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Polycaprolactone and polycaprolactone/chitosan nanofibres functionalised with the pH-sensitive dye Nitrazine Yellow

Lien Van der Schueren[a], Thierry De Meyer[a,b], Iline Steyaert[a], Özgür Ceylan[a], Karen Hemelsoet[b], Veronique Van Speybroeck[b], Karen De Clerck[a,*]

[a] Ghent University, Department of Textiles, Technologiepark 907, 9052 Zwijnaarde (Ghent), Belgium
[b] Ghent University, Center for Molecular Modeling, Technologiepark 903, 9052 Zwijnaarde (Ghent), Belgium

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ABSTRACT

Nanofibres functionalised with pH-sensitive dyes could greatly contribute to the development of stimuli-responsive materials. However, the application of biocompatible polymers is vital to allow for their use in (bio)medical applications. Therefore, this paper focuses on the development and characterisation of pH-sensitive polycaprolactone (PCL) nanofibrous structures and PCL/chitosan nanofibrous blends with 20% chitosan. Electrospinning with added Nitrazine Yellow molecules proved to be an excellent method resulting in pH-responsive non-wovens. Unlike the slow and broad response of PCL nanofibres (time lag of more than 3 h), the use of blends with chitosan led to an increased sensitivity and significantly reduced response time (time lag of 5 min). These important effects are attributed to the increased hydrophilic nature of the nanofibres containing chitosan. Computational calculations indicated stronger interactions, mainly based on electrostatic interactions, of the dye with chitosan (ΔG of -132.3 kJ/mol) compared to the long-range interactions with PCL (ΔG of -35.6 kJ/mol), thus underpinning our experimental observations. In conclusion, because of the unique characteristics of chitosan, the use of PCL/chitosan blends in pH-sensitive biocompatible nanofibrous sensors is crucial.

1. Introduction

Polycaprolactone (PCL) is an aliphatic polyester, often used in (bio)medical applications because of its biocompatibility, slow biodegradability, low-cost, non-toxicity and good mechanical properties (Moghe, Hufenus, Hudson, & Gupta, 2009; Van der Schueren, De Schoenmaker, Kalaoglu, & De Clerck, 2011). However, PCL is hydrophobic (Prabhakaran et al., 2008) which may severely limit its use in certain applications. Combining PCL with a hydrophilic polymer might significantly improve the overall material characteristics. Chitosan, a natural polysaccharide derived from chitin, can provide this hydrophilicity and moreover shows antimicrobial activity and the ability to support the biocompatibility of PCL (Bhattarai et al., 2009; Cooper, Bhattarai, & Zhang, 2011; Hong & Kim, 2011; Muzzarelli, 2011; Prabhakaran et al., 2008; Yang, Chen, & Wang, 2009). The combination of PCL with the carbohydrate polymer chitosan may thus be greatly relevant and contribute to the development of innovative materials.

Stimuli-responsive polymeric materials belong to a rapidly evolving field of research (Stuart et al., 2010). Within textiles, the research towards chromic fibrous materials is of particular value (Little & Christie, 2010; Matilla, 2006, part 3). These materials, which reversibly change colour due to an external stimulus, are prime candidates for sensor systems because of their simplicity and flexibility. Halochromic or pH-sensitive textiles offer major potential within these chromic materials. Numerous possible applications for pH-sensitive textiles exist including protective clothing, filtration, wound dressings, etc. (Van der Schueren & De Clerck, 2012a). Yet, for future practice, also the choice of polymer type is essential to address as this may greatly influence the functioning of the obtained materials (Lloyd, Kennedy, Methacanon, Paterson, & Knill, 1998).

Because of the small diameter of nanofibres, nanofibrous structures show unique characteristics such as small pore sizes, high porosity, high specific surface area and high absorbance capacity (Muzzarelli, 2012; Ramakrishna, Fujihara, Teo, Lim, & Ma, 2005, chap. 2; Greiner & Wendorff, 2007). The latter properties render them highly suited to be used in chromic sensors. A nanofibrous structure may indeed increase the sensitivity and reduce the response time, both being important demands for sensor systems (Fengyu et al., 2009; Liu, Tan, Hu, Li, & Chen, 2010; Malherbe,

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* Corresponding author. Tel.: +32 9 264 57 40; fax: +32 9 264 58 46.
E-mail addresses: lien.vanderschueren@ugent.be (L. Van der Schueren), thierry.demeyer@ugent.be (T. De Meyer), iline.steyaert@ugent.be (I. Steyaert), ozgur.ceylan@ugent.be (Ö. Ceylan), karen.hemelsoet@ugent.be (K. Hemelsoet), veronique.vanspeybroeck@ugent.be (V. Van Speybroeck), karen.declerck@ugent.be (K. De Clerck).

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Sanderson, & Smit, 2010; Mao et al., 2009). Our previous study on halochromic polyamide nanofibres proved the potential of pH-responsive nanofibrous structures (Van der Schueren, Mollet, Ceylan, & De Clerck, 2010). Though, the studied polyamides show limitations for use in (bio)medical applications. As a consequence, further research towards pH-sensitive biocompatible nanofibres is key to fully exploit their potential. PCL can contribute to this development but its hydrophobic nature may need to be tackled since this may impact the sensor properties. The important effect of the speed of sample wetting on the resulting time lag of the sensor has indeed been demonstrated (Van der Schueren & De Clerck, 2010a; Van der Schueren, Mollet, et al., 2010). Therefore, the addition of chitosan is studied as a potential solution for this hydrophobic nature, thereby also providing antimicrobial properties (Muzzarelli, 2008).

To validate the experimental results and to understand the nature of the interactions between dyes and polymeric chains, molecular modelling can be applied. With the fast evolution of computational protocols it is possible to study the effect of the molecular environment on the behaviour of a molecule (Catak, D'hooghe, De Kimpe, Waroquier, & Van Speybroeck, 2010; Catak, Hemelsoet, Hermosilla, Waroquier, & Van Speybroeck, 2011). More in specific, molecular modelling showed to be promising for studying interactions between molecules (Gu, Wang, Leszczynski, Xie, & Schaefer, 2008).

