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ABSTRACT: The absorptivities of polydiacetylenes (PDA) used in Langmuir films or vesicles for the development of PDA sensors films or other applications such as in non linear optics or for field-effect transistors are not known, so polymer contents cannot be deduced from experimental spectra. Here we introduce a novel method, using Nuclear Reaction Analysis (NRA) that allows quantitative determination of the polymer content X proportion of monomers that have been incorporated into PDA chains. We apply it to pentacosadiynoic acid (PCDA) evaporated microcrystalline films. A calibration curve giving X as a function of the area under an absorption spectrum normalized to the monomer areal

density is obtained for blue and red PCDA. The method is applicable to all kinds of films, and to other PDA, provided films with known molecular areal density are available. An example of application to a PCDA Langmuir film is given.

MAIN_TEXT

1 Introduction: motivation for this study.

The development of this method was initially motivated by the extensive current work on possible biological or environmental sensors based on the color transition of polydiacetylenes (PDA) in Langmuir films or vesicles, as discussed in several recent reviews [1-4]. Its application to Langmuir films will be illustrated in § 3-4. But it should be stressed that the method is general, applicable in principle to other types of films used in other types of studies or applications. For instance, PDA films and in particular films of PCDA or similar molecules, have been used in Field-Effect Transistors using Langmuir Blodgett (LB) films [5], or evaporated films [6] and even monomolecular films [5]. The present results can be used to determine the actual polymer content in such devices.

PDA chains, of monomer general formula R'C≡C-C≡CR where R and R' are side groups which may have widely different molecular formulas, can exist in at least two different electronic structures conventionally named the Blue (B) and Red (R) forms. B-type chains have a maximum absorption near $\lambda_{max} \approx 640$ nm and are not fluorescent (with a quantum yield at room temperature of the order of $10^{-5}$ [7]), while R-type chains have a $\lambda_{max}$ often near 540 nm, but it may be as high as 580 nm [10] or as low as 520 nm [11], and they are fluorescent, albeit weakly (quoted yield [10] 0.02), at room temperature [11-13].

A first order transition between B and R forms is observed in many crystalline DA and in Langmuirs films or vesicules. This transition is called ''color transition'' and it is the basis of sensor operation. This transition is often, but not always, irreversible. It can be triggered by various means, such as temperature or pressure. It also sometimes occurs during polymerization.

In the case of sensor applications, it is triggered by the binding of the species to be detected (molecule, ion, or a biological macromolecule) onto a selective group bound at the end of some of the DA molecules forming the film. For instance, in the paper which, as far as we know, initiated this type of research, an influenza virus was detected [14]. Note that the microscopic mechanism by which the transition is produced remains under debate. It was initially thought that the color change which results from a geometrical change of the PDA chain, was an order-disorder transition. However, perfectly ordered red chains are now known [15] with a room temperature absorption spectrum identical to that of red Langmuir films. So it seems more likely that red chains are ordered but not planar, having a twist similar to that known in several red PDA single crystals such as poly-THD [16, 17]. This is discussed in [18] following previous similar proposals [19, 20], but the question is not completely settled.

The sensor can be used to simply verify the presence of an analyte or for quantitative measurements. In film form, they are generally prepared as monolayer Langmuir films of the monomer which are then transferred onto a suitable solid substrate by various methods [21, 22]. Photopolymerization can be carried out either in the Langmuir trough or after transfer, and can easily be monitored spectroscopically, as can the color transition, since PDA of both B and R forms strongly absorb in the visible (examples of spectra are given Figure 1). It is often observed that the B-R transition occurs spontaneously at some stage during photopolymerization [23-25] while for sensor use it is obviously required that the film still be in the blue phase, so for use as a sensor irradiation must be stopped before onset of the color transition, at partial polymerization. Moreover the question of the polymer content is usually not mentioned, and when it is, the polymerization is assumed to stop at completion [26, 27]. Unfortunately, we have shown

previously that, at least in the case of poly4BCMU, the reaction practically stops around 50% [28].

It would be useful to be able to deduce a film's polymer content at any stage of the polymerization, for any polymer color, from an experimental absorption spectrum. Unfortunately, the absorptivity of the PDA used in these applications is generally unknown (this is so for the most used materials in sensors, pentacosadiynoic acid or tricosadiynoic acid and their derivatives), so absorption spectra do not allow calculation of the polymer content X of the film. X is the fraction of the total mass which is polymerized. This is also the ratio of number of monomers that have been incorporated in a chain to the initial total number of monomer, since the reaction occurs with no loss of atoms. Indeed, these PDAs are generally totally insoluble, so the method that has been used to determine the absorptivities of the few soluble PDAs and which requires calibration via a solution [28,29], cannot be used.

The motivation of the present work is therefore to develop a method, applicable to any PDA, for determination of absolute absorptivity, so as to provide a simple method for deducing X in any type of film from quantities easily read on an absorption spectrum, both in the B and R forms. This method uses NRA (Nuclear Reaction Analysis see Supporting information F) in a way that has never been used before. This method allows a quantitative measurement of the areal density of one type of atoms (in our case Carbon) in a film. It is generally used for determination of areal densities of other types of atoms such as oxygen in inorganic films for instance in microelectronics.

The paper is organized as follows: in §2 the sample preparation and the different techniques used are described. In §3.1 the principle of the NRA method and its implementation are presented. In order to validate the method, it is applied in §3.2 to a soluble PDA, poly-4BCMU,

for which absorptivity has been determined previously [28, 29], showing that the method indeed yields the correct absorptivities. Blue and red pentacosadiynoïc acid (referred to below as PCDA, an often used acronym in the literature) is then studied in §3.3, and in §3.4 means are given to deduce a polymer content X from quantities read on an experimental absorption spectrum obtained in the case of PCDA Langmuir films.

## 2 Materials and methods

### 2.1 Materials

4BCMU monomer was synthesized in the laboratory by the standard method [30] and purified by column chromatography. 10-12 pentacosadiynoic acid was purchased from Aldrich and used without further purification except removal of residual polymer by filtration of solutions used for Langmuir films preparation. The molecular structure and the polymerization scheme are given in Supporting Information A and B.

### 2.2 Sample preparation.

*Polycrystalline thin films:* (thickness from 30 to 250 nm) were prepared by vacuum evaporation of the monomer from a quartz crucible on 15mm x15mm precleaned glass substrates using a home-made evaporation chamber at a base pressure of $1.0 \pm 0.2\ 10^{-7}$ Torr. Glass was cleaned using the previously described protocol [28]. Deposited mass was measured by a quartz microbalance (Maxtek, model TM-350). Since the quartz is not positioned exactly at the same place as the substrates, the ratio of the deposition rates (tooling factor) was calculated geometrically.

In each evaporation 4 films were simultaneously prepared. To make sure that they would be identical, the sample holder was rotated around a central vertical axis (period ≈ 2s). Similar deposition rates of 14 ± 2 Å/s and substrate temperatures of 20°C were chosen in all cases. This

ensures the same balance between nucleation and growth of the crystallites, leading to the same crystallite size range. An AFM image is shown in Supporting Information C. After preparation the samples were kept at -20°C in the dark until use.

In each set of 4 films, one was kept unirradiated to serve as a reference for film thickness as measured by Nuclear Reaction Analysis NRA (see below) and the others were photopolymerized.

