

Phosphorimetry and its potential for the study of surface heterogeneity of chromatographic silica-based stationary phases

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Abstract

Phosphorimetry was applied to the study of silica-based chromatographic phase surface heterogeneity by determining the phosphorescence response of a phenanthrene probe as a function of loading on underivatized silica and reversed and ion-exchange phases. The phosphorescence response was used to “titrate” and estimate the number of strong adsorption sites on underivatized silica. By incorporating covalent vs. ionic heavy atom/ion enhancers onto derivatized silicas, it was demonstrated that the probe molecules adsorb at residual silanols at lower loadings but associate with alkyl chains at higher loadings. Suggestions for further phosphorimetric studies are given.

1. Introduction

The understanding of adsorption and partitioning processes for chromatographic surfaces is critical to their continued development and use. Silica-based stationary phases continue to function as the workhorse tool for all analytical separations as evidenced by both user testimony [1] and column manufacturers' new product introductions [2]. Adsorption and partitioning processes for silica-based phases are a function of many factors including the nature of bare and derivatized silica surfaces and their interactions with adsorbates, solvents and solutes. A key question concerning silica-based phases is the degree of surface heterogeneity associated with adsorption and partitioning sites.

A variety of spectroscopic techniques are used for the study of silica-based stationary phases beyond characterization of the phases for analytical or quality control purposes. Fourier transform (FT) IR spectroscopy has been used to observe hydrogen bonding interactions between some population of covalently bound ligands and residual silanols on the silica surface for acetoacetamide [3] and cyanoalkyl [4,5] derivatized phases. FT-IR has also been used to study alkyl chain conformations as a function of temperature for reversed phases of varying length by determining the number of infrared “gauche defects” [6].

²⁹Si Cross polarization magic angle spinning (CP-MAS) solid-state NMR has been used to examine bare silica surfaces as a function of dehydration and rehydration [7]. In all cases, the spectra showed a heterogeneous distribution of silanols (Si–OH), geminal silanols [Si = (OH)₂] and siloxanes (Si–O–Si). ¹³C FT-NMR has been

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used to study the mobility of reversed-phase bonded alkyl chains in the solvated state via line shape analysis or measurement of the spin-lattice relaxation time, t_1 , for various moieties or portions of the chain [8–14]. These experiments have been conducted as a function of position along the chain [8,9], bonding density [9,12], solvent viscosity [10], mobile phase composition [11,12] and temperature [13] and the results have been related to retention data [12,14].

Deuterium NMR line shape analysis has also been used for the study of alkyl chain mobility for C_{18} reversed phases [15] and alkoxy silane phases ranging from C_1 to C_{16} [16] in both dry and solvated states. Deuterium NMR of 2H_2O -methanol and 2H_2O -acetonitrile mobile phases in contact with C_{18} stationary phases has been used to probe the degree of water association as a function of mobile phase composition via the change in t_1 [17,18]. A similar study was conducted in which the degree of association of methanol or acetonitrile with the phase was probed by using deuterated organic modifiers [19].

Much recent information concerning the heterogeneity of silica-based stationary phase surfaces has come from studies using fluorescent probe molecules which are either physisorbed or covalently bound to the phase. Lochmuller et al. [20,21] covalently attached the fluorescent probe, dansyl amide, to study the surfaces of LiChrospher Si 100 and reversed-phase silicas. Bauer et al. [22] studied the adsorption of pyrene on Merck 60 silica gel both in the absence and presence of long-chain alcohol coadsorbates using fluorescence excitation, emission and decay techniques. Lochmuller et al. [23] determined the proximity and distribution of silica surface silanols by observing the fluorescence of chemically bound pyrene as a function of pyrene-ligand bonding density. In a later study [24], the same authors used time-dependent fluorescence to resolve the pyrene fluorophores into three distinct populations and estimated the relative amount of each population. Oelkrug et al. [25] studied the adsorption of N-heterocycles onto silica from either the gas phase or solution as a function of temperature and surface hydroxyl concentration using a combination of transient

absorption, fluorescence and phosphorescence decay measurements.

In separate studies, Stahlberg and Almgren [26] and Carr and Harris [27] used the fluorescence vibronic structure of pyrene to probe the polarity of a C_{18} -derivatized silica surface as a function of mobile phase composition (methanol-water). Further sorption site information was obtained by Carr and Harris [28] from studies in which ionic (prefers mobile phase) vs. non-ionic (prefers stationary phase) fluorescence quenchers were added.

These studies reveal that the surface of silica-based phases is comprised of a heterogeneous distribution of sites, whether they be adsorptive or chemically reactive sites for underivatized silica [22–25], reacted vs. residual sites for derivatized silicas [20,21], or partitioning sites for reversed phases [28]. There are a small percentage of strongly adsorptive/reactive sites [22] and there is a tendency for adsorption or reaction to occur in clusters even at low surface coverages [23,24]. The non-uniform interactions of silica-based phases with solvents and solutes is attributed, at least in part, to the surface heterogeneity [26–28]. There remains considerable controversy as to the specific “structure” of the various adsorption sites on silica as detailed in two comprehensive reviews by Nawrocki [29,30].