The main objective of this paper is to study pH-sensitive PCL and PCL/chitosan blend nanofibrous structures and to compare their performance. The aimed nanofibres can be obtained via the electrospinning of polymer solutions as suggested by the results of our previous studies on the development of PCL and PCL/chitosan nanofibres (Van der Schueren et al., 2011; Van der Schueren, Steyaert, De Schoenmaker, & De Clerck, 2012). The functionalisation of the nanofibres is to be realised by adding the pH-sensitive agent to the polymer solutions, prior to the electrospinning process. The influence of this dye addition on the electrospinning and fibre morphology is examined followed by an analysis of possible dye leaching. Finally, the halochromic behaviour of the nanofibres is studied based on spectroscopic measurements. Nitrazine Yellow (NY) is selected as pH-sensitive dye as it is proven to be successful on polyamide nanofibres (Van der Schueren, Hemelsoet, Van Speybroeck, & De Clerck, 2012). Computational calculations on the interactions between NY and the polymers PCL and chitosan are performed as to obtain more insight into the experimental observations.

2. Materials and methods

2.1. Materials

Medium molecular weight chitosan and PCL (M_n 70,000–90,000) were supplied by Sigma Aldrich. Also the solvents 98 v% formic acid and 99.8 v% acetic acid, hydrochloric acid, sodium hydroxide and potassium nitrate were supplied by Sigma Aldrich. The pH-sensitive dye Nitrazine Yellow (NY), which changes colour from yellow to blue in aqueous solution in the pH range 6.0–7.0, was obtained from Sigma Aldrich as well. The complexing agent poly(diallyldimethylammonium chloride) (Perfixan RDV) was kindly supplied by Chemotex (Kortrijk, Belgium).

2.2. Preparation and characterisation of the electrospinning solutions

The 14 wt% PCL electrospinning solutions were prepared in a 1:9 acetic acid–formic acid solvent system since our previous study showed that this system results in reproducible nanofibres with a small fibre diameter distribution (Van der Schueren et al., 2011). For the PCL/chitosan blend nanofibres, a polymer blend containing 6 wt% PCL and 20% chitosan in 3:7 acetic acid–formic acid was chosen since this allows for reproducible nanofibres with a considerable amount of chitosan as was also discussed in an earlier research (Van der Schueren, Steyaert, et al., 2012). A certain amount of the dye NY, expressed in % on mass of fibre (% omf) was added to the polymer solutions. In some cases, also the complexing agent poly(diallyldimethylammonium chloride), which is a polyquaternary ammonium compound, was added to the solutions at a concentration of 4% omf. Next, the solutions were magnetically stirred at room temperature for three-and-a-half hours, time needed for complete dissolution. The viscosity of the solutions obtained was measured using a Brookfield viscometer LVDV-II. The conductivity was measured with a CDM210 conductivity meter (Radiometer Analytical).

2.3. Electrospinning of PCL and PCL/chitosan nanofibres

During the electrospinning process, the polymer solutions were pumped from a 20 ml syringe into a 15.24 cm long needle with an inner diameter of 1.024 mm. A KD Scientific Syringe Pump Series 100 regulated the flow rate of the solution. The voltage was adjusted using a Glassman High Voltage Series EH 30P3 source (voltage range 0–30 kV). Electrospinning was carried out at room temperature (22 ± 2 °C) and room humidity (40 ± 5%). The processing parameters were set based on the literature focusing on the electrospinning of pure PCL and PCL/chitosan nanofibres (Van der Schueren et al., 2011; Van der Schueren & De Clerck, 2012a; Van der Schueren, Steyaert, et al., 2012). The tip to collector distance was thus set at 12.5 cm. To electrospin PCL, the flow rate was set at 1 ml h^{-1} and the voltage at 18 kV while this was 0.6 ml h^{-1} and 26 kV for electrospinning the polymer blend.

2.4. Characterisation of electrospun samples

The morphology of the electrospun structures was examined using a Scanning Electron Microscope (SEM) (FEI QUANTA 200 F). Prior to SEM-measurements, the sample was coated with gold using a sputter coater (Balzers Union SCD 030). Fifty diameter measurements on each sample using Cell D software (Olympus) determined the average fibre diameter.

To study the hydrophilic nature of the nanofibrous structures, contact angle measurements were carried out with the drop-shape analysis system DSA 10-Mk2, coupled to a control unit G120 Mk1/G140-Mk1 and with the drop-shape analysis software DSA1 (v1.80, Krüss). The variation coefficient of the contact angle measurements typically was 2.5%. In addition, dynamic Vapour Sorption (DVS) measurements were conducted in a Q-5000SA instrument (TA-instruments). All measurements were performed at 23 ± 0.1 °C. Deliquescent salts (sodium bromide and potassium chloride) were used to verify the humidity of the instrument. 4 mg of nanofibres were placed in the quartz sample pans. At the start of each moisture sorption cycle, the fibres were dried at 0% relative humidity (RH) until the weight change was stabilised to be less than 0.05% for a period of 15 min. After the stabilisation, the moisture sorption cycle was started and the humidity was increased stepwise, with steps of 10% RH from 5% till 95%. At every RH, the equilibrium moisture concentration is monitored after reaching equilibrium, or thus when the weight change is less than 0.05% over a time period of 15 min.

Dye leaching of the dye-loaded electrospun samples was analysed by placing 0.012 g of the nanofibrous samples in 10 ml of demineralised water at pH 8. After 24 h the absorbance of the water solution with possible released dye was measured by UV–vis spectroscopy and finally the dye release was converted into percentage

Table 1
Conductivity, average fibre diameter and dye release of PCL and PCL/chitosan solutions as a function of the NY concentration and presence of complexing agent (CA).

% omf NY	% omf CA	PCL			PCL/chitosan		
		Conductivity (mS/cm)	Fibre diameter (nm)	Dye release (%)	Conductivity (mS/cm)	Fibre diameter (nm)	Dye release (%)
0	0	0.089	364 ± 83	–	0.888	196 ± 46	–
0.5	0	0.216	337 ± 53	4.8	0.928	183 ± 45	57.1
0.25	4	0.480	310 ± 55	0.0	1.239	167 ± 47	0.0
0.5	4	0.502	343 ± 45	0.0	1.250	168 ± 43	0.0
1	4	0.530	374 ± 71	1.2	1.280	184 ± 48	7.1

release with respect to the original amount of dye present in the samples.

2.5. Characterisation of halochromic behaviour

The halochromic behaviour of the samples was analysed by immersing them into aqueous pH baths with a pH from 2 to 11, after which their colour was determined. pH measurements of the baths were executed with a combined reference and glass electrode (SympHony Meters VMR). Potassium nitrate with a concentration of 10^{-2} mol l^{-1} was added to ensure a constant activity coefficient during measurements. Hydrochloric acid and sodium hydroxide were used to adjust the pH.