*Langmuir films:* Concentration of spreading solutions is typically $2 \times 10^{-3}$ mol/L in chloroform. A few microliters of the solution were deposited onto the air/liquid interface of a Langmuir trough equipped with a double movable barrier for film compression. The surface pressure was measured according to the Wilhelmy plate method. The plate was made of filter paper and the measurement device was a microbalance from Riegler&Kirstein GmbH (Germany). The subphase consisted of Milli-Q Millipore ultrapure water (18.2 MΩ·cm). The temperature was regulated at ± 0.5°C using a circulating water bath. All isotherms were performed at a constant compression speed of 9 $Å^2$/mol/min. The typical duration of a compression is about 40 min.

*Polymerization:* For evaporated films, photopolymerization was carried out either at 254 nm (bandwidth 5 nm) in a Cary 5000 spectrometer for small polymer content, or in a home-made irradiator using a high pressure Xe lamp (Oriel), a water filter to absorb unwanted IR light and a 45° dielectric mirror for unpolarized light (Melles Griot type KRF-2037-45-UNP) to preferentially select the range 230-270 nm. The measured ratio of the polymerization efficiencies of the two methods is about 60, with 1 hour in the Cary is approximately equivalent to 1 minute in front of the Xe lamp. A low pressure Hg lamp (100W – Ulice optronique) essentially emitting the 254 nm line was used for in situ polymerization of Langmuir films. The used wavelengths

are exclusively absorbed by the DA group, other parts of the molecules only absorb at significantly shorter wavelengths.

*Removal of residual monomer:* At the end of the irradiation, films still contain between 30 and 90 % monomer, which has to be removed before the NRA experiment, without removing any polymer. Two methods were tested: high temperature evaporation under vacuum or selective dissolution at room temperature in an appropriate solvent. They gave identical results, but there is a risk of incipient degradation of the polymer at high temperature which would affect the use of red spectra for determination of polymer as described below. So all data presented here used the dissolution method. Note that dissolution of residual monomer has been a standard method for determining the polymer concentration in a partially polymerized samples (crystal, powdered film) [31-35], and therefore the polymerization kinetics. Nevertheless, since the method has not been specifically validated for PCDA, its validity was checked as detailed in Supporting Information D.

Removal of some polymer (for instance relatively short oligomers if any are formed) could be a problem with the dissolution method. But comparison with the results of removal by evaporation, where no polymer is evaporated, showed this not to be the case. Also, the solvents used for dissolution remained colorless.

For dissolution, a glass slide covered with a film was put in a Petri dish and solvent was carefully added until the slide was covered under several millimeters of solvent. To determine the optimum procedure leading to total dissolution of the monomer several groups of identical samples were tested varying one parameter at a time: time of contact with the solvent and choice of the solvent. Acetone is a good solvent of both monomers, but it does not wet 4BCMU well, so capillary forces might degrade the film; therefore the following procedure was used. The slide is

first immersed in n-Hexane, a solvent of both monomers chosen for its low surface energy; about twice as much acetone is then carefully added and the dish is repeatedly gently agitated for 1 hour at room temperature. It was checked that the same results were found after 10 minutes of contact and this was taken as indicating that at 1 hour complete monomer dissolution is achieved. More details are given in Supporting Information D.

Before dissolution of residual monomer all polymer chains were in the blue phase, whatever the polymer content. After, they were all red (see Figure 1), in the process the polymer chains undergo the blue to red phase transition. In fact in 4BCMU and PCDA the blue polymer phase is a metastable one which transforms into the stable red one thanks to the free volume generated by dissolution of the monomer. As we will see in §2.3 this conformational transition has no influence on the NRA experiment, only the number of carbon atoms is determined and this transition does not change this number. The aim of the method is to create a calibration curve giving the polymer content versus an easy to read parameter on an absorption spectrum. Thanks to this color transition occurring when the monomer is removed, with the same NRA experiment two calibration curves can be obtained, one for the B conformation, the other for the R conformation as described below in §3.1.

2.3 Techniques

*Absorption spectra:* All spectra of evaporated films were taken using a double beam Cary 5000 spectrometer, typically between 350 and 800 nm: polymer films are transparent beyond 800 nm, and glass would begin to absorb below 320 nm. Blue polymer absorption is very small below 350 nm, and red polymers only show a weak residual absorption there. Since spectra are broad a 2 nm resolution was chosen, but it was checked that higher resolution leads to identical

spectra (but with more noise). The final spectrum corresponds to the polymer content to be measured by NRA.

For Langmuir films, absorption spectra are obtained in-situ using a bifurcated fiber made of 6 illumination fibers connected to a halogen light source (HL2000 Ocean Optics) around a read fiber connected to a UV-Vis spectrometer (Avantes avaspec-2048x64). A mirror is placed in the Langmuir trough prior to deposition. The transmitted light is collected, after reflection on the mirror, via the same optical fiber and analyzed by the spectrometer (see scheme in Supporting Information E). The reference spectrum is taken on the compressed monomer just before irradiation.

*Nuclear Reaction Analysis (NRA):* A general introduction to the method is provided in Supporting Information F. Measurements were performed in a home-made dedicated vacuum chamber, using dry pumping to avoid carbon contamination during the experiments, installed at the Tandem van de Graaff accelerator ALTAIS of the Laboratoire d'Analyses par Réactions Nucléaires at the University of Namur, Belgium. It delivered stable deuteron beams in the energy range 850-970 keV used in the experiments. Films were mounted with their surface perpendicular to the deuteron beam direction, on a sample holder containing typically 20 to 25 samples which could be studied in the same run. NRA is a method for counting carbon atoms, irrespective of their chemical state. It is also insensitive to the organization and structure of the studied material. In this way one obtains an areal density of carbon atoms $N_C(cm^{-2})$ and molecules $N_m$ $(cm^{-2})$ in a film. Once this is known, the absorption spectrum of the film can be used to compute absolute absorptivities, or the area of the absorption spectrum, which is related to the oscillator strength of the electronic transition corresponding to the absorption.

Here we use the $^{12}C(d,p_0)^{13}C$ nuclear reaction, meaning that the reaction of a deuteron with a $^{12}C$ nucleus generates a high energy proton which is counted by a suitable detector, and a stable $^{13}C$ nucleus. Practically, absolute numbers are obtained by comparing the number of detected events for a given deuteron fluence to that of a suitable reference containing a known areal density of atoms, for the same fluence and in the same geometry. But carbon references are not stable enough (they can lose carbon by radiation damage or gain carbon via contamination), so a very stable $^{16}O$ reference is chosen, using the $^{16}O(d,p_1)^{17}O$ reaction.

The cross sections of the two reactions have been determined; they depend on the incident deuteron energy and the geometry used for detection [36]. Here we used the following conditions: energy of the incident deuterons 854 keV for the $^{16}O$ reaction used for reference, and 968 keV for the $^{12}C$ reaction used for studying the film. These energies were chosen so that the corresponding reaction cross section is only slowly varying in the vicinity of the chosen values, so that small calibration errors of the accelerator energies are irrelevant. The ratio of cross section of these carbon and oxygen nuclear reactions is in these conditions 5.56 [37].