We recently investigated the feasibility of designing substrates for room temperature phosphorimetry (RTP) based on the coderivatization of silica with analyte- and heavy ion-binding ligands [31]. (Certain heavy ions are known to enhance phosphorescence quantum yields). In these experiments, we measured the phosphorescence of a model analyte (phenanthrene) as a function of amount analyte deposited on both derivatized and underivatized silica thin-layer phases. While the approach yielded limited success for the design of analytically useful RTP substrates, our research demonstrated that phosphorimetry is a powerful tool for studying adsorption processes on silica and derivatized silicas and should also be applicable to the study of partitioning processes.

Because of the inherently long lifetimes of excited triplet states, phosphorescence is extremely sensitive to the microenvironment of the

phosphor including its state of rigidity and number of collisions it experiences. A strong surface–phosphor interaction results in a rigidly held phosphor experiencing less motion and providing a higher phosphorescence yield. By using a low-surface-area base silica (ca. 2 m²/g), even higher phosphorescence signals are obtained, making the use of conventional room temperature fluorescence (RTF)/phosphorescence instrumentation feasible for these studies. Our results confirmed the heterogeneous nature of silica-based phases, but also provided specific information concerning the adsorption site of phenanthrene as a function of the amount deposited on underivatized, reversed-phase and ion-exchange phases. Based on these studies, phosphorimetry should be pursued as a fundamental tool for the study of chromatographic surface processes.

2. Experimental

2.1. Reagents

Phenanthrene (Sigma, >96%), sodium dodecyl sulfate (SDS; Fluka, >98%) and thallium nitrate (Alfa, 99.5%) were used as received. Sodium iodide (Fisher) was recrystallized twice from acetone–diethyl ether and dried under vacuum at 50°C before use. Dichloromethane and toluene (Fisher) were distilled under nitrogen over P₂O₅ and CaCl₂, respectively. All silanes were purchased from Huls America, desiccated under nitrogen and used as needed. Dimethylaminopyridine (Nepera) was dried

overnight at 80°C and desiccated. Peroxyoctanoic acid was prepared as needed from caproic acid (Sigma) and 30% hydrogen peroxide (Fisher) in concentrated sulfuric acid and extracted into diethyl ether for immediate use according to Parker et al. [32] and Fazio et al. [33].

2.2. Derivatized silicas

The base silica used for all RTP studies was a 1.5- μ m non-porous silica donated by Keystone Scientific. This silica has a low specific surface area of 2.04 m²/g (BET analysis, krypton adsorption, Micromeritics Materials Analysis Laboratory) which allows high sensitivity for phosphorescence measurements. *n*-Butyl-, *n*-octyl-, *n*-octadecyl-, 2-(3-cyclohexenyl)ethyl-, 3-iodopropyl- and thallium propylsulfonate-derivatized Keystone silica phases were prepared in our laboratory as described below. Because of the low surface area of the Keystone silica, elemental analysis and other conventional techniques were not sensitive enough to characterize derivatized phases made using this silica. Therefore, derivatization conditions were developed and tested using higher surface silicas and then applied to the Keystone silica. Silicas used to develop derivatization conditions were Waters Nova 5- μ m (114 m²/g) and 12- μ m (119 m²/g) silicas, Waters Resolve 5- μ m silica (175 m²/g) and Davisil 20–30- μ m silica (300 m²/g). Bonding densities obtained for the higher surface area silicas are given in Table 1.

Table 1
Bonding densities obtained for derivatized silicas

Ligand	Silica	Huls silane	Bonding density (μ mol/m ²)
<i>n</i> -Butyl	Waters Nova 12 μ m	<i>n</i> -Butyldimethylchlorosilane	3.5 ^a
<i>n</i> -Octyl	Waters Nova 5 μ m	<i>n</i> -Octyldimethylchlorosilane	3.2 ^a
<i>n</i> -Octadecyl	Waters Nova 12 μ m	Dimethyl- <i>n</i> -octadecylchlorosilane	3.3 ^a
Cyclohexenyl	Waters Nova 12 μ m	[2-(3-Cyclohexenyl)ethyl]dimethylchlorosilane	3.2 ^a
Iodopropyl	Waters Resolve 5 μ m	3-Iodopropyltrimethoxysilane	2.6 ^a
Propylsulfonic acid	Davisil 20–30 μ m		0.94 ^b

^a Calculated from %C analysis.

^b Active capacity based on acid–base titration. Total bonding density is 1.2 μ mol/m² based on %S.