To quantify the colour, UV–vis spectra were recorded with a Perkin-Elmer Lambda 900 spectrophotometer. For the transmission spectra of solutions 1 cm matched quartz cells were used, for the reflection measurements on fabrics an integrated sphere (Spectralon Labsphere 150 mm) was used. The spectra were recorded from 380 nm to 780 nm with a data interval of 1 nm (transmission) and 4 nm (reflection). The resulting absorbance (for solutions) and Kubelka–Munk (for fabrics) spectra were normalised to a value of one at the peak maximum to account for possible artefacts due to dye leaching for some of the samples.

2.6. Computational details

For the molecular modelling calculations, Density Functional Theory (DFT) was applied since this provides a valid compromise between computational resources and chemical accuracy. All computations in this work were carried out in the Gaussian09 software package, using the M06-2X electronic structure method in combination with a 6-311G(d,p) basis set (Frisch et al., 2009; Zhao & Truhlar, 2008). The DFT-functional M06-2X is a meta hybrid high-nonlocality functional with double the amount of non-local exchange (2X), which has proven to be a good method to model systems where dispersion interactions are important (Catak et al., 2010, 2011; Gu et al., 2008; Zhao & Truhlar, 2008). Frequency calculations were performed at the same level of theory as the geometry optimisation to validate that all points are true minima on the potential energy surface and to obtain the necessary temperature corrections of the Gibbs free energies.

3. Results and discussion

3.1. Electrospinning of pH-sensitive PCL and PCL/chitosan nanofibrous structures

Adding components such as dyes to the electrospinning solutions might significantly affect the solution parameters and thus the electrospinning process (Li & Xia, 2004). To determine this effect, different amounts of NY were added to the polymer solutions after which the viscosity and conductivity were determined. Moreover, also the addition of poly(diallyldimethylammonium chloride) as dye complexing agent was studied to avoid possible dye leaching. The viscosity of the solutions was not significantly influenced by the addition of the components. The viscosity was on average 2667 mPa s for the PCL solutions and 7188 mPa s for the PCL/chitosan solutions. In contrast to the viscosity, the conductivity was affected by the dye addition, the effect being more prominent for pure PCL solutions (Table 1). The conductivity increases with increasing NY concentration for both polymer systems due to the charges brought into the solution by the dye molecules. However, because of the polycationic nature of chitosan, the intrinsic conductivity of the blend polymer solutions is much higher compared to the pure PCL solutions and their conductivity is thus less influenced by the dye addition. Also the complexing agent has an effect on the conductivity of PCL and PCL/chitosan solutions, the influence being again less pronounced for the polymer blend. The complexing agent is indeed a polycationic molecule, increasing the solution's conductivity.

All solutions stated in Table 1 could be successfully electrospun using the parameters stated in Section 2. The processing conditions and stability of the electrospinning process were not affected by the addition of NY, nor by the addition of the complexing agent. The results hence indicate that electrospinning with added components is an adequate method giving functionalised PCL and PCL/chitosan nanofibres.

3.2. Characterisation of pH-sensitive PCL and PCL/chitosan nanofibrous structures

The fibre morphology of the electrospun samples with added pH-sensitive dye was similar to the morphology of reference blank nanofibrous structures as demonstrated by the SEM images in Fig. 1. A uniform, beadless non-woven was obtained at each dye concentration. In addition, the PCL/chitosan blends all showed an ultrafine nanofibrous web formed in between the main fibres while this web was not present in PCL nanofibrous structures. This phenomenon is attributed to the presence of chitosan (De Vrieze, Westbroek, Van Camp, & Van Langenhove, 2007; Geng, Kwon, & Jang, 2005; Nirmala, Baek Woo, Navamathavan, El-Newehy, & Kim, 2011; Van der Schueren, Steyaert, et al., 2012) and the added components thus did not affect the ultrafine web formation. Moreover, the average fibre diameters of PCL and PCL/chitosan nanofibres (Table 1) indicate that the diameter is not significantly influenced by the addition of NY, nor by the addition of the complexing agent. Yet, a pronounced diameter decrease was noticed for the PCL/chitosan blend nanofibres compared to the PCL nanofibres due to the polycationic nature of chitosan and the lower total polymer concentration of the PCL/chitosan blends (Van der Schueren, Steyaert, et al., 2012) and is consistent with the literature (Shalumon et al., 2010).

After characterisation of the fibre morphology, the hydrophilic nature of the PCL and PCL/chitosan samples was examined. Contact angle measurements, being a useful indicator of wettability of substrates, showed a decrease in contact angle from 129°

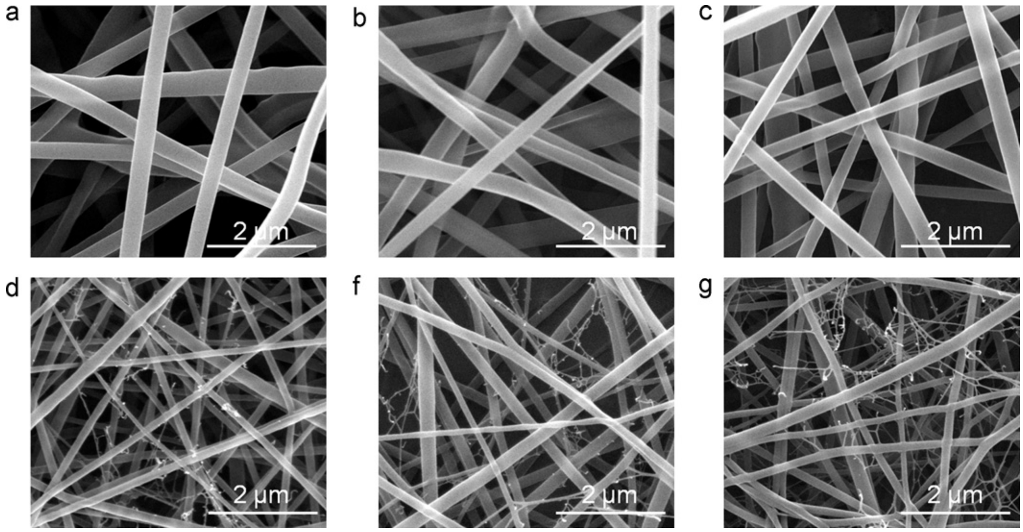

Fig. 1. SEM images of PCL blank nanofibres (a), PCL 0.5% omf NY nanofibres (b), PCL 0.5% omf NY, 4% omf complexing agent nanofibres (c), PCL/chitosan blank nanofibres (d), PCL/chitosan 0.5% omf NY nanofibres (f), PCL/chitosan 0.5% omf NY, 4% omf complexing agent nanofibres (g).