Emitted protons (which have high energies) were detected at a 150° scattering angle. Other particles are also emitted in that direction, for instance elastically backscattered deuterons, but their energy is lower. All such particles, but not the protons, were stopped in a 13 microns Mylar film placed in front of the detector. For each measurement, a complete energy spectrum of the detected protons was recorded, and the peak corresponding to the reaction of interest was selected and integrated. In all cases, background counts at the peak positions were negligible.

*Application to the present study:* to avoid heating the films, the deuteron beam current was limited to 2-4 nA in a rectangular beam of cross section 2 mm x 2 mm. Usually, the total deuteron fluence ranging from less than 1 to about 2 μC, was given in fractions of 0.2 or 0.5 μC

with a short waiting time in between. There are two reasons for fractionating the fluence: to limit heating, and to allow evaluation of possible degradation, which may be corrected for it where necessary by a linear regression of the data to zero dose. Note that, since the chemical nature of the carbon atom is irrelevant in NRA, only degradation processes leading to evaporation of a carbon-containing fragment are detected here. Other degradation processes, which indeed occur, are unimportant. These precautions were unnecessary for the oxygen reference, for which a fluence of 10 μC was usually chosen in a single step.

However, carbon contamination being omnipresent, it has to be taken into account. Contamination was measured in two ways. First, carbon atoms were counted on a part of the slide not covered with the material during evaporation. Second, counts on monomer films of different thicknesses were compared. The mass measurement by the quartz balance is very accurate, so since the raw counts are not exactly in the ratio of the thicknesses, it was assumed that the difference came from contamination. Both methods gave reproducible and consistent results, leading to a contribution from contamination to the measured NRA signal of ≈ 150 counts per μC. This was subtracted from all experimental counts.

*Uncertainties:* the nuclear reaction being a statistical process, there is a statistical uncertainty on the count values recorded. In our experimental conditions, these were 1 to 2 %, except for films of small thickness and low polymer content where it could reach 3 %. Since X is simply the ratio of two counts obtained in identical conditions, the uncertainty on it has no other source.

However, other factors enter in the determination of $N_C$. There is a 1.5 % statistical uncertainty on the areal density of $^{16}O$ atoms in the reference. There are also a number of non statistical uncertainties which identically affect these two quantities. The factor 5.56 is only known to ± 3

%. The number of molecules per unit area (the so-called areal density) N is calculated as follow equation 1:

$$N = \frac{N_{C\_cor}}{N_0} . \frac{\sigma_0}{5.56} . \frac{1}{n_c} \text{N} \quad (1)$$

$N_{C\_cor}$ is $N_C$ corrected by removing the carbon contamination (150 counts/μC) and multiplying by 1.011 to take into account the natural abundance of the $^{13}C$ isotope, since only $^{12}C$ is determined in the NRA measurements. $N_O$ is the number density of oxygen atoms per unit area counted by NRA, $\sigma_O$ the areal density of $^{16}O$ reference and $n_C$ the number of C atoms in the molecule of interest.

3 Results and discussion

3.1 NRA method to determine the polymer content-calibration curve

The polymer content obtained by NRA $X_{NRA}$ is the ratio $N_p/N_m$, where $N_m$ and $N_p$ are the molecular areal densities (number of molecules per unit area in the total thickness of the film) in a film before and after removal of the monomer. In the polymer we mean by "molecule" a repeat unit of the polymer chain which has the same molecular formula and therefore the same number of carbon atoms as the monomer molecule. Rather than performing the two NRA measurements on the same film (with the risk of unwanted degradation on handling, particularly during the dissolution step) we chose to proceed as follows: as mentioned before, four films are simultaneously prepared. They should be identical, and this was indeed checked in two ways: they give the same NRA results, and after UV irradiation for the same duration their absorption spectra are identical. Therefore, in each series of 4 films one was kept intact and its NRA result gives the areal density $N_m$ valid for the four films. The three others were irradiated and their spectra recorded just after irradiation and before removal of residual monomer (a blue spectrum is obtained). The residual monomer was then removed leaving a red, pure polymer film and the

corresponding red spectrum was recorded. This film was then studied by NRA giving a molecular (repeat unit) areal density $N_p(cm^{-2})$.

The next step is to determine a quantity, related to the polymer content, easily measurable on such absorption spectra. The maximum of absorption or the second lower peak corresponding to the vibron associated to the double bond stretch vibration could be chosen. However these quantities may be affected by changes in absorption lineshape. Indeed, it is observed that the halfwidth of the longer wavelength band increases with polymer content, so its height becomes correspondingly smaller. So we shall consider a more global quantity: the area Σ under the spectrum plotted in wavenumbers. This area is proportional to the oscillator strength, a quantity characteristic of the electronic transition (it is well established that all the absorption in the visible and near UV of blue as well as red PDA corresponds to a single excitonic transition [38-39]), so it is a physically meaningful quantity independent of variations in spectral shape. This would not be the case for a spectrum plotted in wavelengths. Considering that the majority of published spectra are given between 400 and 800 nm this spectral interval was chosen here for the determination of Σ. Finally the calibration curve is obtained plotting $X_{NRA}$ as a function of $\Sigma/N_m$, where Σ is normalized to the molecular areal density which is directly related to the thickness of the sample.

3.2 Validation of the NRA method on 4BCMU

The diacetylene (DA) chosen to test the NRA method is known as 4BCMU (of side group formula $-(CH_2)_4$-OCONH-$CH_2$-$COOC_4H_9$ see Supporting Information A). The corresponding PDA has been much studied (for reviews see [38-39]), partly because it is among the few soluble PDAs [29]. Thanks to this property a method to determine the polymer content $X_{abs}$, knowing an absorption spectrum has already been described in [28], where the peak absorptivity of blue

poly-4BCMU crystalline films has been determined: $\alpha_B = 2.62 \pm 0.13\ 10^5\ cm^{-1}$ at room temperature.

In §3.1 an alternative and independent method giving the polymer content $X_{NRA}$ has been described. This method is not based on the solubility of the PDA. In the present work twelve 4BCMU films, of thicknesses 100 to 250 nm, were prepared (plus 4 pure monomer reference films) and photopolymerized. Irradiation durations were from 1.4 to 60 minutes, corresponding to polymer contents $X_{abs}$ from 0.08 to 0.42; as shown previously, 4BCMU photopolymerization practically stops near $X_{abs} \approx 0.5$. Spectra up to the end of irradiation are typical blue ones (Figure 1).

The residual monomer was extracted as described above. The absorption spectra recorded after extraction are typical red ones (Figure 1). We have prepared and polymerized, but not studied by NRA, several films with thicknesses between 2.7 nm (one monolayer) [40] and 350 nm. For all thicknesses absorption spectra are very similar so the conclusions drawn from the NRA will be valid for films of all thicknesses.

Figure 1 shows that the maximum absorbance of the same film is much smaller in the red phase than in the blue one. The ratio of red to blue absorbance is 1.8 so the maximum absorptivity of red poly-4BCMU is $\alpha_R = 1.45\ 10^5\ cm^{-1}$ at room temperature. However, the areas $\Sigma$ under the two spectra, calculated between 12500 and 25000 $cm^{-1}$, are almost equal, meaning that the oscillator strengths of the two transitions are almost equal, but this does not imply that the peak absorptivity will be the same, since the spectra have different shapes.

From the NRA results, $X_{NRA}$ values for all samples could be calculated as the ratio of the counting rate on the film to that on the corresponding reference monomer film. The statistical uncertainties are from about 1 % at high polymer content to about 3.5 % for the smallest one.

