Study of Dye Diffusion in Fibers by Laser Scanning Confocal Microscopy

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Summary

The diffusion of a fluorescent dye in nylon 66 fibers was studied by using a laser scanning confocal microscope (LSCM) and by a conventional method. The diffusion coefficients were determined by both methods and they were in close agreement. The study of dye diffusion in fibers by LSCM is simple, quick and also able to provide high resolution images of fiber cross-sections and 3D images of the dye distribution in fibers. To our knowledge this is the first report of using a LSCM to study dye diffusion in polymeric fibers.

Introduction

LSCM has recently emerged as a significant new technique that exhibits several advantages over conventional optical microscopy. One of the key advantages of an LSCM is its ability to eliminate out-of-focus light that obscures the possibility of obtaining a three-dimensional view of the object being imaged in a normal light microscope. This ability to reject out-of-focus light is clearly demonstrated by a sharp decrease in the total integrated intensity as the image is defocused in an LSCM, while the total intensity appears to be a constant with defocus in a standard microscope. This property allows non-invasive serial optical sectioning of intact specimens and leads to the possibility of generating 3D images of thick transparent objects such as biological tissues. LSCM is also compatible with computer image storage techniques, allowing, for example, generation of high resolution digitized data sets of the 3D distribution of fluorescent labels within specimens, or of the topography of a surface, suitable for subsequent image processing. Although the principle of confocal microscopy was first described by Young and Roberts in 1951 [1] and the details of imaging were first described by Minsky in 1961 [2], commercial LSCM systems were produced only in 1987. During the last decade the availability of LSCMs of ever-increasing power and sophistication has revolutionized the science of microscopy as applied to cell and developmental biology, physiology, cytogenetics, and diagnostic pathology [3].

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Although LSCM has been a very powerful and popular tool in biological science for over a decade, the application of LSCM in material science has begun only recently. A number of papers have appeared in the literature that use LSCM for a variety of applications, including 3D imaging of colloidal crystals [4-7], defects in crystals [8,9], matrices surface damage in composites [10], morphology of polymer coatings [11], phase separation of polymer blends [12, 13], and characterization of fiber direction in reinforced polymer composites [14, 15]. However, its application in fiber science is almost nonexistent with the exception of a study by Clerck and Oostveldt [16] whose preliminary imaging consisted of obtaining cross sections of polyester microfibers from LSCM.

Study of diffusion processes using a LSCM is still in its infancy. Recently Blonk [17], Cutts [18], Wedekind, Kubischeck and Peters [19-22] have reported their studies of lateral diffusions in bio-membranes by performing a fluorescence recovery after photobleaching (FRAP) experiment using a LSCM. Kim et al [23] investigated the diffusion of tracer molecules into polymer particles by using a LSCM. Here we report on the diffusion of fluorescent dye molecules into nylon 66 fibers.

The process of fiber dyeing can be divided into three stages: the diffusion of dye molecules from the dyebath to the surface of a fiber, the adsorption of the dye molecules on the surface of a fiber and the diffusion of dye molecules towards the center of the fiber. Of the three stages, diffusion of dye molecules inside the fiber is the rate-determining step, so that the study of fiber dyeing is concentrated on the dye diffusion in fibers. One of the most common methods to study dye diffusion is to perform an infinite dyebath dyeing in an well-agitated dyebath, in which the dye concentration is constant. By determining the amount of dye uptake at given intervals of time, the diffusion coefficient can be calculated from Hill's solution of Fick's second law of diffusion, written as [24]

$$\frac{C_t}{C_\infty} = 1 - Ae^{-BK} - Ce^{-FK} - Ge^{-HK} \cdots\cdots$$

where C_t is the dye concentration in the fiber at time t, C_∞ is the dye concentration in the fiber at the equilibrium state, A, B, C, etc., are known numerical constants, and $K = Dt/r^2$, with D as the diffusion coefficient and r as the radius of the fiber. The diffusion coefficient D can be calculated from Hill's solution if the ratio of C_t/C_∞ is obtained. Traditionally C_t and C_∞ are determined individually by the measurement of the absorbance from the solutions containing the dissolved fiber. This process is tedious, time consuming and is unable to provide the spatial distribution of dye in a fiber. In our newly developed method, both the spatial distribution of dye molecules in fibers and the diffusion coefficient can be quickly

determined. To our knowledge this is the first report of using a LSCM to study dye diffusion in polymeric fibers.

