Metal-enhanced fluorescence from silver nanoparticle-deposited polycarbonate substrates†

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Silver nanoparticles were deposited onto polycarbonate (PC) films by employing sequential surface modification methods. In the first step, PC films were etched either by exposure to ultra-violet radiation or by etching with sodium hydroxide at 70 °C, which resulted in the formation of a mixture of hydroxyl and carboxyl groups on the surface of the films. Next, the PC films were silanized with 3-(aminopropyl) triethoxysilane (APS) in order to introduce amine terminal groups to the surface. Lastly, silver nanoparticles were deposited onto APS-modified PC films, which occurred due to the well-known affinity of silver nanoparticles towards amine groups. Subsequently, the potential of silver deposited-PC films for metal-enhanced fluorescence applications has been demonstrated.

1. Introduction

Metal-enhanced fluorescence (MEF), a phenomenon where the quantum yield and photostability of weakly fluorescing species are dramatically increased, is becoming a powerful tool for the fluorescence-based applications of drug discovery,[1,2] high-throughput screening,[3,4] immunoassays[5] and protein–protein detection.[1,4,6] In all of these examples of MEF,[1–6] primarily quartz or glass substrates with deposited silver nanostructures have been used. This has been because the chemistries of the surface of glass (and quartz) are well established and therefore the covalent immobilization of silver nanostructures onto glass less arduous and is indeed reproducibly reliable.[1–6]

Polymeric substrates may, however, provide a suitable, cheap and versatile alternative to the use of chemically benign glass substrates.[7] Although often unsuitable for short-wavelength fluorescence-based detection strategies due to intrinsic polymer fluorescence, several polymers, such as poly(methyl methacrylate) and polycarbonate (PC), typically do not suffer from these detractions and are suitable for use in longer-wavelength ($\lambda > 520$ nm) fluorescence-based detection schemes.[8] However, most plastics do not possess a suitable interfacial chemical structure required to immobilize biomolecules and metal nanoparticles on their surfaces. The practical approaches to polymer surface modification for the deposition of biomolecules have been corona discharge treatment, plasma, surface grafting, light and chemical modification.[9] In some cases, further functionalization of the

polymer substrate with silanes containing terminal functional groups, is used to enhance the deposition of biomolecules while still retaining the properties of the polymer.[7] In this regard, the polymer substrates when functionalized with silanes containing amine terminal groups allow the deposition of metal nanoparticles due to affinity of noble metals (gold and silver in particular) towards amine groups.[10]
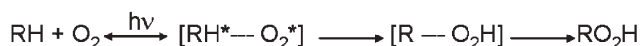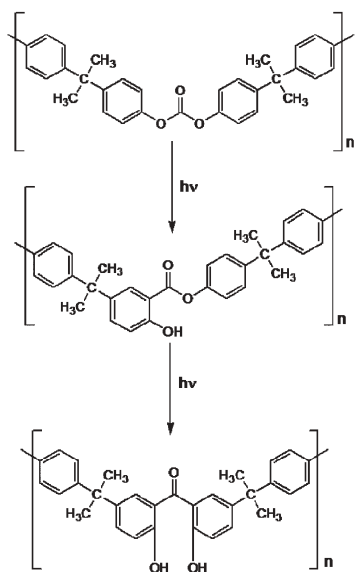
In this paper, polycarbonate was chosen as the polymer of interest due to its widespread use in biotechnology.[9,11,12] The deposition of silver on polycarbonate films was performed using both chemical and light modification (UV-radiation) of the surface. Polycarbonate (bisphenol A) is known to undergo a photo-Fries reaction with a relatively high quantum yield (values between 0.03 and 0.4) at wavelengths between 254 and 300 nm as shown in Scheme 1.[13] The photo-Fries reaction results in the formation of phenyl salicylates and hydroxy-benzophenones (Scheme 1, top). The occurrence of an O_2 contact charge-transfer complex as an initiation step in the photooxidation of both aliphatic and aromatic hydrocarbons has been demonstrated by Chien.[14] One potential reaction of the excited O_2 complex is formation of hydroperoxides via a caged mechanism, and this complex may go onto form carboxylic acids, as illustrated in Scheme 1, bottom. Thus, the direct exposure of PC to UV radiation creates a hydroxylated/carboxylated surface that can be functionalized with silanes to introduce amine groups. Similarly, acid or base catalyzed hydrolysis of the PC film readily creates additional surface functionality for silver island film deposition. Silver island films (SiFs)[1–4] have been widely used on glass slides for a variety of applications in MEF typically producing >5 fold enhancements in fluorescein emission intensity, as compared to a control sample containing no silver.[1–4]