for PCL to 120° for PCL/chitosan nanofibres. This thus already shows a higher hydrophilicity for the blend nanofibrous structure. Moreover, the contact angle of PCL nanofibres remained constant during 20 s after droplet formation while the angle of PCL/chitosan nanofibres decreased to 116° after 20 s. Hence, the blend nanofibres absorb water more efficiently. To support this theory, DVS experiments were carried out. DVS is a well-suited technique to study the moisture sorption of a compound as it determines the equilibrium amount of moisture sorbed as a function of the humidity (Markova, Sparr, & Wadso, 2001). DVS is thus applied to study the intrinsic moisture sorption of the nanofibrous structures. A clear difference between both samples was found as demonstrated in Fig. 2. The PCL/chitosan structures absorb more water, resulting in a greater weight change (5.6% weight change compared to 0.9% for PCL at 95% RH). Moreover, adsorption isotherms are classified in five types according to the IUPAC classification. Types II and III describe adsorption on macroporous and non-porous adsorbents with strong and weak adsorbate–adsorbent interactions, respectively (Sangwichien, Aranovich, & Donohue, 2002). With the presence of chitosan, the shape of the curve changes from a rather type III-isotherm to a rather type II-isotherm as seen in Fig. 2. Thus, also the shape of the isotherms strongly suggests an increased interaction with water when chitosan blend nanofibres are used.

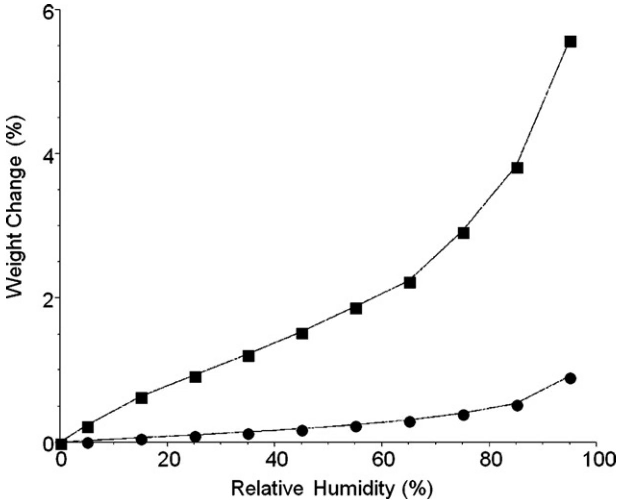

Fig. 2. DVS measurement of PCL (●) and PCL/chitosan (■) nanofibrous structure.

As a final step prior to the halochromic study, the dye leaching of the samples was characterised (Table 1), following the procedure explained in Section 2. A clear difference between dye leaching of PCL and PCL/chitosan samples with 0.5% omf NY was noticed (4.8% for PCL and 57.1% for PCL/chitosan). The relatively low dye release of the PCL nanofibrous structures is probably attributed to a combined effect of the material's hydrophobicity and an interaction between PCL and NY. Due to the hydrophobic nature of PCL, the samples are hardly wetted, minimizing contact between water and NY molecules and hence dye leaching. To increase the wettability, addition of chitosan is vital as shown in Fig. 2. However, owing to the increased hydrophilicity, the dye release increases as well. Therefore, a dye complexing agent was added resulting in a decreased dye release for both PCL and PCL/chitosan. The applied complexing agent poly(diallyldimethylammonium chloride) is indeed known to form a complex with anionic dyes, the complex being less subject to dye diffusion (Clark, 2011). Samples with 0.25 and 0.5% omf NY and 4% omf complexing agent did not show any dye release. These data demonstrate the promising potential of PCL and PCL/chitosan pH-sensitive nanofibres and prove the need of the complexing agent addition to avoid dye leaching of the blend nanofibres.

3.3. Halochromic behaviour of pH-sensitive PCL and PCL/chitosan nanofibrous structures

The nanofibrous samples were all yellow just after the electrospinning process, in agreement with the acidic conditions during their production (acetic acid–formic acid solvent system). However, after conditioning the samples during 24 h in a neutral air atmosphere, their visual aspect showed a clear variation. The PCL nanofibrous structure only containing NY remained yellow while the PCL samples with the addition of complexing agent changed to green. On the other hand, all PCL/chitosan nanofibres obtained a blue colour, in agreement with the colour of a neutral aqueous NY solution.

Even after immersion in a strongly alkaline pH bath (pH 11), the PCL sample with only NY did not present a colour shift whereas

