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Effect of ethanol variation on the internal environment of sol–gel bulk and thin films with aging

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Abstract

Sol–gel derived bulk and thin films were prepared from different compositions at low pH (∼2.0) containing varying concentrations of ethanol from 15 to 60% at constant water (H_2O)/tetraethyl-orthosilicate (TEOS) ratio ($R = 4$). The fluorescence microscopic and spectroscopic measurements on fluorescent probe, Hoechst 33258 (H258) entrapped in these compositions were carried out at different days of storage to monitor the effects of concentration of ethanol on the internal environment of sol–gel materials. Fluorescence microscopic observations on sol–gel thin films, prepared by dip coating technique depicted uniform and cracked surface at withdrawal speed 1 cm/min (high speed) and 0.1 cm/min (low speed) respectively, which did not change during aging. Fluorescence spectral measurements showed emission maximum of H258 at ∼535 nm in fresh sols at all concentrations of ethanol which depicted slight blue shift to 512 nm during aging in bulk. No such spectral shift has been observed in sol–gel thin films coated at high speed whereas thin films coated at low speed clearly showed an additional band at ∼404 nm at 45 and 60% concentration of ethanol after about one month of storage. Analysis of the fluorescence lifetime data indicated single exponential decay (1.6–1.8 ns) in fresh sol and from third day onwards, invariably double exponential decay with a short (τ_1) and a long (τ_2) component were observed in sol–gel bulk with a dominant τ_1 at ∼1.2 ns at all concentrations of ethanol. A double exponential decay consisting of a short component (τ_1) at ∼0.2 ns and a long component (τ_2) at ∼3.5 ns were observed at all ethanol concentrations in both fresh and aged sol–gel thin films. Further, distribution analysis of lifetimes of H258 showed two mean lifetimes with increased width in aged bulk and thin films. These results are likely to have strong implications in designing the internal environment for applications in biosensors.

Keywords: Tetraethyl-orthosilicate; Sol–gel; Hoechst 33258; Fluorescence spectroscopy; Optical biosensors

1. Introduction

The sol–gel process is a convenient and versatile method of preparing transparent optical materials at low temperature (Dorothy et al., 1996). Ambient processing conditions also enable one to encapsulate numerous organic, organometallic and biological molecules within these sol–gel derived optical materials (Dunn and Jeffrey, 1997). The physical and chemical properties of the materials are strongly influenced by the composition of sols as well as the nature of dopant molecules (Zusman et al., 1990). The synthetic approach for sol–gels can be used for designing sensor materials for biosensors (MacCraith et al., 1991).

Most sol–gel techniques use water and low molecular weight alkoxide such as tetramethyl-orthosilicate (TMOS), tetraethyl-orthosilicate (TEOS) or an equivalent organometallic alkoxide (such as tetra-isopropoxytitanium or tri-isopropoxyaluminium) as sol–gel precursors (Lev et al., 1995). Most commonly, alkoxide precursors are used for preparation of glassy matrix (Kaufman and Avnir, 1988; Lin and Brown, 1997; Dunn and Jeffrey, 1997). Hydrolysis and condensation of tetra-alkoxysilanes during the sol–gel process help to form solid silicate around the dopant or sensing molecule, which is dissolved in the liquid phase (Dorothy et al., 1996). The nature of the local environment around the

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sensing molecule is critical in achieving the desired property for applications such as detection of analytes using biosensors. Sol–gel thin films are prerequisite for biosensors applications which could be based on either optical or electrochemical methods (Dave et al., 1997). Although, the physical characteristics of sol–gel thin films can be effectively measured and controlled by tailoring the preparative conditions but very little is known about the internal environment of these films (Malins et al., 2000).