Metal-enhanced fluorescence is known to be a through space phenomenon, thus, when fluorophores are placed within 4–10 nm of the surface of the silver nanostructures, the emission of the fluorophores is enhanced.[15,16] Subsequently, in this paper, the fluorophore was positioned a mean 4 nm from the surface using a labelled protein, namely FITC–HSA. The

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Scheme 2 Deposition of silver island films (SiFs) on a UV-etched polycarbonate (PC).

2% *v/v* solution of 3-(aminopropyl) triethoxysilane (APS) in denatured ethanol for 2 h. The APS-coated PC films were removed from the solution and rinsed several times with ethanol and deionized water to remove the unbound APS. SiFs were formed on half of the silanized PC films (the non-silvered half is used as control by which to compare the benefits of using MEF phenomenon) similar to the procedure found in literature.[10] SiFs, in low yield, were also formed on virgin PC films to demonstrate the need for surface modification.

Base-etching of polycarbonate. Polycarbonate (PC) films were cut into 75 × 25 mm pieces and were placed onto glass microscope slides in order to provide support for the films. PC films were hydrolyzed in 2 M aqueous NaOH solution for 30 min at 70 °C, then rinsed with deionized water (Scheme 3). PC films were transferred onto new glass slides and hydrolyzed further in acetic acid for 15 min at room temperature, rinsed with deionized water and finally dried under a stream of cool air (Scheme 3). This procedure results in the formation of a mixture of hydroxyl and carboxylic acid groups on the PC films. The hydrolyzed PC films were silanized with a 2% *v/v* solution of APS in denatured ethanol for 2 h. The APS-coated PC films were then removed from the solution and rinsed several times with ethanol and deionized water to remove the unbound APS. SiFs were formed on half of the silanized PC films (the non-silvered half is used as control) similar to the procedure found in literature.[10] SiFs were also formed on

Scheme 1 Top: the photo-Fries reaction scheme for polycarbonate; bottom: formation of hydroperoxides *via* a caged mechanism. Adapted from ref. 12.

results clearly show that plastics can indeed be modified for silver deposition and notable enhancements in fluorescence emission can be achieved from the plastic substrates, similar to that observed from glass as previously reported by our groups.[1–6]
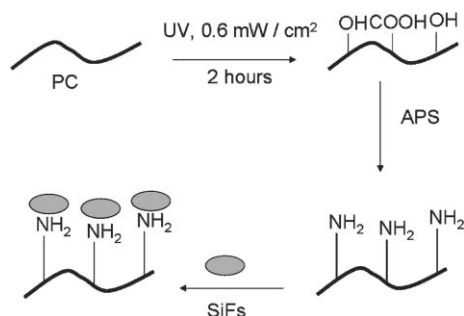
2. Materials and methods

2.1. Materials

Silver nitrate (AgNO$_3$, 99.9%), sodium hydroxide (NaOH, 99.9%), ammonium hydroxide (NH$_4$OH, 30%), trisodium citrate, D-glucose and premium quality glass slides (75 × 25 mm), FITC-labelled human serum albumin (FITC–HSA, 10 moles FITC per mole of albumin) were obtained from Sigma-Aldrich. Polycarbonate films with *ca.* 1 mm thickness (PC thick films) were provided by Innovative Microplate, Inc. (Chicopee, MA). All chemicals were used as received.