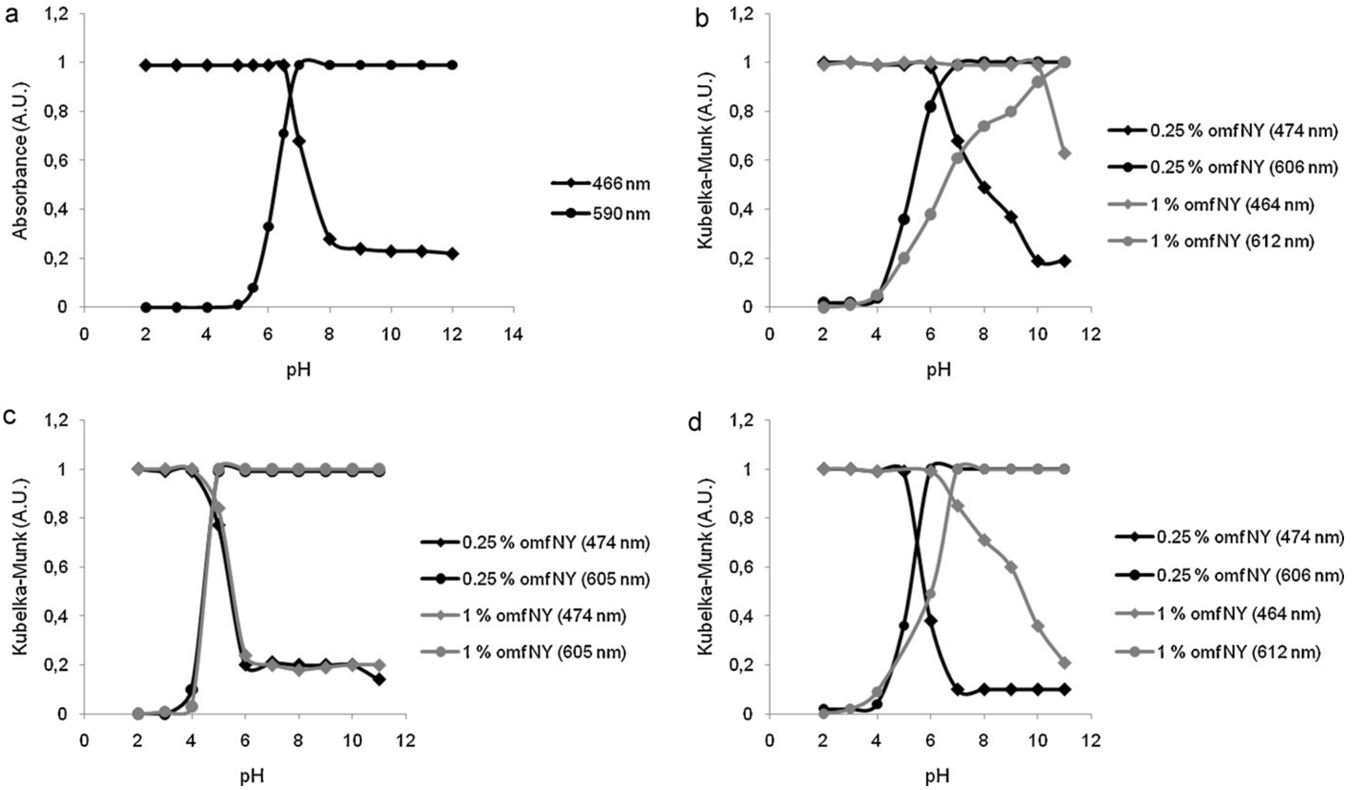

Fig. 3. Normalised absorbance of NY in aqueous solutions (a), normalised Kubelka–Munk value of PCL nanofibrous structures with 0.25 and 1% omf NY and 4% omf complexing agent (b), normalised Kubelka–Munk value of PCL/chitosan nanofibrous structure with 0.25 and 1% omf NY and 4% omf complexing agent (c), normalised Kubelka–Munk value of PCL nanofibrous structure with 0.25 and 1% omf NY and 4% omf complexing agent after 24 h (d).

the PCL/chitosan samples as well as the PCL sample with complexing agent showed a clear shift to a blue colour. This different halochromism may be caused by different interactions of NY with PCL, chitosan and the complexing agent. To study these interactions, molecular modelling was performed and will be discussed in a later section.

Since PCL samples with only NY do not show a halochromic response and PCL/chitosan samples with NY suffer from a high dye release (Table 1), the discussion on the halochromic behaviour further focuses on the samples to which also the complexing agent is added. The PCL nanofibrous samples showed a reversible colour transition from yellow over green to blue. The analysis of the colour was performed after immersion of the samples during 3 h in the pH baths, time during which the colour of the immersed nanofibres still changed. The time lag for the halochromic transition of the PCL nanofibrous structures was thus large limiting the

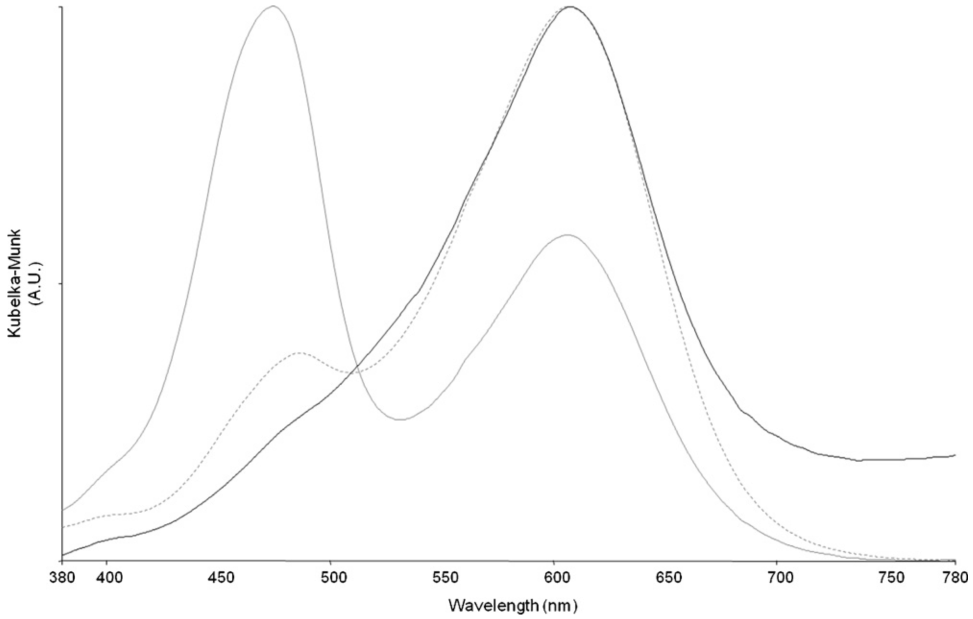

Fig. 4. Normalised Kubelka–Munk of PCL/chitosan (black), PCL recorded after 3 h (grey) and after 24 h (grey, dashed) with 0.5% omf NY and 4% omf complexing agent at pH 6.