The values of $X_{NRA}$ obtained in this way are compared on Figure 2 to those calculated from the absorption spectra using the value of $\alpha_B$ known with ± 5% uncertainty and the carbon atom areal densities measured by NRA with uncertainty ± 1%. They are equal within the experimental uncertainties: as shown on Figure 2, the results are well fitted by a straight line of slope 1.01 ± 0.02 with intercept at zero. This validates the NRA method for determining polymer content.

A calibration curve can thus be obtained for 4BCMU. Figure 3 shows $\Sigma/N_m$, $\Sigma$ is normalized by the surface density $N_m$, to obtain a curve useful whatever the thickness of the samples, versus the polymer content. $N_m$ varies from 14 to 32 $10^{15}$ molecules/cm$^2$. Data have been fitted by a straight line of slope 228 ± 7 $10^{-15}$ cm/molecule with intercept at zero. Such a curve is not necessary in the case of 4BCMU because it is easier to use the peak absorptivity $\alpha$, but in many PDA $\alpha$ is not known because the polymer is not soluble [28]. A similar data treatment can be done on red chains. The red and blue slopes are equal when the same frequencies range is used [12500-25000 cm$^{-1}$]. Since the red spectrum doesn't decrease to zero at 12500 cm$^{-1}$, the slope is slightly underestimated. If a larger range [12500-28500 cm$^{-1}$] is used, the new slope becomes: 250 ± 20 $10^{-15}$ cm/molecule. The NRA method can be used on insoluble PDA, and we shall use it on one of the most studied PDA, 10-12 pentacosadiynoic acid (PCDA) in the next section.

3.3 Polymer content in PCDA thin films: calibration curve

The same procedure as described on 4BCMU is performed on PCDA films. The blue and red spectra of evaporated PCDA film are shown in Figure 1. Again, the value of $\Sigma$ is obtained by integration from 800 nm (12500 cm$^{-1}$) to 400 nm (25000 cm$^{-1}$). The values of the molecular areal density of the samples vary between 5 and 16 $10^{15}$ molecules/cm$^2$. The result $\Sigma/N_m$ function of $X_{NRA}$ for blue chains is given Figure 4, the data are fitted by a line of slope 197 ± 3 $10^{-15}$

cm/molecule and zero intercept. The slope is a little bit smaller than that obtained for 4BCMU suggesting that the oscillator strength of the transition is slightly smaller.

It is now possible to determine the polymer content of B type chains for a given absorption spectrum and obtain polymerization kinetics. Figure 5a shows a series of absorption spectra recorded during photopolymerization of a 150 nm thick film in the same conditions as in [28], and Figure 5b shows the time variation of the deduced polymer content, compared to the peak absorption variation. An integration extending further in the UV would slightly increase the difference.

Often one wants to know the polymer content at the end of an experiment where all chains are in the red phase. The method described for the blue chains can be used exactly in the same way. However, the red absorption spectrum does not fall to zero at 400 nm. To draw a calibration curve for red chains we have chosen to calculate $\Sigma$ between 12500 and 25000 $cm^{-1}$ as for the blue chains, since this is the range almost always used in the literature, so the calculated values slightly underestimate the total area and oscillator strength. The result is given Figure 6. The slope of the line obtained is $216 \pm 4\ 10^{-15}$ cm/molecule, a little bit higher than that obtained in the blue case. When the experimental absorption is the sum of both blue and red chain spectra an approximate value of the total polymer content can be deduced using the average of the slopes of the two calibration curves.

3.4 An example of application to PCDA Langmuir films.

The calibration curves have been obtained using evaporated thin films; these curves can be used on other kind of PCDA samples provided that the absorption spectra have the same shape. The photopolymerization of a trilayer of PCDA at 25mN/m corresponding to an area of 0.08 $nm^2$/molecule and so an areal density $N_m = 1.25\ 10^{15}$ molecules/$cm^2$, on pure water subphase has

been studied by absorption. These conditions are identical to those used by Lifshitz et al. [41] in their determination of the crystal structure of a PCDA trilayer. Experimental details of our experiment are given in Supporting Information E. An example is given Figure 5a, added in bold for comparison to a series of PCDA microcrystalline film absorption spectra. From this spectrum an X of 30% is deduced using the calibration obtained above. We note in passing that the reactivity for photopolymerization is very similar on microcrystalline and Langmuir films (see supporting information E Figure E3). From these two data points, the use of the color ratio and the problem of the apparent concentration can be discussed.

To quantify the color transition, a quantity called the color ratio (CR) is generally used. It was initially introduced by Charych [14,42] as equation 2:

$$CR = \frac{B_0 - B_V}{B_0} \times 100 \quad (2)$$

where $B_0 = \frac{I_0(blue)}{I_0(blue)+I_0(red)}$ and $B_V = \frac{I_V(blue)}{I_V(blue)+I_V(red)}$ , where $I_0$(blue) and $I_V$(blue) are the absorbances at the absorption maximum of blue type polymer chains before and after a color transition, with $I_0$(red) and $I_V$(red) for the red type polymer chains. It is an empirical quantity introduced for its large dynamic range between 0 and 100%. This quantity has to be experimentally calibrated (and it was in these papers). However it has sometimes been equated by others to the relative concentration of red chains, which is not generally true except that it is indeed 0 for a pure blue film and 1 for a pure red one. This is because the maximal absorbance of the two types of chains may not be equal, and in fact they are not in PCDA (and 4BCMU) as may be seen in Figure 1 and the blue absorbance at the wavelength of maximal red absorption is not negligible; in fact it amounts to ≈ 40 % of the maximal blue absorption in the same spectrum.

As already shown [28] a strong slowing down of the photopolymerization rate (Figure 5b) occurs, so that an apparent saturation of the reaction may not correspond to a complete

polymerization. It is indeed the case for PCDA crystal or Langmuir film (see Supporting Information E). The discussions on the blue to red transition in sensors have to take into account the fact that polymer films are potentially constituted of up to 40-50% of monomer.

## 4 Conclusion

This paper introduces a new method for quantifying the polymer content X in a PDA film, using numbers directly given by its absorption spectrum (it should be noted that the peak absorbance of a spectrum does not give a good quantitative measure of X, except possibly at small X. In view of the current activity in PDA sensor development, we have therefore applied the method to obtain a calibration curve for PCDA, a much studied material in the sensor context, however the method is more general.

The method makes it possible to determine X at any stage of the polymerization. It has often been reported in the literature that a spontaneous, unwanted (in fact detrimental), blue to red color transition occurs [23, 27] during photopolymerization. The question of the polymer content at which this transition occurs is never mentioned. The PCDA calibration ($\Sigma/N_m$ as a function of X) obtained with the novel method presented here our method allows determination of X at the stage where this transition occurs. Such a calibration curve can be obtained for other polydiacetylenes using NRA and used for other types of films. Calibration was obtained for both blue and red PDA films. For each of the two materials studied here, 4BCMU and PCDA, the blue and red results are very close despite the large difference in spectral shapes, indicating very similar values of the total oscillator strengths of the excitonic transitions.