2.2. Methods

2.2.1. Modification of polycarbonate and preparation of silver island films on modified polycarbonate. Surface modification of polycarbonate films were performed using two methods: (1) UV-etching, and (2) base-etching.
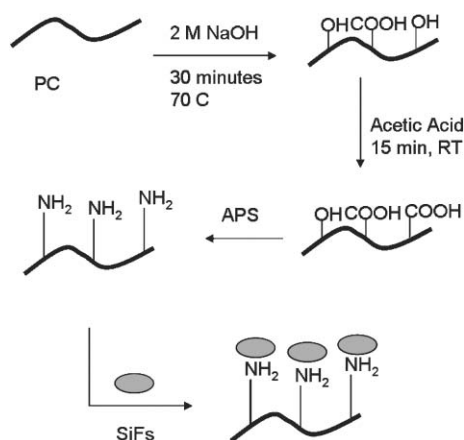
UV-etching of polycarbonate. Polycarbonate (PC) films were cut into 75 × 25 mm pieces and were placed onto glass microscope slides in order to provide support for the films. PC films were then irradiated with a UV-light (254 nm, 0.6 mW cm^{-2}) for 2 h (Scheme 2). This results in the formation of hydroxyl groups on the PC films (photo-Fries reaction, Scheme 1). The UV-light-etched PC films were subsequently silanized with a

Scheme 3 Deposition of silver island films (SiFs) on a NaOH-etched polycarbonate (PC).

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virgin PC films. The total immersion time for the silanized PC films in the medium, where the SiFs are formed, was kept constant for all the samples. This provided an indirect means of depositing the SiFs with similar densities on the surface of the PC films.

2.2.2. Characterization of samples. All absorption measurements were performed using a HP 8453 UV-Vis spectrophotometer. Fluorescence measurements on PC films were performed by placing the films on a stationary stage equipped with the fiber-optics mount on a 15 cm-long arm (normal to sample). The output of the fiber was connected to an Ocean Optics HD2000 spectrofluorometer for the emission spectra. The excitation was from the second harmonic (473 nm) of the diode-pumped Nd:YVO4 laser (compact laser pointer design, maximal output power 30 mW) at an angle of $45°$. The emission was observed through a 488 nm long-wave pass filter. 473-nm excitation was used to achieve better wavelength separation between the excitation and emission.

The *real color* photographs of FITC–HSA on non-silvered PC films and PC films with SiFs were taken with a Olympus Digital camera (C-740, 3.2 megapixel, $10\times$ optical zoom) using the same long wave-pass filter that is used for the emission spectra.

AFM images were collected with an atomic force microscope (TMX 2100 Explorer SPM, Veeco) equipped with an AFM dry scanner (the scanning area was 100×100 μm). Surfaces were imaged in air, in a tapping mode of operation, using SFM non contact mode cantilevers (Veeco). The AFM scanner was calibrated using a standard calibration grid as well as by using gold nanoparticles, 100 nm in diameter from Ted Pella. Images were analyzed using SPMLab software.

2.2.3. Metal-enhanced fluorescence from modified polycarbonate. In previous reports of metal-enhanced fluorescence (MEF), silvered surfaces were coated with fluorophore labelled protein.[10,17,18] This experimental format has been adopted for two main reasons, the first, being that the protein coverage with human serum albumin (HSA) is known to bind to silvered surfaces and indeed forms a monolayer, and secondly, the dimensions of the protein being such that the protein allows for a mean separation of the silver and the fluorophore, MEF being a through space phenomenon, as demonstrated by the late T. Cotton.[19]

Binding the FITC–HSA to the PC films was accomplished by soaking in a 10 μM FITC–HSA solution for 2 h, followed by rinsing with water to remove the unbound material. PC films were transferred onto new glass slides again. Both the non-silvered and SiFs surfaces on PC films were coated with the labelled HSA, which is known to passively absorb to noble metal surfaces and form an *ca.* 4 nm thick protein monolayer, allowing us to study the fluorescence spectral properties of noncovalent FITC–HSA complexes in the absence and presence of SiFs. By equally coating a PC film with FITC–HSA we were also able to determine the enhancement factor (benefit) obtained from using the silver, *i.e.* intensity on silver/intensity on PC film, given that both surfaces are known to have an approximately equal monolayer coverage.[1–4,10]

3. Results and discussion

The deposition of SiFs on 1 mm-thick polycarbonate (PC) films were performed using two different surface modification procedures as explained in the Experimental section: (1) base-etching and (2) UV-etching.