Experimental

Nylon 66 fibers were dyed with fluorescein in an infinite dyebath operated at pH 6, 95°C. The concentration of fluorescein in the fiber was determined by the measurement of the absorbance of the formic acid solutions of the dyed fibers with an UV spectrophotometer. The maximum excitation wavelength of fluorescein is 490 nm and maximum emission is 515 nm. The fiber were imaged by a Leica DMRBE LSCM equipped with 488 nm laser line from an Argon ion laser and a 40 x NA 1.25 oil objective. The images were quantified and analyzed into fluorescent intensity profiles by using Leica TCS NT software. In order to reduce the spherical aberrations and obtain good quality images, the refractive index of the object needs to be matched closely with that of the immersion oil for the objective and mounting medium for the specimen. Since nylon 66 fiber is anisotropic and the refractive index perpendicular to the fiber axis was 1.522, the same immersion oil, with refractive index of 1.518, was used for immersing the objective and mounting the fiber specimen.

Results

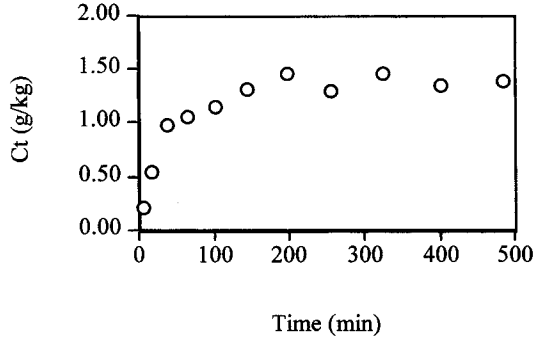

Figure 1. Plot of dye concentration in the fiber as a function of the time of dyeing. Nylon 66 fiber dyed with fluorescein in an infinite dyebath at pH 6, 95°C and dye concentrations in the dyebath was 0.1 g/l.

In a fiber dyeing process, the amount of dye molecules transferred from the dyebath solution into the fiber is a function of dyeing time, as shown in Figure 1. Dye concentration

in the fiber increased quickly at the beginning of dyeing and gradually reached the equilibrium. The images of cross-section of fibers dyed for different periods of time are shown in Figure 2. The fluorescent intensity was higher at the surface area of fiber in the beginning of the dyeing and became uniformly when dyeing reached the equilibrium. Figure 3 demonstrates the relationship between the integrated fluorescent intensity and the concentration of fluorescein in the fiber. The integrated intensity is linearly proportional to the concentration of fluorescein in the fiber when the concentration is lower than 4 g/kg. The diffusion coefficient was (7.8 ± 1.9) x 10^{-11} cm^2/s measured by the conventional method and it was 6.9 x 10^{-11} cm^2/s determined by using a LSCM.

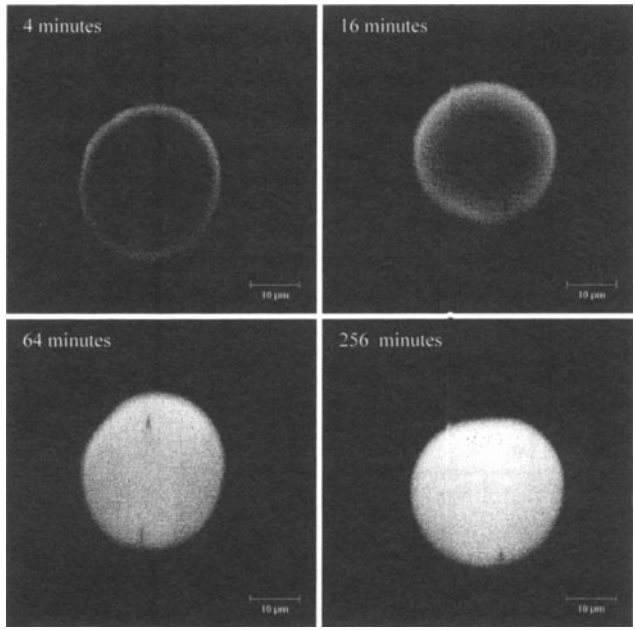

Figure 2 Images of fiber cross sections. The average diameter of the fiber is 30 μm. Nylon 66 fiber was dyed with fluorescein in an infinite dyebath for: 4, 16, 64 and 256 minutes. The dyebath was pH 6, 95°C and the dye concentration was 0.1 g/l.

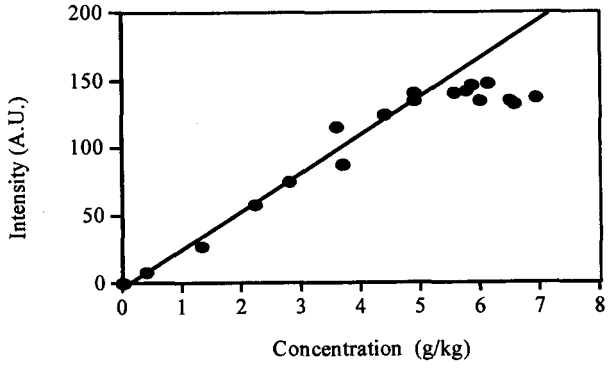

Figure 3. Plot of fluorescence intensity (arbitrary unit) as a function of fluorescein concentration in the fiber (g/kg). Nylon 66 fiber was dyed with fluorescein. Fluorescence intensity is linearly proportional to the fluorescein concentration in the fiber when the concentration is lower than 5 g/kg.

