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Distribution of chlorosulfonyl groups in the subsurface of polystyrene substrates. Analysis by means of vibrational spectroscopy

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ABSTRACT

In this work the activation of transparent PS substrates by chlorosulfonation is described and their distribution in the subsurface region is analyzed using vibrational spectroscopies. Confocal Raman microspectroscopy is shown to be insufficiently surface selective and only the highest modified samples can be analyzed using a mathematical procedure for the correction of diffraction effects on the depth profile. On the other hand, FTIR-ATR spectroscopy carried out using different internal reflection elements and varying angles of incidence allows discrimination between the different modification profiles including those with low modification degrees obtained at low treatment times. The results show that the electrophilic aromatic substitution of polystyrene in pure chlorosulfonic acid is extremely quick with the complete surface covered by chlorosulfonic groups after only 10 min reaction time at −10 °C. It is further demonstrated that the reaction is very surface selective and that even after reaction times as long as 3 h the modification is limited to a layer with a thickness of less than one m.

1. Introduction

Polystyrene is one of the most used polymer materials in the bioanalytical sector because it has excellent optical clarity, is easy to mold and relatively inexpensive. Slides and multiple-well plates from this material have gained widespread acceptance in part because pipetting, washing, and signal detection are easily automated [1–3].

It has recently been shown [4,5] that PS substrates can be activated by a simple and economic wet-chemical treatment in chlorosulfonic acid at low temperatures providing the surface selectively with a tunable number of chlorosulfonic groups that can be used to create or anchor in a second step selectively a great variety of functional groups or biomolecules to the surface without losing its transparency. The pre-activated chlorosulfonated substrates are hydrolytically stable in ambient conditions and can be stored for months without losing their activity.

The approach has been shown to be applicable to commercial multiple-well plates from PS that could be modified selectively with amino or carboxylic groups. These systems have successfully been probed for ELISA (Enzyme-linked Immuno Sorbent Assays) as cheap alternative to commercial materials.

In the present work we have carried out a detailed study of the chlorosulfonation reaction of transparent PS surfaces. Our particular aim here was to gather information concerning the number and distribution of the created functional groups on the air-film interface and in the subsurface region. For this purpose we used on the one hand a Raman microscope in the confocal mode [6–9] and on the other hand an FTIR spectrometer equipped with an ATR device that allowed changing the angle of incidence and the total reflection element.

2. Materials and methods

2.1. Materials

Transparent Polystyrene sheets of 1 mm thickness and a surface of 8.0 cm × 2.5 cm were purchased from Resopal S.A., Madrid, Spain.

Chlorosulfonic acid and concentrated sulphuric acid from Sigma–Aldrich were used. Chlorosulphonic acid was distilled under reduced pressure prior to use. Sodium p-vinylphenylsulfonate was purchased from Sigma–Aldrich and used without further purification.

2.2. Preparative aspects

In a thermostatable reactor rectangular transparent PS samples (dimensions 2.5 cm × 8.0 cm × 0.1 cm) are brought in contact with

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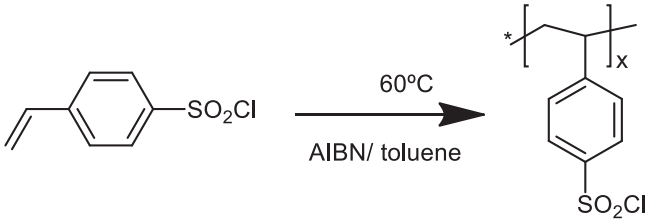

Scheme 1. Preparation of chlorosulfonated styrene homopolymer.

freshly distilled chlorosulphonic acid at a temperature of −10 °C. At different time intervals the simple is taken with teflón coated tweezers, washed for 15 s in cold (0 °C) concentrated sulphuric acid and finally for 30 s in a mixture ice/water. Then the samples are dried at ambient temperature.

The synthesis of 4-vinylbenzenesulfonyl chloride and of the corresponding homo and copolymers is described in detail elsewhere [10].

2.3. ATR-FTIR spectra

ATR-FTIR measurements are carried out using an FTIR spectrometer Spectrum One of Perkin Elmer equipped with a multireflextion ATR device from Pike allowing to measure at different angles of incidence and using as internal reflection elements both a diamond/ZnSe composite or a Ge crystal. This variable set-up allows studying the samples with penetration depths between 0.3 and 2 μm.

To determine quantitatively the relative degree of modification of the functionalized films the bands at 1450 cm^{-1} and 1370 cm^{-1} are used that correspond to the polymeric main chains of PS and the O=S=O valence bond of the SO_2Cl groups, respectively.