These were devised based on the observations that attempts at directly depositing SiFs onto plastic substrates resulted in no silver attachment to the virgin polycarbonate (PC) surface—Fig. 1, top left and Fig. 1, bottom. This is explained by the lack of amine or thiol groups, which have strong affinity towards silver, on the virgin PC films. In this regard, the surface modification methods mentioned above were employed towards creating hydroxyl and carboxyl groups that will allow further introduction of amine groups on the surface of the PC films.

When the PC film is etched with UV-light (creates hydroxyl groups—Scheme 2) and amine groups are introduced through APS coating, there was significant silver attachment to the PC film—Fig. 1, top right and Fig. 1, bottom. The surface plasmon peak and the optical density of the SiFs were 430 nm
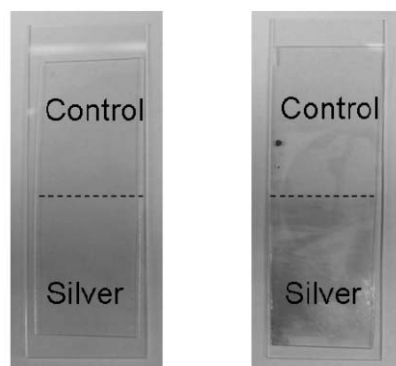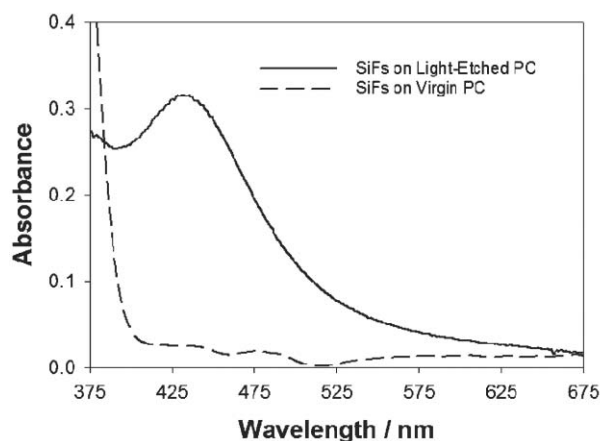

Fig. 1 Photograph of 2 PC films mounted on glass slides: SiFs deposited on unmodified PC (top left) and SiFs deposited on UV-etched PC (top right). Silver is intentionally deposited only on the bottom half of the PC. Bottom: absorption spectra of silver island films on virgin PC and UV-etched PC.

and 0.3, respectively, which are consistent with the reports found in literature.[20,21] These observations indicate that the silver deposited uniformly on UV-light etched PC films.

In a similar fashion to UV-etching of PC films in creating hydroxyl groups, when the PC film is etched with sodium hydroxide and amine groups are introduced through a surface APS coating, there was significant silver attachment to the PC film—Fig. 2, top and Fig. 2, bottom. The surface plasmon peak was at around 450 nm, red-shifted as compared to 430 nm observed from SiFs on UV-light etched PC, and the spectrum was broader, indicating that the presence of larger silver particles and the slightly more heterogeneity of the deposition of silver on the NaOH-etched PC film.

Atomic force microscopy (AFM) was employed to characterize the PC films before and during the etching process, and also after the deposition of SiFs. Fig. 3 shows the AFM images of a virgin PC film, NaOH-etched PC film and a SiFs-deposited NaOH-etched PC film. The virgin PC has a relatively smooth surface; however, ridges and grooves appeared when imaged immediately after etching with sodium hydroxide (Fig. 3, top). The height analysis of the AFM image of the SiFs on NaOH-etched PC films revealed that the average size of the silver islands were 50–120 nm (Fig. 3, bottom), which is relatively larger than the silver deposited on UV-light etched PC, or SiFs deposited on glass substrates, where the size of the silver can vary between 30–80 nm.[4,6] This was also supported by the fact that the surface plasmon peaks for SiFs deposited on UV-light etched PC and glass around 430 nm and not broadened as compared to the broadened

Fig. 3 AFM images of virgin PC film (top left), NaOH-etched PC film (top right), SiFs-deposited NaOH-etched PC film and size analysis (bottom).