It had already been shown [28] that in evaporated films of 4BCMU, photopolymerization does not go to completion. This had been explained by efficient quenching of a photoexcited monomer via energy transfer to a neighboring existing chain. In the present work it is observed

that photopolymerization remains also incomplete in PCDA evaporated films, as shown in Figure E3 in Supporting Information E. These photopolymerisation kinetics were obtained from the polymer contents calculated using the calibration for PCDA obtained in the present work (Figure 4). Since the quenching mechanism present in 4BCMU is in principle a general process, we suggest that it also applies to explain the incomplete polymerization of PCDA. Moreover, a similarly obtained polymerization kinetics of a Langmuir trilayer film is also shown in Figure E3 with the same saturation as for evaporated films, presumably due to the same mechanism.

FIGURES

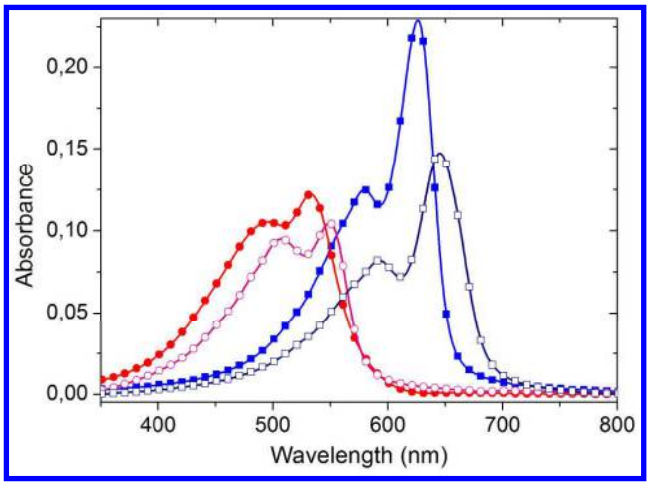


**Figure 1**: Absorption spectra for evaporated polycrystalline films of 4BCMU and PCDA of the blue and red polymer chain type, with $N_m$(4BCMU)=14.$10^{15}$ molecules/$cm^2$ (~100nm) and $N_m$(PCDA)=5.3 $10^{15}$molecules/$cm^2$ (~35nm). Solid and open squares: blue 4BCMU and PCDA respectively; solid and open circles: red 4BCMU and PCDA respectively.

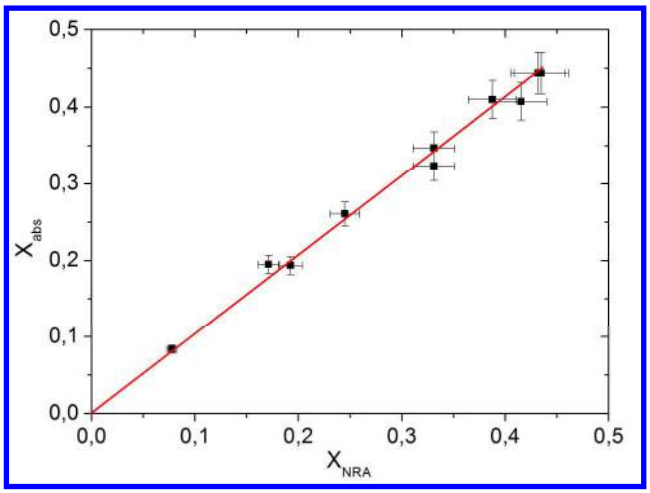


**Figure 2**: comparison of the polymer content determined using the absorption spectra: $X_{abs}$ and using the NRA method: $X_{NRA}$ in evaporated 4BCMU polycrystalline films. Data are fitted with a straight line of slope 1.01 ± 0.02 with intercept at zero.

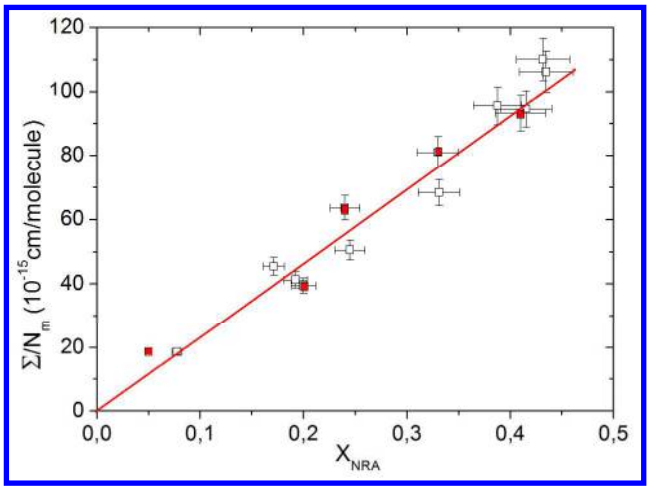


**Figure 3**: 4BCMU calibration curve using the NRA method: $\Sigma/N_m$ as a function of $X_{NRA}$ for the blue and red chains. When the same wavenumber range (12500-25000 $cm^{-1}$) is used to calculate $\Sigma$, similar data are obtained for blue (open squares) and red (solid squares) chains: slope 232 ± 9 $10^{-15}$ cm/molecule and intercept zero.

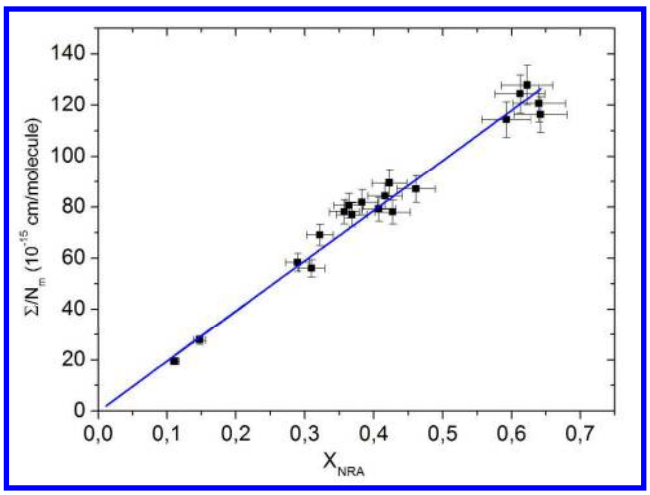


**Figure 4**: PCDA calibration curve using the NRA method: $\Sigma/N_m$ function of $X_{NRA}$ for the blue chains. Slope 197 ± 3 $10^{-15}$ cm/molecule and intercept zero.