Prior to derivatization, all silicas were acid washed by refluxing in 0.1 M HNO₃ overnight and rinsing to neutrality with water and subsequently dried at 105–110°C overnight. Reversed-phase derivatized silicas were prepared by reacting the silica with the appropriate alkyldimethylmonochlorosilane overnight in refluxing dichloromethane, using dimethylaminopyridine (DMAP) as catalyst. A 4:1 molar ratio of silane and 6:1 molar ratio of DMAP were used assuming a reactive silanol surface density of 5 μmol/m². Iodopropyl-derivatized silicas were prepared by reacting the silica with a 4:1 molar ratio of 3-iodopropyltrimethoxysilane overnight in refluxing toluene. Propylsulfonic acid-derivatized Keystone silica was prepared by reacting the silica with a 4:1 molar ratio of 3-mercaptopropyltrimethoxysilane overnight in refluxing toluene, followed by peroxyoctanoic acid oxidation of the thiol to the sulfonic acid following the basic procedure of Fazio et al. [33]. Bonding densities obtained for the higher-surface-area silicas are given in Table 1. The propylsulfonic acid-derivatized silica was converted to its TI⁺ form by washing 6 g of the phase with 100-ml portions of 0.01 M TiNO₃ (6 ×), water (6 ×), methanol (1 ×) and then air drying.

2.3. Sample spotting and phosphorescence measurement

For use as RTP substrates, the silica and derivatized silicas were cast into 200-μm thickness thin-layer phases using a low level of cornstarch binder. Details of the procedures for thin-layer phase casting are reported elsewhere [31]. Thin-layer phases were prepared for spotting by marking off a 6.25 mm circular area (hole punch size) using a technical drawing pen (no ink) and a template. A 1-μl volume of the appropriate solution was then spotted (3–4 replicates per sample load) onto the center of the area using a Hamilton microsyringe. The spotting volume was chosen so that the liquid would spread to fill the marked area without bleeding onto the surrounding phase. The spotting solution used for all experiments was either methanol (for bare

substrate experiments) or 0.08 M SDS–0.024 M TiNO₃ (for coadsorbed surfactant experiments). After spotting, the sample was dried and mounted onto a sample holder. The area of illumination was reduced to a 5 mm circular area by using a flat black mask.

The instrument used for all phosphorescence measurements was a Hitachi F-4010 fluorescence spectrophotometer equipped with a phosphorescence accessory capable of resolving signals with lifetimes ≥ 1 ms. A semi-closed system comprising the optical Dewar assembly normally used for low-temperature measurements was used for inert gas purging with high-purity nitrogen passed through a high-capacity drying tube (Fig. 1). With this purging system, the signal increases initially and then reaches a steady state which is stable for several minutes or more at nitrogen flow-rates of 2–3 l/min. The signal reported is the ratio of the sample steady-state signal to the blank silica or derivatized silica phase steady-state signal. Although the residual humidity and oxygen levels present during purging were not measured in the sample chamber, the same purging conditions (pressure and flow-rate) were used for all measurements in a given study. λ_{ex} was 295 nm for all phosphorescence measurements. λ_{em} varied from 502 to 507 nm as noted in the figure captions.

The relative standard deviation of the average phosphorescence intensity for a given sample load ranged from 5 to 50%. Despite the large

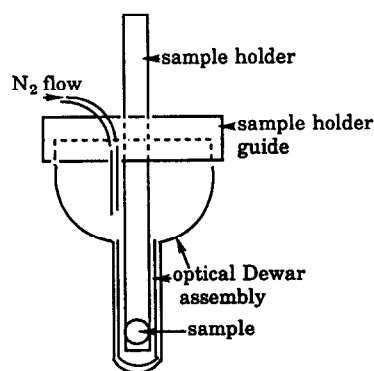


Fig. 1. Inert gas purging design. The sample is positioned in the optical path. Source and detector geometry is 45° from surface normal.

variation among individual measurements, the average phosphorescence intensity for a given sample load was reproducible. While the surface heterogeneity of silica makes a large contribution to the measurement variability, the presence of binder in the thin-layer phases is another source of variability. Other researchers are advised against the use of binder-containing thin-layer phases in fundamental studies.

3. Results and discussion

Phenanthrene was chosen as the probe for several reasons. Phenanthrene is a strong phosphor which previously has been studied in several different sampling modes including low-temperature phosphorimetry [34,35], micelle-stabilized RTP [36,37] and solid-substrate RTP, both with filter paper [38–40] and commercial silica thin-layer phase [41] substrates. Because it is a neutral molecule and has no functional groups, it enables the study of surface–adsorbate interactions uncomplicated by pH effects or specific functional group interactions. Also, because of its non-polar nature, phenanthrene represents a good test case for interaction with surfactant alkyl chains and reversed phases.