Fig. 4 Top: emission spectra of FITC–HSA monolayers on UV-etched PC with and without SiFs and on virgin PC (unmodified); middle: photographs of SiF coated light-etched PC; bottom: the emission of FITCHSA on the unsilvered light-etched PC and on the silvered and UV-etched PC.
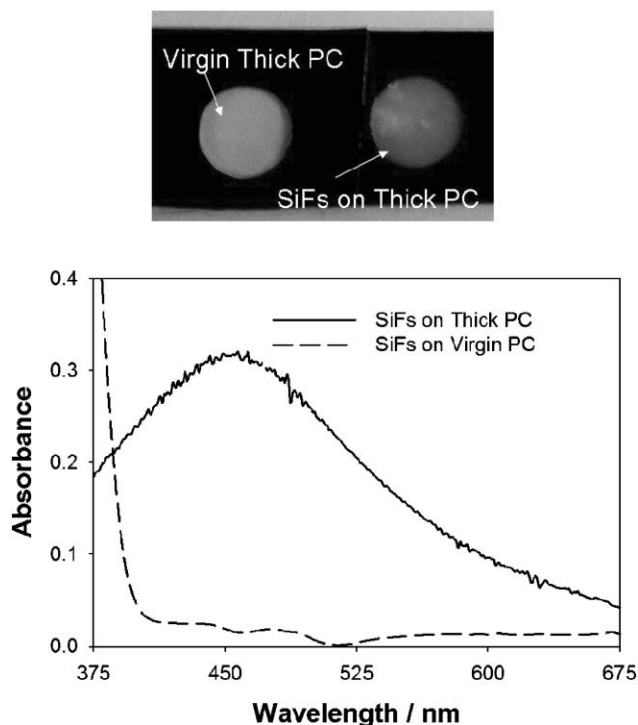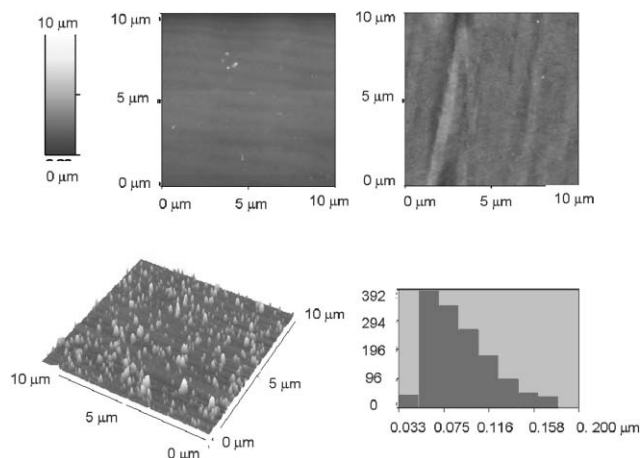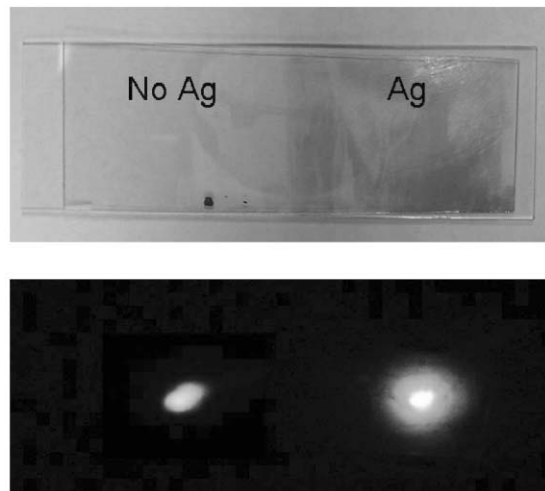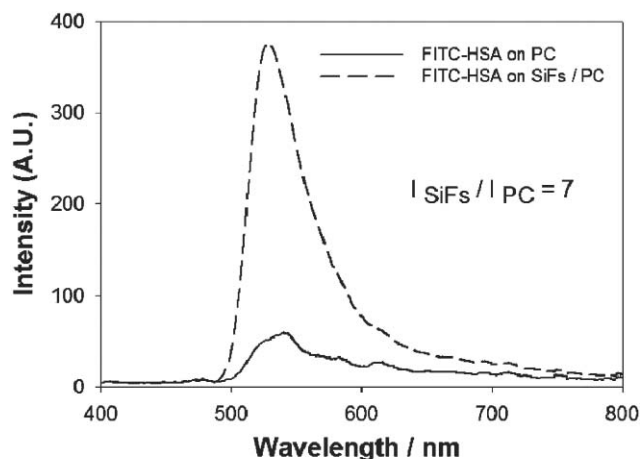

Fig. 2 Top left: photograph of virgin PC film mounted on glass slides; top right: SiFs deposited on NaOH-etched PC; bottom: absorption spectra of silver island films on virgin PC and NaOH-etched PC.

