%), and (b) $Eu(CF_3SO_3)_3$ (13.04 wt.%).

to gelatin [27]. The gelatin-related emission is superimposed on a series of straight lines assigned to the Eu^{3+} intra-$4f^6$ $^5D_0 \rightarrow {}^7F_{0-4}$ transitions. The emission energy dependence on the excitation wavelength was modeled as radiative recombinations involving thermal relaxation within localized states in the framework of the extended multiple trapping approach [28,29]. Focusing our analysis on the intra-f^6 lines, we observe that the energy, full width at half maximum (fwhm), and number of Stark components of the $^5D_0 \rightarrow {}^7F_{1-4}$ transitions are almost independent of the selected excitation wavelength, suggesting that the Eu^{3+} occupy a single average local environment. Moreover, the presence of the $^5D_0 \rightarrow {}^7F_0$ line and the Stark splitting of the $^7F_{1,2}$ levels in three and four components, respectively,

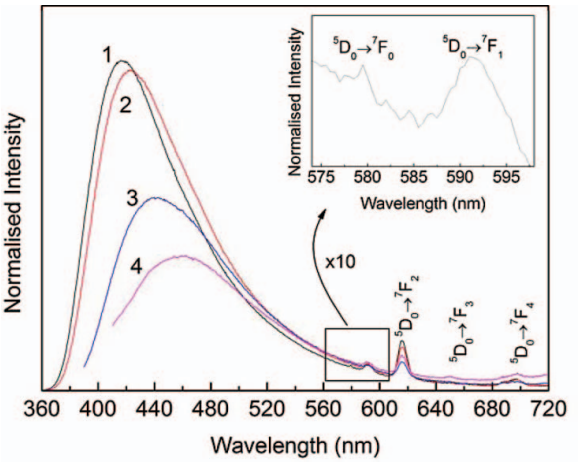

Figure 5. Room temperature emission spectra of the $Eu(CF_3SO_3)_3$ (4.76 wt.%), excited at (1) 330 nm, (2) 350 nm, (3) 375 nm and 395 nm. The inset shows a magnification of the $^5D_0 \rightarrow {}^7F_{0,1}$ transitions for 330 nm.

points out a low local-symmetry for the Eu^{3+}-coordination site with the absence of an inversion center, in agreement with the high relative intensity of the $^5D_0 \rightarrow {}^7F_2$ transition.

Figure 6 shows the gelatin $Eu(CF_3SO_3)_3$ (4.76 wt.%) excitation spectra under different monitoring wavelengths, in order to selectively monitor the excitation paths associated with the broad band emission and the intra-$4f^6$ transition. The former spectra reveal a gelatin-related broad band peaking at 350 nm, [27], which is also present in the excitation spectrum monitored within the Eu^{3+} lines, which also presents a series of straight lines ascribed to the $^7F_0 \rightarrow {}^5D_{3,2}$, $^5L_6$, $^5G_{2-6}$ transitions. The high relative intensity of the broad band emission, when compared with the intra-$4f^6$ lines, points out that the Eu^{3+} ions are essentially populated via a sensitization process rather than by direct excitation.

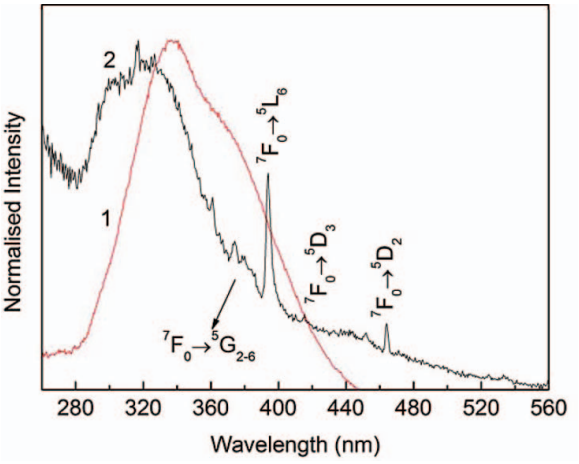

Figure 6. Excitation spectra of the $Eu(CF_3SO_3)_3$ (4.76 wt.%), monitored at (1) 480 nm and (2) 616 nm.

4. Conclusions

Lanthanide ions possess fascinating optical properties as well as responsive luminescent stains for biomedical analysis; medical diagnosis and cell imaging rely heavily on lanthanide ions.

The SEM results allow us to perform a surface analysis of the sample with $Eu(CF_3SO_3)_3$. It allows us to conclude that the doped system has a uniform surface and that separation of phases does not occur.