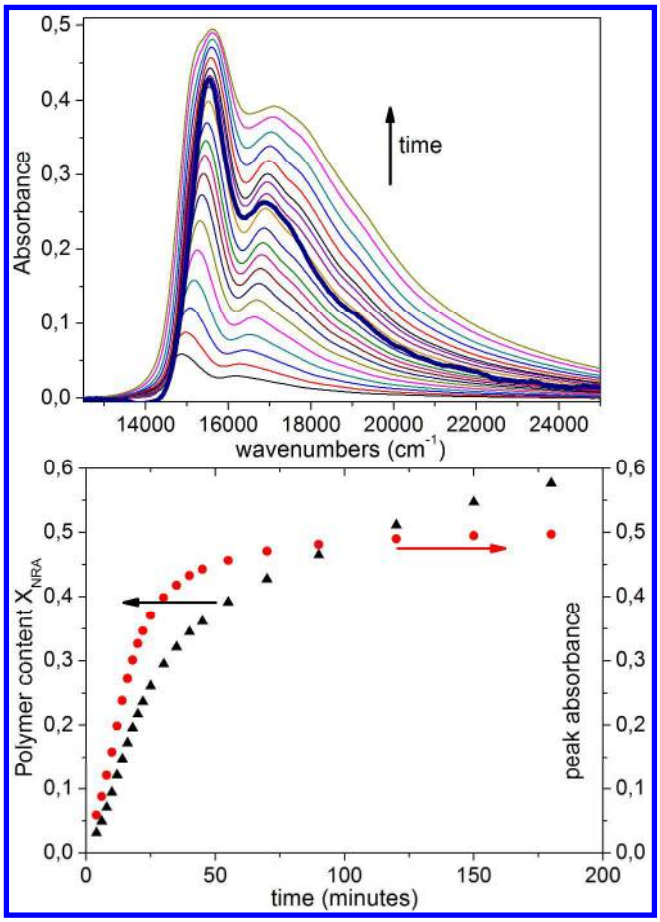


**Figure 5**: a) absorption spectra of microcrystalline PCDA film (150 nm) as a function of UV polymerization time. In bold the absorption spectrum of a trilayer Langmuir film of PCDA corresponding to a polymer content of 30% is added. This spectrum has been multiplied by a factor 20 b) PCDA microcrystalline film: polymerization kinetics using the peak absorption (closed circles) and the polymer content (closed triangles).

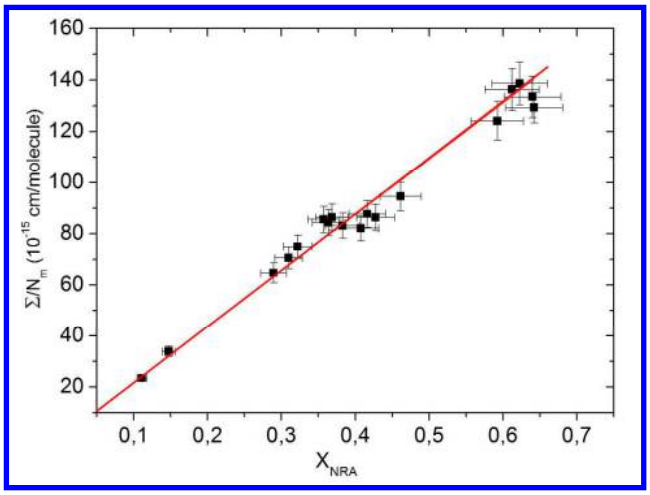


**Figure 6**: PCDA calibration curve using the NRA method: $\Sigma/N_m$ as a function of $X_{NRA}$ for the red chains. Slope 216 ± 4 $10^{-15}$ cm/molecule and intercept zero.

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**Author Contributions**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ASSOCIATED CONTENT

Supporting Information

A- Molecular structure of 4BCMU and PCDA

B- Scheme of topochemical polymerization of diacetylenes

C- AFM of PCDA microcrystalline films

D- Dissolution method to remove the monomer.

E- Isotherms and in situ absorption of PCDA Langmuir film.

F- Nuclear Reaction Analysis (NRA).

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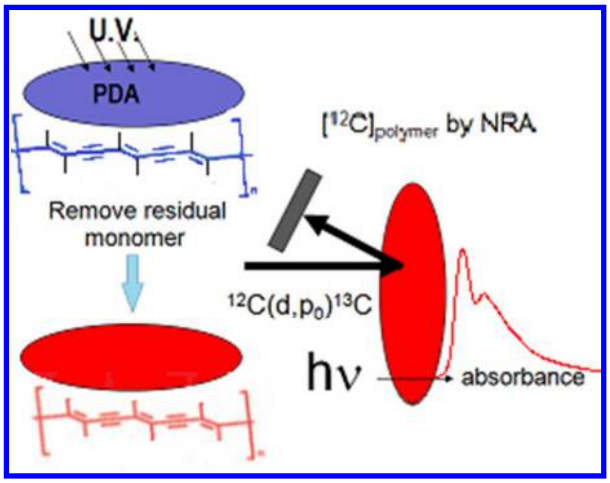
Table of Contents Graphic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hν
absorbance

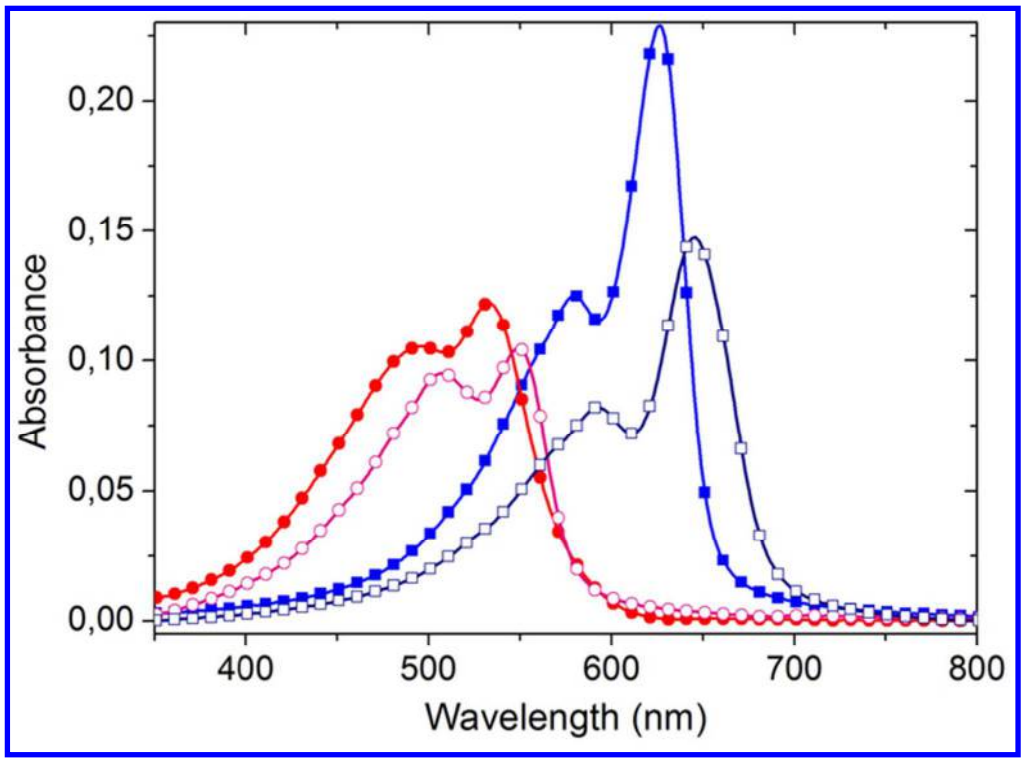


Figure 1: Absorption spectra for evaporated polycrystalline films of 4BCMU and PCDA of the blue and red polymer chain type, with $N_m$(4BCMU)=14.$10^{15}$ molecules/$cm^2$ (∽100nm) and Nm(PCDA)=5.3 $10^{15}$molecules/$cm^2$ (∽35nm). Solid and open squares: blue 4BCMU and PCDA respectively; solid and open circles: red 4BCMU and PCDA respectively.