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surface plasmon peak of SiFs on NaOH-etched PC films at 450 nm.

In recent years the laboratories at University of Maryland at Baltimore and University of Maryland Biotechnology Institute have reported the favorable effects for fluorophores conjugated to proteins placed in close proximity to surface immobilized silver nanostructures (greater quantum yields (increased emission), reduced lifetimes and/or increased photostability).[1–6,10,17,18,21]

In this regard, the usefulness of SiFs-deposited plastic surfaces for metal-enhanced fluorescence was tested by equally coating the unsilvered and silver-deposited sides of the PC films with fluorescein labelled HSA (human serum albumin), where the FITC–HSA has been shown to be an ideal labelled protein,[10,17,18,21] with regard to positioning the fluorophore a couple of nanometers from the silver nanoparticles to facilitate metal-enhanced fluorescence.

Fig. 4, top shows that the emission spectrum of FITC–HSA monolayers on UV-etched PC with SiFs is substantially greater (ca. 7-fold) than the emission spectrum of FITC–HSA on UV-etched PC but without silver. This relatively large increase in emission intensity could also be seen visually, Fig. 4, bottom, through the same long pass filter as used in Fig. 4, top. As the
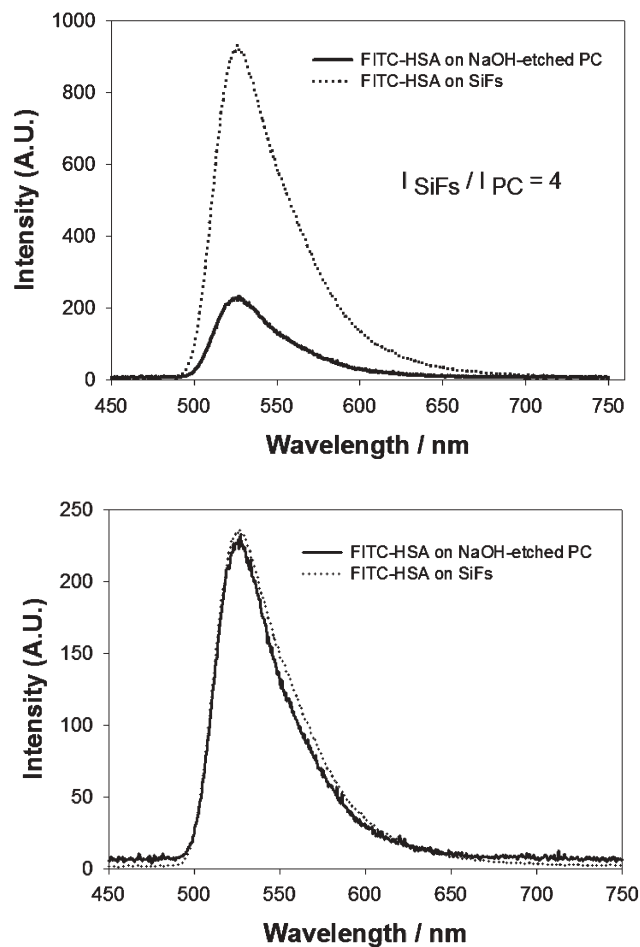
473 nm laser excitation is moved from the unsilvered plastic to the silvered plastic side, a dramatic increase in fluorescein fluorescence emission is observed, Fig. 4, bottom.