The results of DSC and TGA analysis are consistent with a minimum thermal stability of about 220°C for the 7.02 wt.% electrolyte composition, a value considered acceptable for applications under normal operating conditions. The present work demonstrated that gelatin europium-based membranes are very promising materials to be applied in electrochemical devices as well as to diagnose methods for medical applications.

Acknowledgments

The authors gratefully acknowledge the financial support provided by the University of Minho and the Fundação para a Ciência e Tecnologia (contracts POCTI/SFA/3/686 and SFRH/BD/38616/2007) for laboratory equipment and research staff. The authors are also indebted to CNPq and CAPES for the financial support given to this research.

References

[1] Al-Kahlout, A., & Vieira, D. (2010). *Ionics*, *16*, 13–19.
[2] Vieira, D., Avellaneda, C., & Pawlicka, A. (2007). *Electrochim. Acta*, *53*, 1404.
[3] Raphael, E., Avellaneda, C. O., Manzolli, B., & Pawlicka, A. (2010). *Electrochim. Acta*, *55*, 1455.
[4] Sung, J., Kim, S., & Lee, K. (2004). *J. Power Sources*, *126*, 258.
[5] Vidinha, P., et al. (2008). *Chem. Commun.*, 5842.
[6] Rinaud, M. (2006). *Prog. Polym. Sci.*, *31*, 603.
[7] Mourya, V. (2008). *React. Funct. Polym.*, *68*, 1013.
[8] Ikada, Y. (1994). *Biomaterials*, *15*(10), 725.
[9] Sell, S. A., Wolfe, P. S., Garg, K., McCool, J. M., Rodriguez, I. A., & Bowlin, G. L. (2010). *Polymers*, *2*(4), 522.
[10] Shen, M., Yoshimoto, H., & Vacanti, J. P. (2004). *Tissue Eng.*, *10*(1–2), 33.
[11] Kim, I.-Y., Seo, S.-J., Moon, H.-S., Yoo, M.-Y., Park, I.-Y., Kim, B.-C., & Cho, C.-S. (2008). *Biotechnol. Adv.*, *26*, 1.
[12] Sashiwa, H., & Aiba, S.-I. (2004). *Prog. Polym. Sci.*, *29*, 887.
[13] Yoshikawaa, M., Higuchi, A., Ishikawa, M., Guiver, M. D., & Robertson, G. P. (2004). *J. Membr. Sci.*, *243*, 89.
[14] Maquet, J., Théveneau, H., Djabourov, M., Lebloud, J., & Papon, P. (1986). *Polymer*, *27*, 1103.
[15] Patil, R. D., Mark, J. E., Apostolov, A., Vassileva, E., & Fakirov, S. (2000). *Eur. Polym. J.*, *36*, 1055.
[16] Rivero, S., García, M. A., & Pinotti, A. (2010). *Innov. Food Sci. Emerg. Technol.*, *11*, 369.
[17] Nunes, S. C., de Zea Bermudez, V., Cybinska, J., Sá Ferreira, R. A., Carlos, L. D., Legendziewicz, J., Silva, M. M., Smith, M. J., & Ostrovskii, D. (2008). *J. Alloy. Compd.*, *451*, 510.
[18] Silva, M. M., Barbosa, P., Evans, A., & Smith, M. J. (2006). *Solid State Sci.*, *8*, 1318.
[19] Yi, C., Tang, Y., Liu, W., & Tan, M. (2007). *Inorg. Chem. Commun.*, *10*, 1505.
[20] Fangfang, C., Zuqiang, B., & Chunhui, H. (2009). *J. Rare Earth*, *27*, 345.
[21] Allred, C., & Menzel, E. (1997). *Forensic Sci. Int.*, *85*, 83.
[22] Bünzli, J., Comby, S., Chauvin, A. S., & Vandevyver, D. B. (2007). *J. Rare Earth*, *25*, 257.
[23] Bünzli, J., & Eliseeva, S. (2010). *J. Rare Earth*, *28*(6), 824.

[24] Evans, C. H. (1990). *Biochemistry of the Lanthanides*, Plenum Press: New York.
[25] Basu, B., & Vasantharajan, N. (2008). *J. Lumin.*, *128*, 1701.
[26] Guo, C., Lang, A., Wang, L., & Jiang, W. (2010). *J. Lumin.*, *130*, 591.
[27] Cole, C. G. B., & Roberts, J. J. (1996). *J. Food Sci. Nutr.*, *8*(4), 139.
[28] Ferreira, R. A. S., Ferreira, A. L., & Carlos, L. D. (2006). *Eur. Phys. J.*, *B 50*, 371.
[29] Ferreira, R. A. S., Ferreira, A. L., & Carlos, L. D. (2006). *J. Non-Cryst. Solids*, *352*, 1225.