2.4. Confocal Raman microscopy

Raman spectra were recorded on a Renishaw Ramascope 2000 spectrometer using the 632.8-nm line of a He-Ne laser. This instrument was equipped with a Peltier-cooled charge-coupled device (CCD) detector, a holographic grating (1800 grooves/mm), and a Raman holographic edge filter, which prevented the backscattered laser radiation from entering the spectrograph. The stigmatic single spectrograph was attached to an Olympus BH2 microscope. The Ramascope was set up in the confocal mode with a 100× short-working-length objective (numerical aperture (NA) value of 0.95), a slit width of about 15 μm in one dimension, and a CCD of 576 pixels (pixel size 22 μm) in the other. The arrangement of the CCD and the slit acted as a synthetic confocal aperture. The depth resolution of the confocal arrangement in air has previously been checked, using a silicon sample as a reference material and has been determined as 2 μm by the full width at half-maximum criterion.

3. Results and discussion

3.1. Depth profiling by confocal Raman microscopy

Before measuring a depth profile of the modified PS substrate the most characteristic differences in the Raman response of PS and chlorosulfonated PS films have been studied using unmodified PS and the chlorosulfonated model obtained by homopolymerization of 4-chlorosulfonyl styrene [10] (Scheme 1).

In the window between 1700 and 950 cm^{-1} (Fig. 1) both spectra show a characteristic band around 1450 cm^{-1} that corresponds to the C-H vibrations of the polymeric chain. As the main chain is not affected by the modification reaction this band does not change its intensity and is used for normalization of the spectra. The main

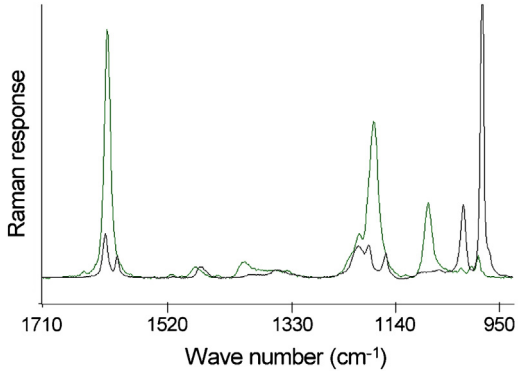

Fig. 1. Raman spectra of pure PS (black) and PS-SO_2Cl (green) homopolymer. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

difference in both spectra are the two new bands at 1597 cm^{-1} and 1170 cm^{-1} due to the change of the symmetry of the aromatic ring and the O=S=O vibration of the chlorosulfonyl group, respectively.

Depth profiles of the modified PS samples were then measured using confocal Raman microspectroscopy. In this technique one focuses the laser light through the lens of a microscope on the sample scanning stepwise in steps of 1 μm through the polymer film and recording a spectrum at each step. Due to the confocal configuration optical sectioning of the system with a depth resolution of 2–5 μm is possible and one can get structural information of virtual planes inside the sample. In the case of our chlorosulfonated PS samples this technique was not able to properly discriminate between samples modified for short times as in all of them no modification could be detected. Actually only samples obtained after a very long treatment time, as the one shown as an example in Fig. 2 obtained after 290 min, rendered Raman spectra in which modifier groups could be detected. A series of Raman spectra in the window from 1000 to 1650 cm^{-1} of a PS substrate chlorosulfonated is shown in Fig. 2. The first spectrum corresponds to the Raman response when the laser beam is focused 4 μm above the surface. Then the lens of the microscope is moved in steps of 1 μm downwards recording a spectrum at each step until a depth of 6 μm below the surface. All spectra are normalized with respect to the Raman band at 1450 cm^{-1}. As the modifier bands are overlapped with bands from unmodified PS, a spectrum of pure PS is first subtracted from each of the spectra in order to facilitate quantification. In this way one obtains two isolated signals at 1170 and 1595 cm^{-1} that can be easily quantified (using the model compounds for calibration) and represented as a function of the depth at which they have been measured. The profile obtained in this way is shown in Fig. 3. Readers should note that the depth scale inside the sample has been corrected [11,12] according to the refractive index difference between sample and air ($d = d_0 \times 1.4$). According to this profile the maximum amount of chlorosulfonyl groups is situated near the surface and apparently, there are SO_2Cl-moieties up to a depth of 5–6 μm below the surface. However the analysis also shows chlorosulfonyl groups 'outside' the sample, what is not physically reasonable and is due to well-known diffraction phenomena of the optical system [13]. The profile does therefore not reflect the true distribution of modifier groups in the film. However, as has been shown in previous work [13], the function that describes the true distribution can be calculated from the measured function taking into consideration the contribution of the neighbour regions. The three functions are related by the Fredholm integral equation

$$f(x) = \int_0^b [I(y)H(x,y)]dy \quad (1)$$