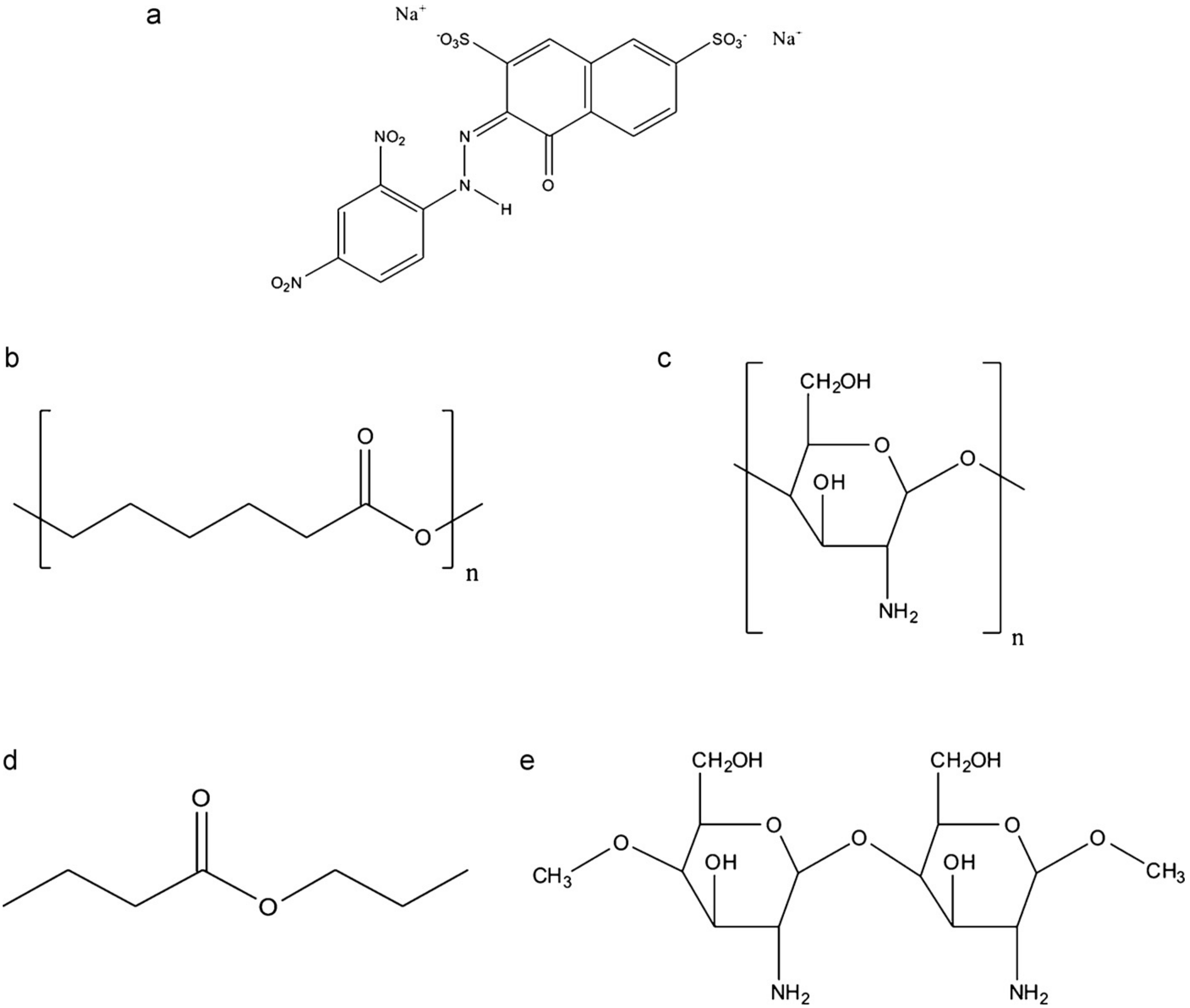

Fig. 5. Structural formulas of NY (a), PCL (b), chitosan (c) and model compounds for PCL (d) and chitosan (e).

practical use of these PCL sensor systems. Similar to the colour transition of PCL nanofibres, PCL/chitosan structures reversibly altered from yellow to blue with a variation in pH. However, the time lag for this halochromic response was 5 min at maximum, this being greatly shorter than the time needed for the pure PCL samples and moreover greatly shorter compared to results previously obtained with conventional fabrics (Van der Schueren & De Clerck, 2010a). This rapid response is caused by the increased hydrophilicity of the blend nanofibres resulting in a fast wetting of the entire nanofibrous structure. Chitosan addition is thus of utmost importance for obtaining a fast sensor system.

To study the effect of dye concentration, the halochromic response was recorded for three different NY concentrations being 0.25, 0.5 and 1% omf NY. The dynamic pH range of PCL structures was found to broaden with increasing dye concentration. This is demonstrated in Fig. 3b which depicts the variation in normalised Kubelka–Munk value at the acidic and alkaline peak maximum as a function of pH and is most likely related to accessibility issues. Owing to the highly hydrophobic nature of PCL (Section 3.2), the NY molecules are difficult to access, leading to a slow and broad response. Indeed, compared to the sharp transition of NY in aqueous solutions, which occurs between pH 6 and 8 (Fig. 3a), the response of PCL nanofibres is less sensitive (response between pH 4 and 10, Fig. 3b). Moreover also the wavelength maxima depend on the NY concentration, again most likely caused by the low water sorption of the samples and hence the different accessibility. The acidic maximum decreases from 474 nm at 0.25% omf NY to 464 nm at 1% omf NY, the alkaline maximum increases from 606 nm at 0.25% omf to 612 nm at 1% omf. In addition, these wavelength maxima differ from the maxima found for NY in solution (466 nm and 590 nm), consistent with numerous studies on the behaviour of dyes in different environments (Ertekin, Karapire, Alp, Yenigul, & Icli, 2003; Garcia-Heras et al., 2005; Jurmanovic, Kordic, Steinberg, & Steinberg, 2010; Van der Schueren & De Clerck, 2012a). Indeed, due to the interactions between NY and the surrounding polymeric PCL matrix, changes to the dye characteristics are very likely.

The augmented hydrophilicity of PCL/chitosan blends led to an easy accessibility of all dye molecules, even at higher NY concentrations. Due to this effect, no differences in halochromic behaviour with varying NY concentration (0.25, 0.5 and 1% omf NY) were observed. All PCL/chitosan samples showed a sharp transition between pH 4 and 6 (Fig. 3c). Also the wavelength maxima did not alter and remained constant at 474 nm and 605 nm in acidic and