The main mode of interaction of phenanthrene with a surface is expected to occur via its aromatic π -electron system. There are a variety of silica surface hydroxyl structures, but in general, they are weak, inorganic acids. Hydrogen bonding between weakly acidic silanols and the electronegative π -system is a favorable interaction. Ramasamy and Hurtubise [42] obtained IR spectra for phenanthrene on polyacrylic acid which were consistent with a hydrogen bonding interaction between phenanthrene π -electrons and the polymer carboxylic acid groups.

The photophysics of phenanthrene adsorbed on bare silica have only recently been studied by Liu et al. [43–45], who studied six polyaromatic hydrocarbons (PAHs), including phenanthrene, adsorbed on silica gel. Because their research has direct bearing on the present work, it is reviewed with respect to phenanthrene in the next section. Liu et al.'s research confirms that

phenanthrene is an ideal probe to study adsorption on silica because its photophysics, including phosphorescence yield, are extremely sensitive to the surface–adsorbate interactions.

3.1. Photophysical studies of Liu et al.

Liu et al. determined the fluorescence lifetime distribution [44], fluorescence quantum yield [45] and rates of total decay, fluorescence decay and non-radiative decay [45] for phenanthrene adsorbed on both “wet” and “dry” Kieselgel-60 silica gel. “Wet” silica refers to a pretreatment of 8 h at 25°C under vacuum; the resultant surface is assumed to be hydroxylated and contain a high density of hydrogen-bonded hydroxyl groups. “Dry” silica refers to the same pretreatment except at a temperature of 800°C; the resultant surface is assumed to be highly dehydroxylated containing significantly fewer hydroxyl groups, of which most or all are isolated hydroxyl groups [44].

The fluorescence spectrum and fluorescence lifetime distribution profile for phenanthrene on wet silica were similar to those obtained in polar solvents. However, a dramatic decrease in the fluorescence lifetime distribution profile [44] and large increases in both the rate of non-radiative decay [45] and the phosphorescence quantum yield relative to the fluorescence quantum yield [44] were observed for phenanthrene in going from wet to dry silica (phosphorescence quantum yields were not actually measured but phosphorescence increased dramatically relative to fluorescence). The fluorescence spectrum was also broadened on the dry silica [44].

The results for phenanthrene and the other PAHs were interpreted in terms of the adsorbate–surface interactions and the symmetry properties of the lowest excited singlet state [44,45]. The PAHs were classified into two groups according to whether or not the S_0 – S_1 transition is symmetry allowed or forbidden. Phenanthrene has a symmetry-forbidden S_0 – S_1 transition with a molar extinction coefficient (ϵ_{\max}) of only 250 [44].

On wet silica, adsorbed phenanthrene ex-

periences an environment similar to polar solvents due to association with hydrogen-bonded hydroxyl groups. The symmetry properties of the lowest excited singlet state are not significantly perturbed relative to polar solvents. The situation changes for dry silica. The authors theorized that formation of a surface complex between phenanthrene and isolated hydroxyls on dry silica causes a perturbation of symmetry-forbidden singlet states leading to a "mixed state" with increased rates of S_1-T_1 intersystem crossing. This perturbation is consistent with all of the observed changes in the measured photophysical parameters of phenanthrene in going from wet to dry silica, including the larger increase in phosphorescence quantum yield relative to fluorescence quantum yield. The photophysical parameters for PAHs with symmetry-allowed S_0-S_1 transitions were similar on wet and dry silica [44,45].

3.2. Adsorption on underivatized silica: bare vs. coadsorbed surfactant studies

The spotting solution used in our early RTP studies contained 0.08 M SDS, a long-chain surfactant. Based on studies with reversed-phase derivatized silicas (discussed in the next section), we suspected that the long-chain surfactant was attenuating adsorbate-silica interactions, resulting in an overall lower phosphorescence yield for the adsorbate. To address this question, we determined the phosphorescence response of phenanthrene as a function of the amount deposited on bare silica (phenanthrene spotted from methanol) and compared the results to the coadsorbed surfactant case (phenanthrene spotted from 0.08 M SDS–0.024 M $TiNO_3$).

The results are plotted in Fig. 2 and clearly demonstrate a higher response on bare silica over the entire range tested (0–180 ng phenanthrene), confirming that the coadsorbed surfactant attenuates the phosphor-silica interactions. Furthermore, the response on bare silica is discontinuous while the response in the presence of coadsorbed surfactant is continuous. The implication is a more heterogeneous adsorption surface for bare silica and a more homogeneous

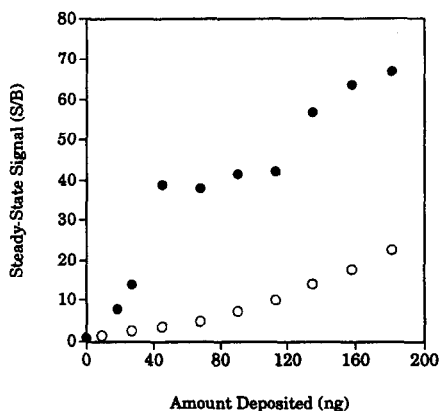


Fig. 2. Phenanthrene phosphorescence response on underivatized Keystone silica phases, bare substrate (●) vs. coadsorbed surfactant case (○). Bare substrate is spotted from methanol, coadsorbed surfactant case is spotted from 0.08 M SDS–0.024 M $TiNO_3$, λ_{em} is 502 nm for both phases.