Fig. 5, top shows that the emission spectrum of FITC–HSA monolayers on NaOH-etched PC with SiFs is substantially greater (ca. 4-fold) than the emission spectrum of FITC–HSA on NaOH-etched PC, but without silver. The emission spectra of FITC–HSA on NaOH-etched PC with and without silver overlap when normalized, indicating that the fluorophore emits with the same photophysical properties both on silver and plastic, Fig. 5, bottom. It was previously shown that when silver is deposited onto a surface in a highly heterogeneous fashion (e.g., fractals), usually a greater spatial distribution of enhancement of fluorescence is observed,[22] where the enhanced fluorescence of fluorescein varied 10^1 to 10^4. In this work, there is no pronounced difference in the heterogenity of the SiFs deposited on the UV-etched NaOH-etched PC films that would produce significantly different enhancement factors as those observed on silver fractals.[22]

Fig. 6 and 7 show the photostability of the FITC–HSA on both the unsilvered and silvered UV-light etched and NaOH-etched PC films, respectively. Using the same laser power,
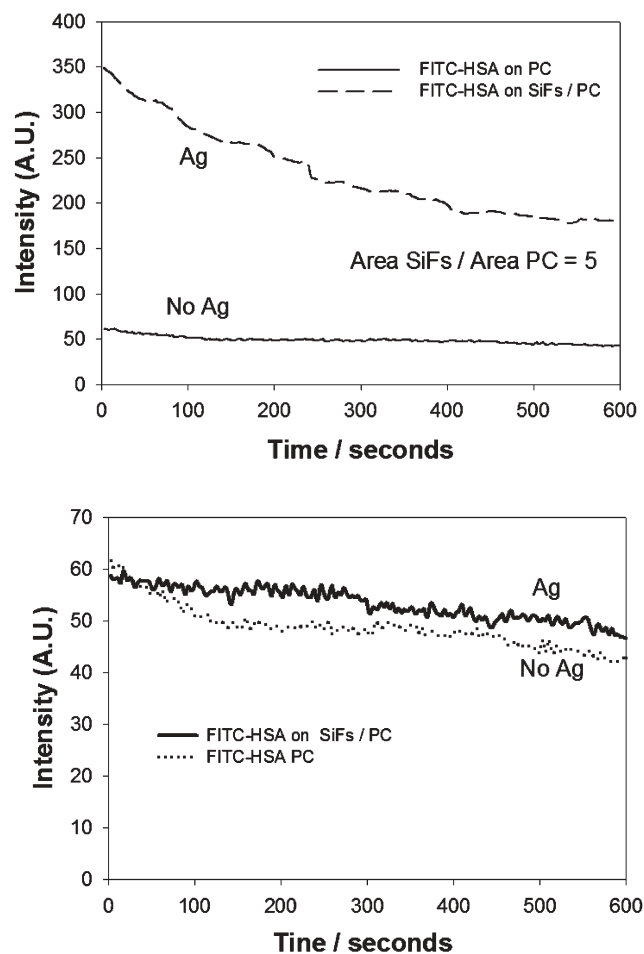

Fig. 5 Emission spectra of FITC–HSA monolayer on NaOH-etched PC with and without SiFs and on virgin (unmodified) PC (top); normalized intensities (bottom).