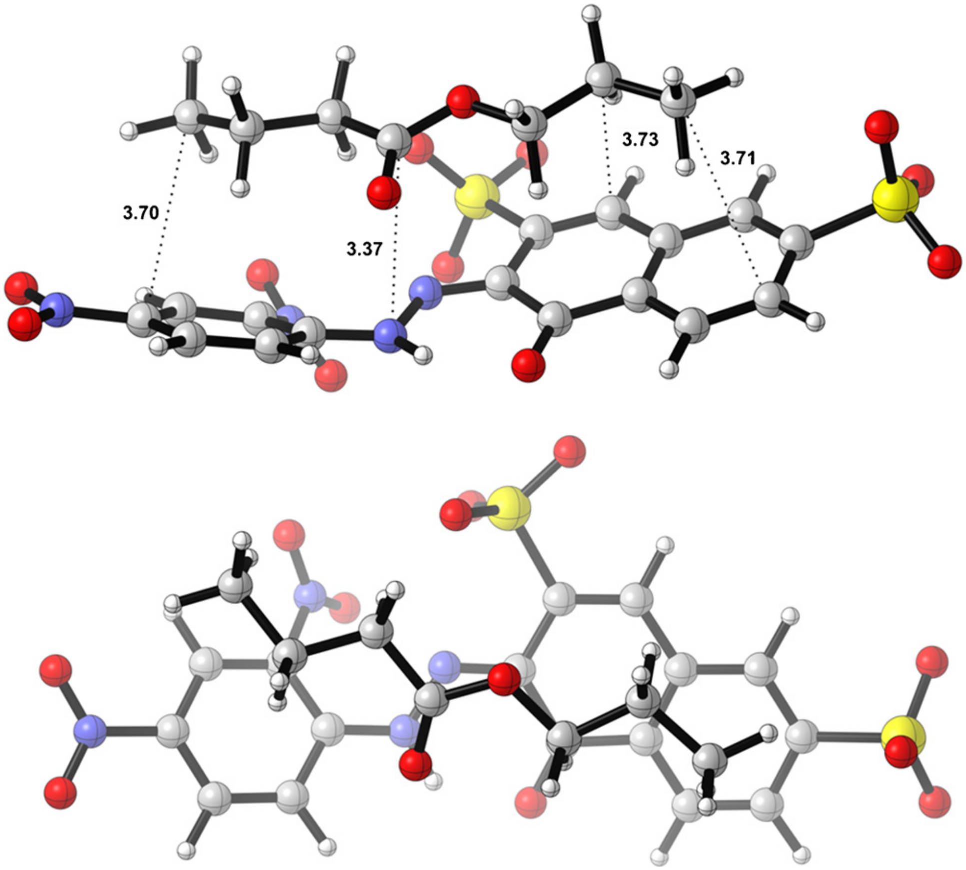

Fig. 6. Optimised structures of the NY–PCL complexes (M06-2X/6-311G(d,p)).

alkaline environment, respectively. Yet, even although no influence of the dye concentration was observed, the halochromism of PCL/chitosan nanofibres differs from the halochromism of NY in solution. Due to interactions between the dye and chitosan, the dynamic pH range, albeit similarly sharp, underwent an acidic shift of two pH units after incorporation in the PCL/chitosan blend. The effective halochromic behaviour of PCL/chitosan nanofibres loaded with NY establishes their major potential to be used as fast and sensitive sensor systems in the slightly acidic pH range.

As a final step of the halochromic study, the colour of the nanofibrous structures after 24 h immersion in the pH baths was analysed. No further change in colour compared to the previously recorded colours (Fig. 3b and c) was observed for the PCL/chitosan samples confirming that all present dye molecules almost immediately assumed their final conformation. The PCL samples, however, did show a further colour change. This is clearly demonstrated in Fig. 4 showing the spectra of PCL nanofibres with 0.5% omf NY at pH 6 recorded after 3 and 24 h. While the dominant peak after 3 h immersion was at 470 nm (the acidic maximum), it is shifted to 607 nm (the alkaline maximum) after 24 h immersion and only a small peak at 470 nm remained. A higher resemblance to the spectrum of PCL/chitosan was hence obtained. Eventually this further colour change resulted in a sharper dynamic pH range after 24 h immersion (Fig. 3d). The low dye concentration (0.25% omf) almost coincides with the graph obtained with PCL/chitosan (Fig. 3c) while also the highest concentration (1% omf) shows a narrower pH range compared to the results after 3 h, even though it is still broader than the range with PCL/chitosan. A long immersion time, dependent on the dye concentration, is thus necessary to allow for a complete conversion of all NY molecules in PCL nanofibres because of their slow wetting. These results underpin the fact that the differences found between PCL and PCL/chitosan nanofibres loaded with the complexing agent are mainly attributable to their different water sorption characteristics. In conclusion, the presence of chitosan is vital as to obtain an adequate sensor with a rapid response.

3.4. Interpretation of halochromic behaviour using molecular modelling

In this section, molecular modelling is used to gain molecular-scale insight into the difference in halochromic behaviour of NY incorporated in PCL and PCL/chitosan. Focus is given to the pure polymers, thus omitting the complexing agent from the discussion. In Fig. 5, the chemical formulas of the molecules relevant to this discussion are displayed. As discussed in previous work (Van der Schueren, Hemelsoet, et al., 2012), the halochromism of NY (Fig. 5a) arises from the removal of the hydrogen atom bonded to the azo group by the alkaline solvent. The sodium ions of NY are omitted from the calculations, as was previously validated to be a good model (De Meyer et al., 2012).

For both PCL and chitosan a valid model system was constructed which allows to perform the molecular modelling calculations and to represent the most essential interactions (Fig. 5d and e). In case of PCL one ester group terminated by two n-propyl groups is taken into account. For chitosan a dimeric model was used consisting of two monomeric units which are terminated by methyl groups. Such a dimeric system has already shown to be a good model for chitosan (Braier & Jishi, 2000). Proton affinities (PA) were calculated for both model structures. The chitosan model (Fig. 5e) has a PA of 908 kJ/mol, which is much higher than the value of

