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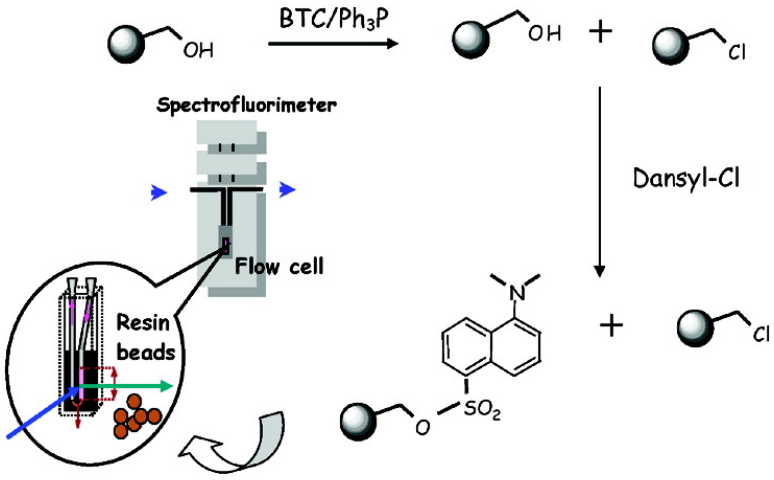

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Fluorometric Monitoring Of Organic Reactions On Solid Phase

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The direct monitoring of reaction progress on solid supports by fluorescence spectroscopy is described. An immobilized fluorescent tracer molecule (dansyl chloride) is used to monitor the reaction on OH resins (Argopore Wang, PS Wang, and Argogel Wang), both in batch and in parallel chemistry. Fluorescence measurements were obtained directly on solid phase. The method demonstrated to be a valuable tool for the quantitative determination of resin-bound hydroxyl groups, to study reaction kinetics and for continuously monitoring the progress of the conversion of the hydroxyl resins into the chlorinated ones. The procedure proposed is highly sensitive compared to the traditional ones. The system can be extended to monitor a variety of reactions on solid supports, and in conjunction with a well-established technique such as flow analysis, basic studies on solid-phase become possible.

Introduction

Solid-phase organic synthesis (SPOS) has become an increasingly important technique for the generation of libraries of small molecules of pharmaceutical and agrochemical interest.[1,2] In addition, reactions on solid supports are of key importance in the development of (bio)chemical sensors.[3,4] The lack of sensitive analytical techniques for on-bead analysis and the interference provided by the solid-phase backbone itself are key drawbacks associated with the chemistry on solid phase making it quite difficult to directly monitor the progress of the reactions and the in situ characterization of intermediates and products. Conventionally, the cleavage of the product from a portion of the solid phase is carried out, and the resulting product is analyzed by standard solution-phase analytical methods. Although it is possible to obtain useful structural and analytical information, the data are not always indicative of the actual on-bead situation, and the approach does not offer a real-time insight into the chemistry taking place on the resin. Titrimetric,[5] combustion elemental analysis,[6] spectrophotometric,[7] and fluorometric[8,9] assays have been employed for screening single bead/single compound libraries. There are also sophisticated strategies to obtain on-resin data, such as FTIR[10] NMR, MAS-NMR[11,12] and confocal Raman[13] that are beginning to be adapted in the area of solid-phase chemistry.

Analytical methods based on fluorescence detection have not been widely exploited in solid-phase chemistry, despite its high sensitivity. On the other hand, the described fluorometric methods were originally developed for peptide and oligonucleotide chemistry, so they were limited to functionalities of concern in this field. In this work, we investigated the analytical potential of a fluorescent probe, dansyl chloride, as a versatile readout output for the development of an accurate and convenient method for determination of the loading of conventional resins (PS Wang, Argogel Wang, and Argopore Wang) used in SPOS. In addition, the kinetics of a chlorination reaction[14] performed on the above-mentioned resins could be followed by incorporation, through a simple protocol, of the dansyl probe onto the final chlorinated resins. On the basis of these experiments, we found that the dansyl fluorometric method with a general calibration curve works well for the quantification of the chlorination reaction and offers a useful tool for determination of hydroxyl resin loading.