adsorption surface with weaker surface-adsorbate interactions in the presence of coadsorbed surfactant. The strong surface-adsorbate interactions for bare silica were also confirmed by the appearance of vibrational fine structure in the phenanthrene phosphorescence spectra (Fig. 3). This fine structure was absent in the spectra for the coadsorbed surfactant case, with the phos-

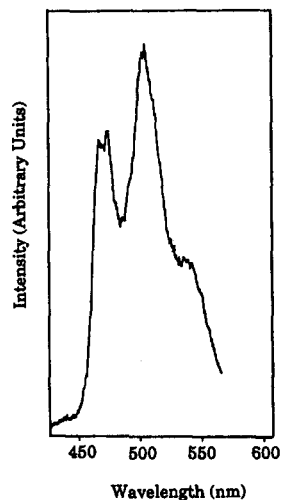


Fig. 3. Phenanthrene phosphorescence spectrum on bare, underivatized silica thin-layer phase. Phenanthrene is deposited from methanol at 180 ng. Excitation and emission bandpasses are both 5 nm.

phorescence emissions centered at 472 and 502 nm appearing as two smooth bands.

These results are wholly consistent with the fluorescence studies conducted by Bauer et al. [22] for pyrene adsorbed on silica. Bauer et al. [22] demonstrated that the surface of silica comprises a heterogeneous distribution of adsorption sites of which a small percentage can be characterized as strong, preferred adsorption sites. They further demonstrated that the adsorption surface becomes more uniform in the presence of glycerol or 1-decanol coadsorbed at greater than 50% surface coverage, evidently via the blockage of the strong adsorption sites. The surface coverage of SDS in our experiments is estimated to be 340% based on a contact surface area of 39 nm² per SDS micelle. It is assumed that the three-dimensional solution micelle structure collapses upon adsorption and drying to give a contact area ($2\pi r^2$) with the silica surface which is equal to one half the micelle surface area. This is a reasonable assumption for two reasons: first, the silica used in the study is non-porous so that penetration into the particle is not expected and second, dodecyl sulfate micelles are known to be “dry” or devoid of water even in solution [46]. Other pertinent parameters used in this calculation are micelle radius of 2.5 nm [47], aggregation number 62 [48], critical micelle concentration of 0.0081 M [48] and surface area available for adsorption on silica of 0.008 m² (calculated from specific surface area of silica and mass of silica in spotted disc). The estimated surface coverage does not include the additional surface area occupied by surfactant monomer.

Examination of the discontinuous response on bare silica reveals three distinct regions: a steep response region from 0–45 ng, a flat response region from 45–115 ng and another dynamic region above 115 ng. Loadings above 180 ng were not tested. The last point for each of the lower two regions represent 0.032 and 0.081 μmol of phenanthrene per m² of silica, respectively. Assuming a molecular area of 150 Å² for phenanthrene based on Snyder's method [49], the corresponding percent surface area coverages are 3 and 8%, respectively. A plausible explana-

tion for the steep response region is that it represents the filling of the strongest adsorption sites. The “titration” of strong adsorption sites using phosphorescence gives similar results to the gas-phase titration technique used by Nawrocki [30]. Nawrocki observed multi-tiered “titration curves” in which the strongest adsorption sites representing the first tier ranged from 0.035–0.095 μmol of diethyl ketone per m² of silica for three different silicas. If the 3% surface area coverage from strong adsorption sites from our study is used to calculate the area density of strong adsorption sites, the result is 0.24 $\mu\text{mol}/\text{m}^2$ based on a total silanol bonding density [50] of 8 $\mu\text{mol}/\text{m}^2$.

The two higher loading regions are more difficult to interpret. A key question is whether adsorption occurs randomly or in aggregates. In general, adsorption from a solution phase occurs favors aggregation [25]. Lochmuller et al. [23] found that pyrene based silanes react on the surface of silica in clusters at surface coverages as low as 1%. If clustered adsorption does occur in our experiments, then the second dynamic range could represent strong interactions among aggregated molecules. It is important to note that the phosphorescence spectra of adsorbed phenanthrene on bare silica did not change to any significant extent as a function of loading.