Fig. 6 Emission intensity vs. time of FITC–HSA on both silvered and unsilvered UV-etched PC with constant 473 nm excitation (top), and with the laser power adjusted to give the same initial steady state fluorescence intensity (bottom).
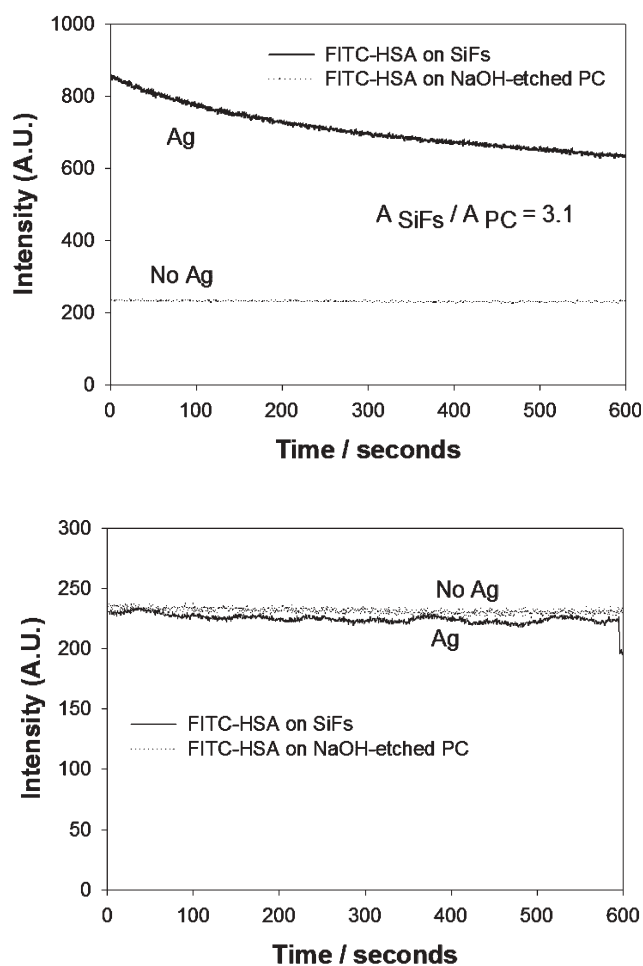
Fig. 7 Emission intensity *vs.* time of FITC–HSA on both silvered and unsilvered NaOH-etched PC with constant 473 nm excitation (top), and with the laser power adjusted to give the same initial steady state fluorescence intensity (bottom).

significantly more fluorescence from the silvered plastic is observed, by simply considering the integrated area under the respective curves, Fig. 6, top and Fig. 7, top, *i.e.*, the photon flux. However, when the laser power is attenuated on the silver surface to give the same initial emission intensity as observed on the unsilvered but modified plastic, similar photostability characteristics are seen, Fig. 6, bottom and Fig. 7, bottom.

Metal-enhanced fluorescence results in both an increased emission intensity, accompanied by a reduction in fluorophore lifetime, *i.e.* a radiative rate modification.[1–5] It was previously shown that the lifetime of FITC was reduced from 2.58 ns on the unsilvered thin plastic to 1.68 ns on the silvered thin plastic.[10] Although not repeated in this work, reduced lifetime of fluophore is expected on SiFs-deposited thick PC films similar to silvered thin plastic films.

4. Conclusions

PC films were chosen to demonstrate the potential of plastics in metal-enhanced fluorescence applications due to their inherent chemical composition that can be modified using relatively simple techniques. When etched with UV-radiation or sodium hydroxide, chemically benign groups such as hydroperoxides are formed on the surface of the PC films. Subsequently, amine terminal groups were introduced to the surface by silanization, which allowed the deposition of the silver nanoparticles onto surface of the PC films. On the other hand, when the PC films were not modified by etching followed by silanization, the deposition of the silver nano-particles was negligible and therefore the surfaces not useful for applications in MEF. It was found that the silver nanoparticles were deposited more homogeneously onto PC films etched by UV-radiation as compared to PC films etched with sodium hydroxide. Enhanced emission of fluorescence from FITC was observed from the silver-deposited side of the PC film as compared to the non-silvered side. It was also found that FITC retained its photophysical characteristics, *i.e.*, same emission spectrum and high photostablity, on the silver-deposited PC films, similar to the observations on silver-deposited glass substrates. These results indicate that PC films are suitable and a cheap alternative to glass for numerous metal-enhanced fluorescence applications.[1–6]

The importance of the current work stems from the implications of the use of silver-deposited PC films produced by the methods employed here in microfluidic devices and other industrial sensors based on fluorescence detection with enhanced sensitivity. Work is currently underway in our laboratories in this regard and will be reported in due course.

Acknowledgements

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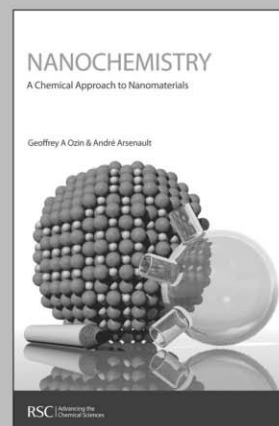22 A. Parfenov, I. Gryczynski, J. Malicka, C. D. Geddes and J. R. Lakowicz, *J. Phys. Chem. B*, 2003, **107**, 8829.