Ethanol is often used for homogenizing the immiscible water and alkoxide precursors in sol–gel processing (Matsui et al., 1989; Severin-Vantilt and Oomen, 1993; Narang et al., 1994; Huang et al., 2000). Sol–gel thin films have been prepared using sols diluted with alcohol and even in pure ethanol to decrease viscosity, enhance sol stability and improve substrate wetting (Brinker and Scherer, 1990; Brinker et al., 1992). In spite of these advantages, the conventional sol–gel procedures for preparing bulk and thin films are not generally suitable for encapsulation of proteins because high concentrations of acid and alcohol needed in these procedures, can lead to denaturation of most proteins (Ellerby et al., 1992). Good optical quality thin films have been reported using methyl alcohol (MeOH), tetramethyl-orthosilicate and buffer mixtures (Dave et al., 1997). Dunn et al. have reported the retention of chemical functionality of the protein in sol–gel bulk containing 60% (v/v) methanol. It was reported that beyond this concentration of methanol, aggregation of protein occurred (Dunn et al., 1998). Strong influence of the solvent type (water or ethyl alcohol) on the material optical properties was reported viz. alcohol-based samples showed stable homogeneous structures, with no cracking and water-based samples demonstrated higher transmittance in the visible region (Lechna et al., 2002). Vasconcelos et al. reported synthesis of colloidal silica particles and obtained different structures due to the variation of molar ratios of ammonium hydroxide (NH_4OH), ethyl alcohol (C_2H_5OH) and water (H_2O) with respect to precursor (Vasconcelos et al., 2002). Thus, it is clear from literature that different molar ratios of water to silica precursor (R) and concentrations of ethanol were used in several studies for preparation of sols but no systematic studies on the effects of ethanol variations on the sol–gel internal environment of bulk and thin films have been reported. Since, elucidation of these characteristics is essential in designing and fabrication of desired sol–gel thin films therefore, we have undertaken systematic fluorescence spectroscopic studies to elucidate the effects of ethanol on the internal environment of sol–gel bulk and thin films with aging.

In the present study, different compositions of sol–gels were prepared at low pH (∼2.0) by varying ethanol concentrations from 15 to 60% at constant H_2O/TEOS ratio ($R = 4$). There are many other factors, e.g. viscosity, dip coating speed and H_2O/TEOS ratio (R) can affect the thin films texture. We have discussed the effects of these factors in our previous study (Gupta et al., 2005). The addition of ethanol is also known to improve the uniformity in sol–gel thin films. A heterocyclic bisbenzimidazole derivative Hoechst 33258 (H258) has been shown to be useful in elucidating the physico-chemical properties of sol–gels (Chaudhury et al., 2003; Gupta et al., 2005). We report the results of fluorescence microscopic and spectroscopic measurements on H258 entrapped in sol–gel bulk and thin films, prepared at different ethanol concentrations as a function of storage time (aging).

2. Materials and methods

2.1. Chemicals

Tetraethyl-orthosilicate was obtained from E.Merck (Germany). Fluorescent probe molecule Hoechst 33258 (2′-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2, 5′-bi-(1H-benzimidazole) was procured from Sigma Chemical Co., USA. All other reagents were of guaranteed grade (GR) from E. Merck and used without further purification. Deionized water of 18 MΩ resistances was obtained from Millipore MQ water purification system.

2.2. Preparation of samples

The sol–gel compositions at different ethanol concentrations from 15 to 60% at constant H_2O/TEOS ratio ($R = 4$) were prepared by adding tetraethyl-orthosilicate, phosphate buffer (0.1 M), ethanol and HCl (pH ∼ 2.0) as catalyst in disposable fluorescent cuvettes. The probe molecule H258 at a final concentration of 20 μM was added prior to sonication. Methodology for preparation of samples, measurement of viscosity of initial sols after sonication, pre-treatment of glass strips for sol–gel coating, masking and coating deposition were discussed in our previous study (Gupta et al., 2005).

2.3. Microscopic and fluorescence spectroscopic investigations

Surface examination of sol–gel coated thin films was performed using Olympus Microscope (Model BX60, Japan) attached with CCD camera and frame grabber for image transfer and visual inspection. The fluorescence emission and lifetime measurements were carried out in a time resolved fluorescence spectrofluorimeter, model FS900CDT and FL900CDT (Edinburgh Analytical Instruments, UK) respectively. Details of fluorescence microscopic and spectroscopic investigations were mentioned in our previous work (Gupta et al., 2005). The distribution analysis of fluorescence lifetimes of H258 in sol–gels was calculated by using level 2 program, available with FL900CDT. The distribution kinetic model fits a decay profile by weighting a series of individual exponential lifetimes instead of only single, double and triple exponentials as usually done in exponential decay analysis. The goodness of fit is simultaneously judged by various statistical parameters provided in the analysis software.