3.3. Adsorption on reversed phases in presence of coadsorbed surfactant

Reversed phases were investigated to determine if alkyl chain–analyte interactions would decrease the rate of phosphorescence-quenching processes for a non-polar analyte such as phenanthrene. The spotting solution used in the reversed-phase experiments was again 0.08 M SDS–0.024 M TiNO₃. However, due to the hydrophobicity of the reversed phases, it was necessary to prespot with methanol to promote wetting. The phosphorescence response of phenanthrene was determined as a function of the amount deposited on *n*-butyl, *n*-octyl and *n*-octadecyl phases. A cyclohexenyl phase in which the cyclohexenyl moiety was attached via

a two-carbon spacer was also tested to determine if the ring structure would result in a higher phosphorescence response for phenanthrene owing to a more rigid environment and/or more favorable interactions.

The results for the *n*-butyl phase are given in Fig. 4 vs. the underivatized phase. For both phases, there is a general increase in phosphorescence intensity with increased phenanthrene loading, but the response on the *n*-butyl phase shows significant scatter, especially at higher phenanthrene loadings. The intensity of the response is about the same in the lower loading region (0–70 ng) for the two phases, but is slightly lower for the *n*-butyl phase in the higher loading region (70–180 ng). Evidence that the lower loading region corresponds to adsorption at unreacted silica surface sites and the higher loading region corresponds to association with the alkyl chains was obtained in subsequent experiments. The higher scatter for the *n*-butyl phase is indicative of a more heterogeneous environment, as is expected for a derivatized silica surface. The scatter may also be due to bulk phase inhomogeneity because it was more difficult to obtain homogeneous casting dispersions for the reversed phases.

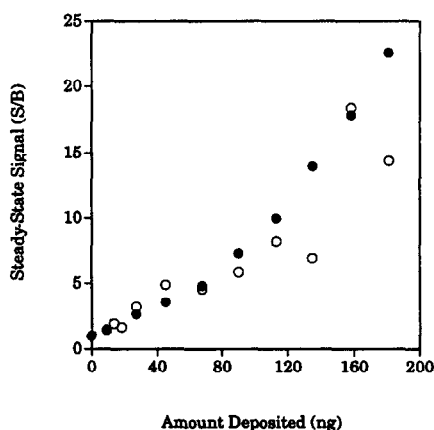


Fig. 4. Phenanthrene phosphorescence response on underivatized (●) and *n*-butyl-derivatized (○) Keystone silica phases. Both phases were spotted from 0.08 *M* SDS–0.024 *M* TINO₃. λ_{em} is 502 nm for the underivatized phase and 504 nm for the *n*-butyl phase.

Results for the *n*-octyl and cyclohexenyl phases are given in Fig. 5 vs. the *n*-butyl phase. For clarity, the *n*-octadecyl phase results are not plotted. The response for the *n*-octyl, cyclohexenyl and *n*-octadecyl phases were lower than the *n*-butyl phase over the entire loading range with the trend *n*-octadecyl \approx cyclohexenyl \ll *n*-octyl $<$ *n*-butyl. Phosphorescence was barely detectable for the *n*-octadecyl phase (signal/background near 1) although the actual spectrum of phenanthrene was distinguishable vs. the blank spectrum even at the lowest loading tested (0.2 ng). These results indicate that the interactions of phenanthrene with reversed phases is weaker than for underivatized silica and the interactions become weaker as chain length increases. The response for the cyclohexenyl phase with a total of 8 carbons is much less than the C₈ straight chain phase and almost as low as the C₁₈ phase. This behavior is actually consistent with a rigid phase and probably indicates that penetration of phenanthrene into the phase did not occur under the conditions of this experiment. Both phase wetting and penetration from an aqueous surfactant solution are expected to decrease as the hydrophobicity of the phase increases. The lower phosphorimetric response on reversed phases

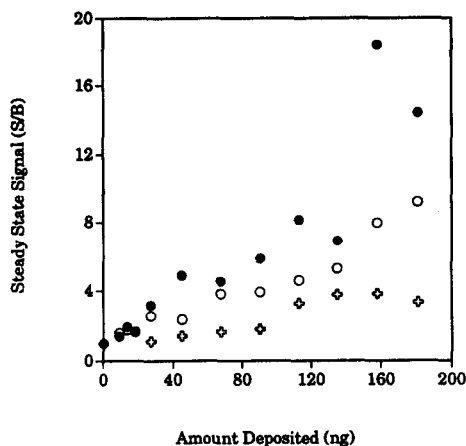


Fig. 5. Phenanthrene phosphorescence response on reversed phases: *n*-butyl (●), *n*-octyl (○) and cyclohexenyl (+). All phases were spotted from 0.08 *M* SDS–0.024 *M* TINO₃. λ_{em} is 504 nm for the *n*-butyl and *n*-octyl phases, and 507 nm for the cyclohexenyl phase.

also represents a further attenuation of the strong phosphor–bare silica interactions.