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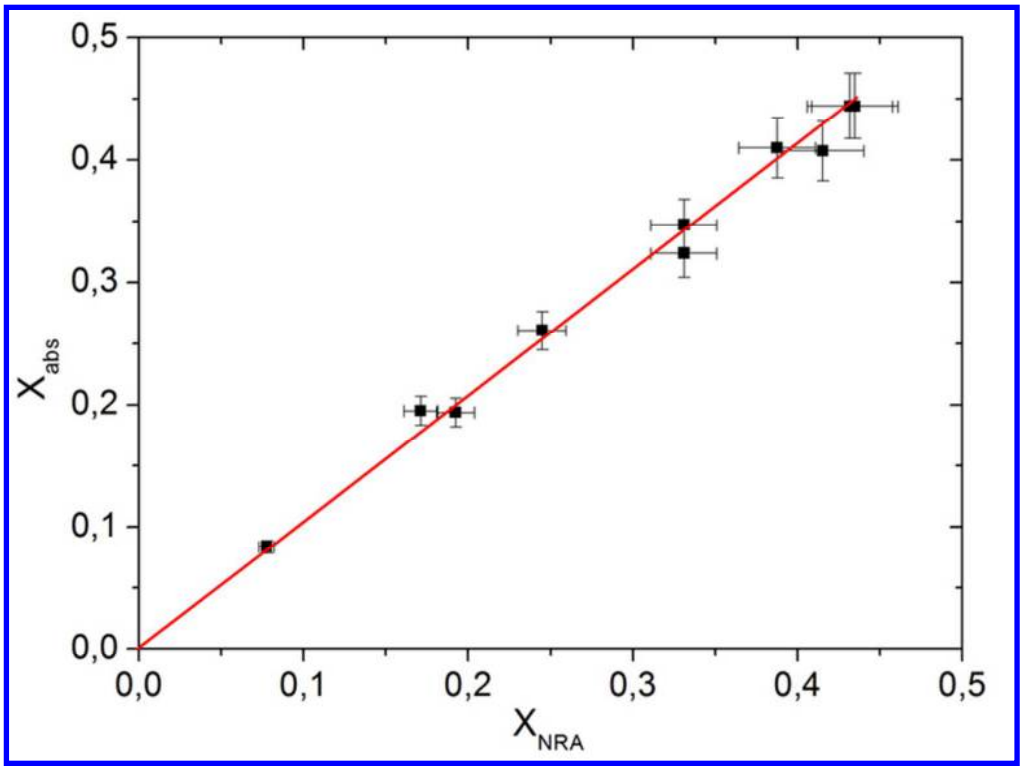


Figure 2: comparison of the polymer content determined using the absorption spectra: Xabs and using the NRA method: XNRA in evaporated 4BCMU polycrystalline films. Data are fitted with a straight line of slope 1.01 ± 0.02 with intercept at zero.

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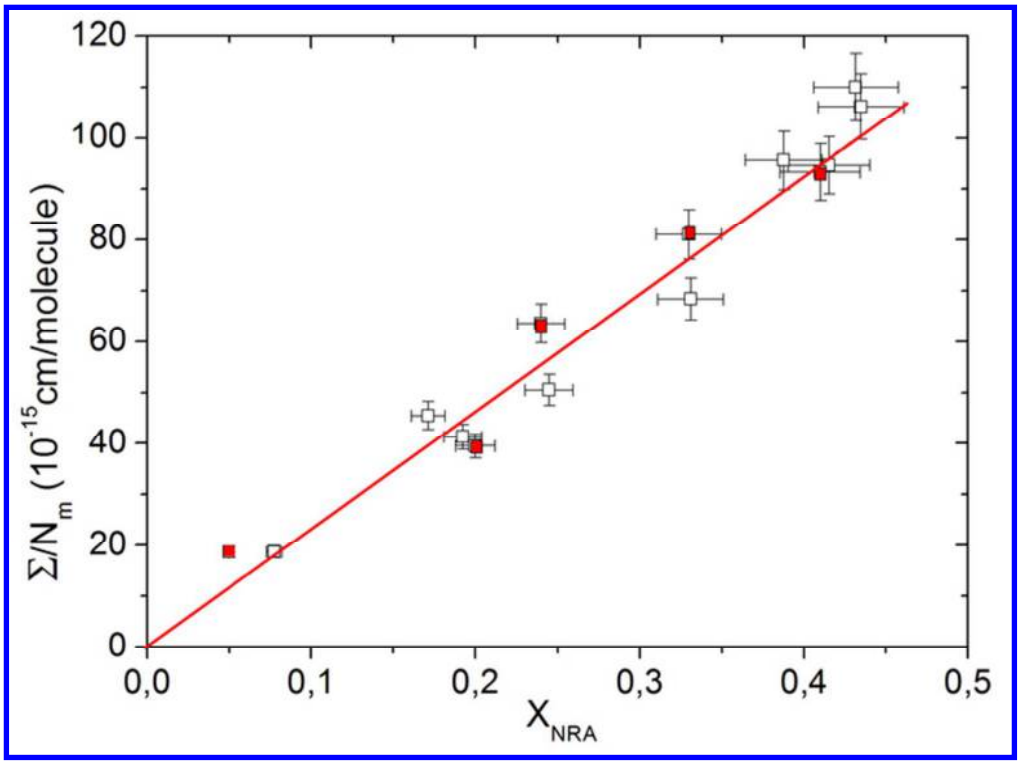


Figure 3: 4BCMU calibration curve using the NRA method: Σ/Nm as a function of $X_{NRA}$ for the blue and red chains. When the same wavenumber range (12500-25000 $cm^{-1}$) is used to calculate Σ, similar data are obtained for blue (open squares) and red (solid squares) chains: slope 232 ± 9 $10^{-15}$ cm/molecule and intercept zero.

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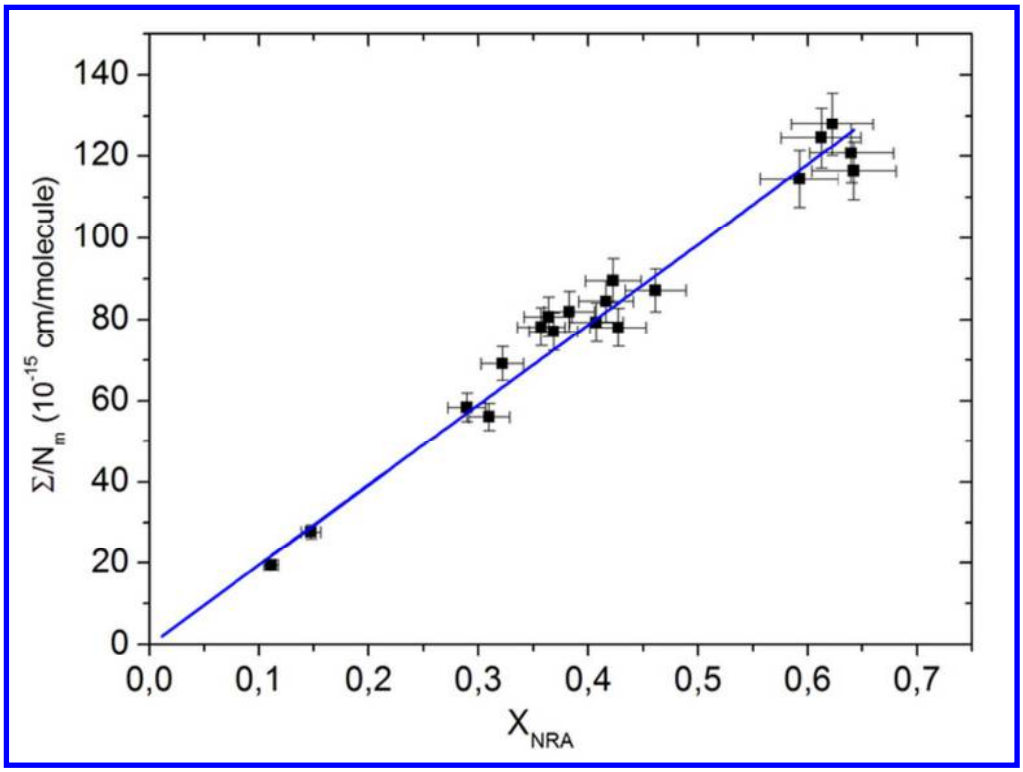


Figure 4: PCDA calibration curve using the NRA method: Σ/Nm function of $X_{NRA}$ for the blue chains. Slope 197 ± 3 $10^{-15}$ cm/molecule and intercept zero.