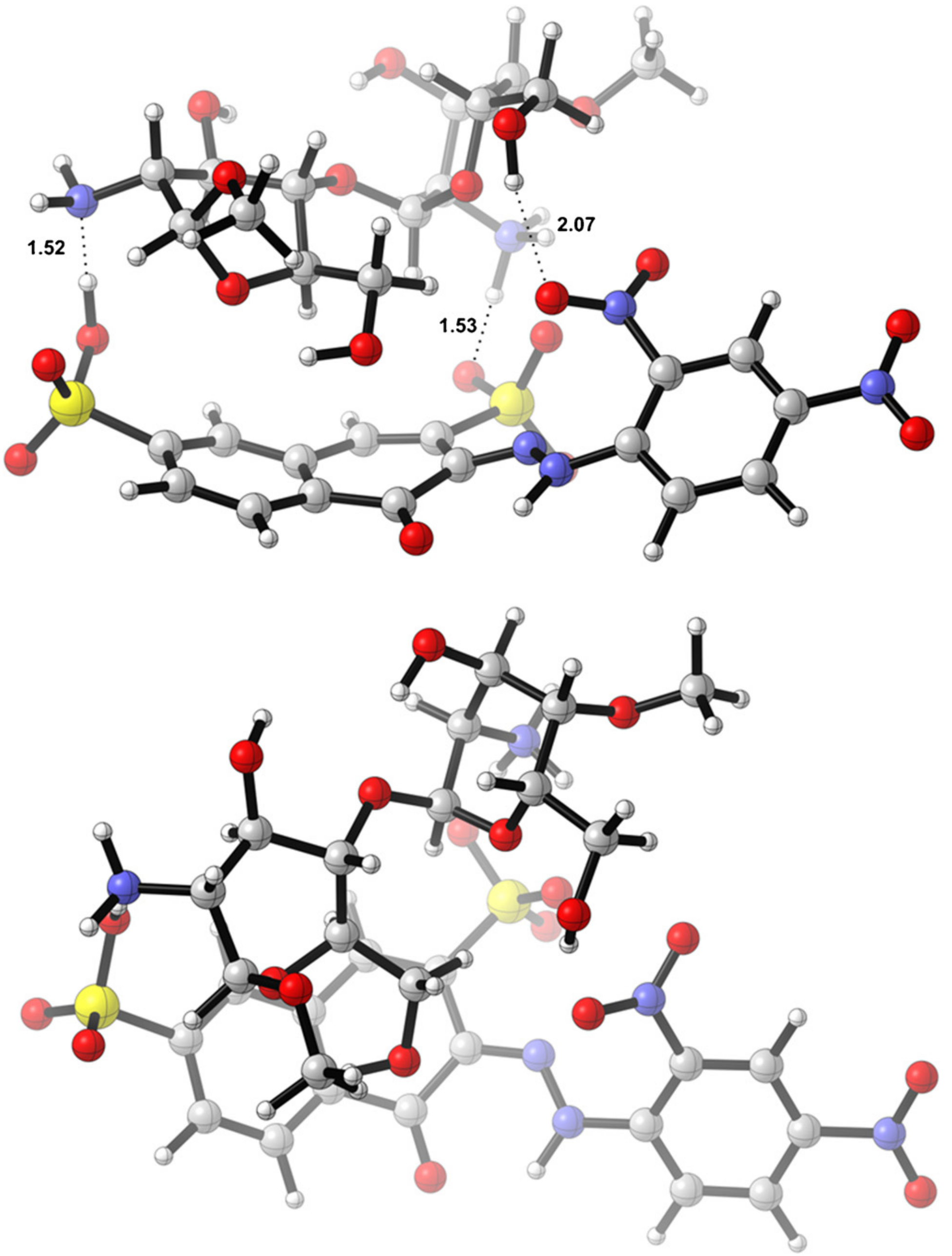

Fig. 7. Optimised structures of the NY–chitosan complexes (M06-2X/6-311G(d,p)).

841 kJ/mol obtained for the PCL model (Fig. 5d). This suggests that the chitosan amino groups get preferentially protonated to NH_3^+ in acidic circumstances and explains why a protonated structure of chitosan is used in our calculations. Optimised structures of NY interacting with the PCL and chitosan model systems are shown in Figs. 6 and 7.

Several interaction patterns of the NY–PCL system were explored, including hydrogen bonding of the PCL ester group oxygen with the NY hydrogen atom on the NY azo bond, but the geometry depicted in Fig. 6 was found to be most optimal. The ester group interacts via long range interactions with the chromophoric unit, thus, in combination with the hydrophobic properties of PCL, shielding the NY azo group from any interaction with water molecules. The hydrogen atom hence remains bonded to the azo group and no colour change can occur when NY is incorporated in PCL. The corresponding interaction Gibbs free energy (ΔG_{298}) was found to be −35.6 kJ/mol.

The ΔG-value in the case of chitosan (Fig. 7) is much higher, −132.3 kJ/mol, which strongly indicates that NY preferentially interacts with chitosan in the PCL/chitosan blend. As seen from Fig. 7, the interaction with chitosan differs from the interaction with PCL as it is a combination of electrostatic interactions between the sulphate groups of NY and the amino groups of chitosan and hydrogen bonds. In this conformation the halochromic azo group is not directly involved in the interaction and NY is thus still susceptible to deprotonation in alkaline environment. This, together with the hydrophilicity of chitosan, explains the present halochromic behaviour of PCL/chitosan nanofibres. The complexing agent used in this work is a polycationic chain. The same ionic bound with NY as in the case of chitosan can therefore be expected, leading to the halochromic response of PCL nanofibres to which not only NY but also the complexing agent is added.

The molecular modelling calculations help to reveal the essential nature of the interactions and to understand the experimental observations. This knowledge leads to a better understanding of the halochromic behaviour in terms of the environment and can eventually lead to a better application of halochromic dyes in the future.

4. Conclusion

Biocompatible PCL and PCL/chitosan nanofibres loaded with the pH-sensitive dye NY could be successfully obtained using the electrospinning technique. To minimize dye leaching, addition of a complexing agent was recommended. The morphology of the functionalised nanofibres was similar to the morphology of blank PCL and PCL/chitosan nanofibres.

Molecular modelling strongly suggested a different interaction of NY with PCL and chitosan polymeric chains. While the interaction with PCL shields the group responsible for the pH-sensitive behaviour, the interaction with chitosan – being stronger than the interaction with PCL – still allows for a halochromic response. Owing to a similar ionic interaction, the addition of the complexing agent also led to a pH-sensitive PCL nanofibrous structure.

The obtained PCL and PCL/chitosan nanofibres (with addition of complexing agent) showed a reversible halochromic transition, but significant differences between both polymeric systems were found. While a slow response was observed for the PCL structures, the PCL/chitosan samples demonstrated a rapid halochromic response. Moreover, the transition occurred in a sharp pH range from pH 4 to 6 for the PCL/chitosan nanofibres while a broad pH range was noticed for PCL. The increased hydrophilicity of the blend nanofibres was found to be responsible for these alterations. Thanks to the presence of chitosan, the interaction with water increases, thus allowing for a significantly faster and more sensitive response. The use of the polymer blend containing chitosan is thus essential for the development of rapid and sensitive pH-sensitive sensors ideally suited for medical applications.

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