3.4. Heavy atom/ion derivatized silica phases

One goal of this research was to determine the feasibility of incorporating heavy ion enhancers onto a phase by derivatizing silica with ion-exchange ligands. Whether or not a given heavy ion will cause net enhancement for a given phosphor is largely empirical and depends on the relative change in rates of intersystem crossing, collisional deactivation and phosphorescence caused by the heavy ion perturber [51] which is a function of the relative amounts of phosphor and heavy ion perturber [52]. Thallous ion was determined to provide net enhancement of phosphorescence for phenanthrene adsorbed on underivatized Keystone silica in previous experiments [31], with loadings of 1–60 $\mu\text{mol}/\text{m}^2$ thallous ion providing a minimum of 2.5 times enhancement.

A thallium propylsulfonate-derivatized silica phase with an estimated thallous ion loading of 0.9 $\mu\text{mol}/\text{m}^2$ was subsequently prepared and the phosphorescence response of phenanthrene as a function of loading on this phase was determined. An iodopropyl-derivatized silica phase with an estimated iodine loading of 2.6 $\mu\text{mol}/\text{m}^2$ was also prepared and tested. Iodine was tested in its covalent form because iodide was determined to be unstable on silica surfaces [31], probably due to the surface acidity.

The results for the thallium propyl sulfonate and iodopropyl phases are given in Fig. 6 vs. the underivatized phase and are split out into the lower loading region (0–45 ng, Fig. 6A) and the higher loading region (45–180 ng, Fig. 6B). All phases were spotted from methanol so that no coadsorbed surfactant was present. The net result for the thallium propylsulfonate phase is almost no difference vs. the underivatized phase in the lower loading region, but a lower response in the higher loading region. The net response for the iodopropyl phase is almost no difference vs. the underivatized phase in the lower loading region, but a higher response in the higher loading region.

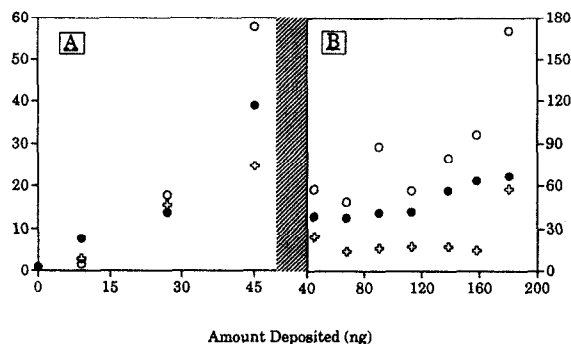


Fig. 6. Phenanthrene phosphorescence response on iodopropyl (○), thallium propylsulfonate (+) and underivatized (●) Keystone silica phases for both low (A) and high (B) loading regions. All phases were spotted from methanol. λ_{em} is 502 nm for the iodopropyl and underivatized phases, and 503 nm for the thallium propylsulfonate phase. Note the difference in both x- and y-axis scales for the two plots. y-Scale is intensity in arbitrary units.

These results provide information regarding the general adsorption sites of the phosphor and provide further evidence that the lower loading region corresponds to unreacted surface sites. Enhancement of phosphorescence should only occur if the analyte and heavy ion/atom are in close enough proximity. The heavy atom for the iodopropyl phase is covalently attached to the alkyl chain ligands. The fact that no enhancement occurs in the lower loading region indicates that the phosphor is associated with unreacted surface silanols. Enhancement in the higher loading region indicates a closer association of the analyte with the alkyl chain ligands for these loadings. The enhancement from the heavy atom is sufficient to provide a net phosphorescence increase despite the attenuation caused by the alkyl chains.

The heavy ion for the thallium propylsulfonate phase is not covalently linked to the propylsulfonate ligand. It is free to migrate according to its relative attraction to the silica surface and the sulfonate counterion and its proximity to the alkyl chains is expected to be much less vs. the iodopropyl phase. The fact that no enhancement occurs in the lower loading region and net attenuation occurs in the higher loading region

for the thallium propylsulfonate phase is again consistent with close association of the phosphor with the unreacted silica surface at low loadings and association with the alkyl chains at higher loadings.

4. Conclusions and suggestions for further study

When comparing results from different researchers, it is imperative to consider the different ranges of surface coverage that the experiments comprise, as well as the mode of attachment (adsorptive or covalent) of the probe molecules to the surface. Our research comprises surface coverages (0–0.14 μmol of phenanthrene per m^2 of silica) that are most similar to Nawrocki's [30]. A summary of the surface coverages and probe conditions used by the other researchers cited in this paper is given in Table 2. Surface coverages for the other researchers' work are estimated from the information given in the references.

It is significant that no decrease in phosphorescence occurred in the lower loading regions for any of the short alkyl chain (C_3 or C_4) derivatized phases (reversed phases, heavy ion/atom phases), providing a consistent picture in which strong residual adsorption sites are present and accessible for these phases. It is also important to emphasize that these experiments were designed to test the analytical utility of silica phases

and not to study fundamental adsorption processes. For fundamental adsorption studies, powdered samples rather than thin-layer phases should be used to eliminate unwanted interferences from binders. Precise measurement as well as control of humidity and oxygen levels should be made in the sample chamber.