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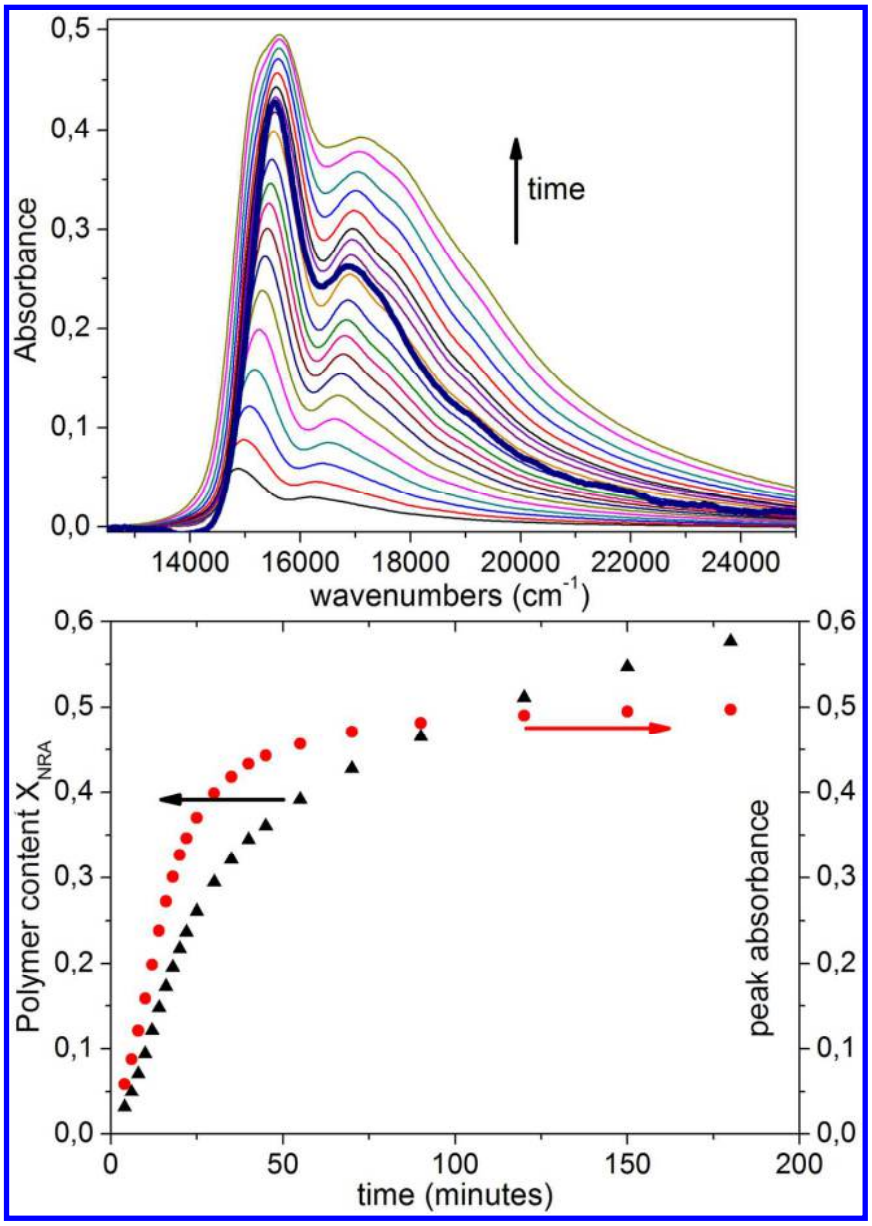


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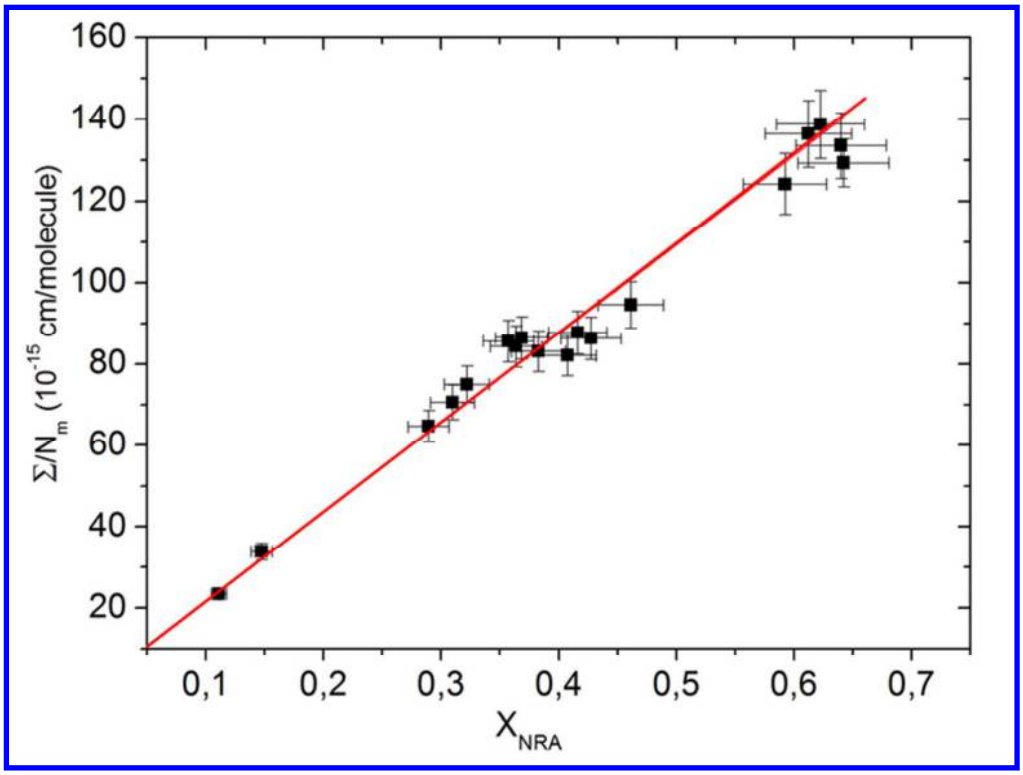


Figure 6: PCDA calibration curve using the NRA method: $\Sigma/N_m$ as a function of $X_{NRA}$ for the red chains. Slope 216 ± 4 $10^{-15}$ cm/molecule and intercept zero.

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