Discussion

Conventional study of dye diffusion determines the total amount of dye uptake or the average dye concentration in fiber without details of the dye diffusion process, such as the local dye concentration or the spatial distribution of dye molecules in the fiber and its changes in the dyeing process, although they are more meaningful in characterizing the dye diffusion process. With the advantages of optical sectioning, dye spatial distribution in the fiber is easily obtained by using LSCM. As shown in Figure 1, the average dye concentrations in the fibers were 0.28 g/kg, 0.55 g/kg, 1.1 g/kg and 1.4 g/kg after the fibers were dyed for 4 minutes, 16 minutes, 64 minutes and 256 minutes respectively. The dye spatial distributions in these fibers are illustrated in Figure 2 by using an LSCM, if the fluorescent intensity is considered linearly proportional to the fluorescein concentration in the fiber. Figure 2 shows that dye concentration in the fiber is not uniform before the dyeing reaches the equilibrium and the dye molecules diffuse into the fiber gradually from the surface into the center of the fiber.

One of the advantages of LSCM is the ability of quantitative measurement under the assumption that the fluorescent intensity is proportional to the fluorescence concentration in the object. Figure 3 shows that the integrated fluorescent intensity is linearly proportional to

the fluorescein concentration in the fiber when the concentration is lower than 5 g/kg. In the experiments reported the maximum dye concentration was 1.4 g/kg, thus providing data in the linear region of Figure 3. When integrated fluorescent intensity is linearly proportional to dye concentration in the fiber, the ratio of concentration C_t/C_∞ will be equal to the ratio of integrated fluorescent intensity, which can be obtained from the total integrated area of the fluorescent intensity profiles. Figure 4 shows the fluorescent intensity profiles of the fibers dyed for 4 minutes and 256 minutes. By calculating the C_t/C_∞ from Figure 4, the diffusion coefficient was found to be 6.9 x 10^{-11}cm^2/s from Hill's solution. This result is in close agreement with the measurements of (7.8 ± 1.9) x 10^{-11}cm^2/s from the conventional method, thus demonstrating the validity and the value of the approach presented here.

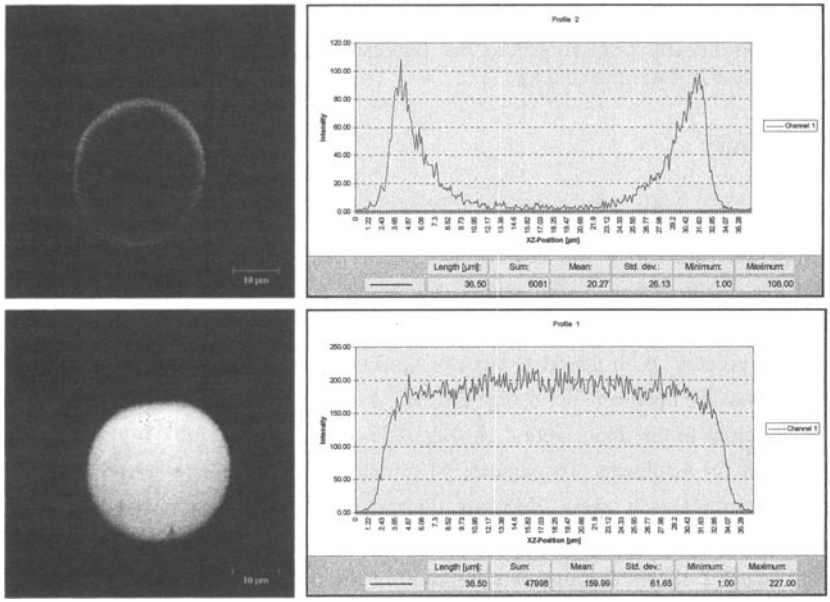

Figure 4 Top: image of fiber cross section and the fluorescence intensity across the fiber diameter when fiber dyed for 4 minutes. Bottom: image of fiber cross section and the fluorescence intensity across the fiber diameter when fiber dyed for 256 minutes. Nylon 66 fiber was dyed in an infinite dyebath at pH6 and 95°C. The dyebath concentration was 0.1g/l.

Conclusions

We have applied LSCM as a new nondestructive method to study dye diffusion behavior in fibers. This technique is simple and quick. It is able to provide high resolution images of the fiber cross-section, quantitative measurement of the diffusion coefficient and 3D images of the dye distribution inside the fiber. The diffusion coefficient of fluorescein in nylon 66 fiber measured by LSCM agreed with the result from conventional method.

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