Despite the limitations of the present study, the use of phosphorimetry for the study of surface heterogeneity and adsorption processes has clearly been demonstrated. The degree of surface heterogeneity is examined by "titrating" the surface with adsorbate and observing the change in phosphorescence signal, which is directly related to the average strength of surface-adsorbate interactions. The adsorption site as a function of adsorbate loading can be probed on derivatized phases by selective incorporation of heavy atom/ion enhancers. These types of studies can be extended to other base silicas, derivatized silicas and adsorbates. The effect of various silica pretreatments and derivatization processes on surface heterogeneity can be determined. It is important to note that phosphorescence sensitivity is also a function of silica specific surface area [31], with lower surface area silicas giving higher sensitivity.

The type of information which can be gained from phosphorimetric studies supplements and/or complements information obtained from IR and fluorescence studies and alleviates some restrictions. The type and strength of bonding

Table 2
Experimental conditions for silica surface studies

Ref.	Probe molecule	Mode of attachment	Surface coverage (reported units) ^a	Surface coverage ($\mu\text{mol}/\text{m}^2$) ^b
[20]	Dansyl amide	Covalent	0.07–0.31 (mmol/g)	0.29–1.3
[21]	Dansyl amide	Covalent	$1.5 \cdot 10^{-11}$ (mol/cm ²)	0.15
[22]	Pyrene	Adsorption	0.2–3 (%)	0.002–0.03
[23,24]	Pyrene	Covalent	0.13–1.10 ($\mu\text{mol}/\text{m}^2$)	0.13–1.10
[30]	Diethyl ketone	Adsorption	0.01–0.18 ($\mu\text{mol}/\text{m}^2$)	0.01–0.18
[44,45]	Phenanthrene and other PAHs	Adsorption	0.22 (mg/g) (phenanthrene)	0.0019
Present work	Phenanthrene	Adsorption	0–180 (ng/spot)	0–0.14

^a Ranges and units reported in cited reference.

^b Converted to μmol of probe molecule per m^2 of silica based on information given in reference.

interactions have been probed by IR, but the large silica background severely limits the accessible IR region and the ability to make quantitative measurements. Surface heterogeneity has been examined using fluorescence spectroscopy, but there is the need to choose probes which have critical interaction distances or whose spectral features are polarity or environmentally sensitive. By comparison, the background phosphorescence of silica is minimal and virtually all phosphorescent probes are sensitive to environment due to the inherent lifetimes of excited triplet states. Fluorescence and phosphorescence studies can easily be combined using today's conventional RTF/RTP instruments to gain the maximum information. Fluorescence quenching and phosphorescence enhancement measurements can be made on the same samples. The phosphorescence behavior of pyrene probes can be examined to determine the relationship between pyrene–pyrene interaction distances and strength of surface–pyrene interactions.

The application of phosphorimetry to the study of partitioning processes is also feasible. It is known that phosphorescence is easily quenched in the fluid state. An exception is micellar solutions in which PAH phosphors are protected from quenching processes by partitioning into the long-chain micelles. (Heavy ion enhancers must also be present.) It is therefore feasible that phosphorimetry be used to probe two types of analogous partitioning processes which are thought to occur in reversed-phase LC: intercalation of flat, rigid aromatics into C_{18} chains [53] and the collapse of C_{18} chains in the presence of high-water mobile phases [54,55].

Both theory [54] and experiment [53,55] support the model that C_{18} chains exist in an extended "bristle" state when they are fully solvated but exist in a collapsed state in the presence of high-polarity, high-water mobile phases. Recently, Montgomery et al. [56] provided evidence that C_{18} chains are wetted but neither fully solvated nor extended in the presence of low organic mobile phases. Shape selectivity is highest for high bonding density and/or polymeric phases in the extended state because only flat, rigid molecules are able to intercalate

between the extended chains [53]. The extended chain state is frequently described [53–55] as being "rigid", "stiff" or "ordered", but Bayer et al.'s [12] ^{13}C NMR studies showed a higher mobility for the extended state than the collapsed state. While shape selectivity is low for phases in the collapsed state, partitioning of solutes into the stationary phase is still possible for these phases and it is predicted that the solute distribution will follow classical liquid–liquid partitioning [54]. Assuming that intercalation and partitioning do occur, the phosphorescence of solute probes will be sensitive to the rigidity of C_{18} chains in the extended or collapsed state. In this regard, phosphorimetric studies would supplement NMR studies of alkyl chain mobility.

Finally, it may be possible to directly study bonded phase rigidity as a function of mobile phase composition by preparing a phosphorescent stationary phase. Such a phase will necessarily contain aromatic moieties, but Lochmuller et al. [55] have shown that a dipentylbiphenyl aromatic phase may be a good model for studying the bonded chain rigidity of C_{18} stationary phases.

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