Results and Discussion

Spectral Characteristics of Dansyl-Labeled Resin Beads. The loading of the dansyl moiety as a fluorescent probe into the OH resins was accomplished by reacting the resins (20 mg) in a DCM solution of the probe for ∼45 min at room temperature using a dansyl/resin molar ratio of 1/1.5.[15] Then the resins were rinsed repeatedly with DCM in order to remove all unreacted dansyl. After a new thoroughly rinsing with methanol, the resins were vacuum-dried and stored protected from room light. The schematic representation of the whole procedure is shown in Scheme 1. The resin beads were held in place inside a flow cell, and emitted light was collected at 90° to the excitation beam (Figure 1). Immobilization of dyes onto solid supports has been studied extensively in the field of optical sensing using solid surface luminescence.[3] Different configurations are possible, among which the one used in this work is one of the more versatile due to the possibility of integration into continuous flow systems[16,17] for obtaining information with simplicity, reliability, and speed.

The excitation and fluorescence spectra of dansyl-modified resins are shown in Figure 2. The spectra markedly differ

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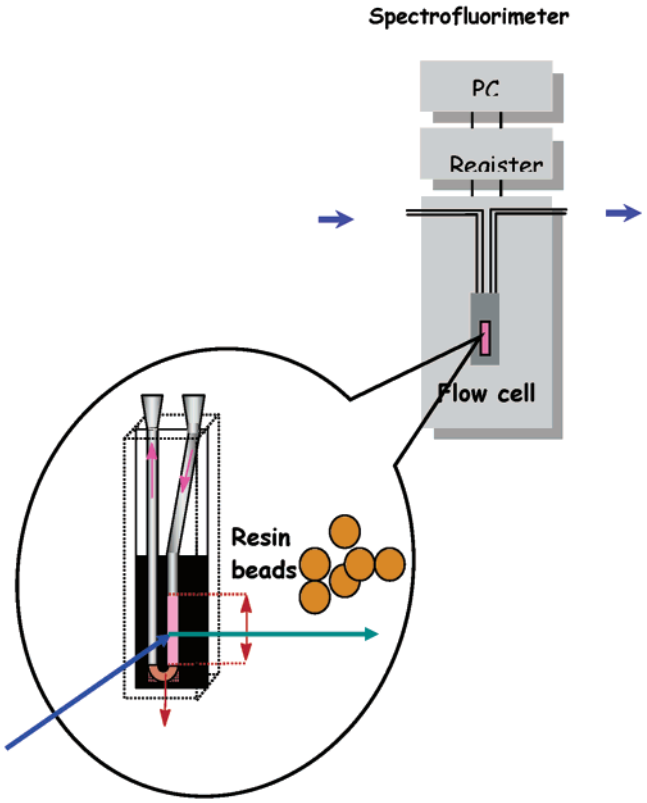
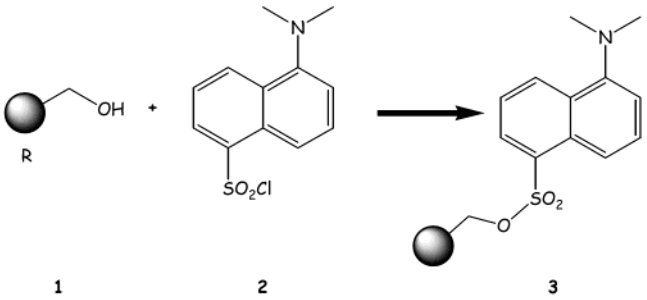


Figure 1. Flow-cell schematic showing the light beam and resin beads.

Scheme 1. Coupling of the Dansyl Chloride Probe with the Hydroxyl Group of a Solid Support or with That of a Molecule in Solution



3a, R: $-H_2C-$⟨benzene⟩

3b, R: Argopore Wang

3c, R: PS Wang

3d, R: Argogel Wang

from that of "blank" (unmodified) resins, indicating an effective incorporation of the dansyl probe onto the resins. The maximum excitation and fluorescence wavelengths of the dansyl label in solution (λ_{exc} = 340 nm, λ_{em} = 525 nm, in methanol) are shifted for the immobilized dansyl measured at the same illumination conditions. Spectral fluorometric data are collected in Table 1. Within the experimental error (±2 nm), a similar emission maximum was observed when comparing the dansyl-Argopore Wang resin with dansyl in methanol homogeneous solution, whereas a blue shift was seen in dansyl-PS Wang and dansyl-Argogel Wang resins. Because the polymers' microenvironments are likely to be more hydrophobic than methanol due to a higher content of

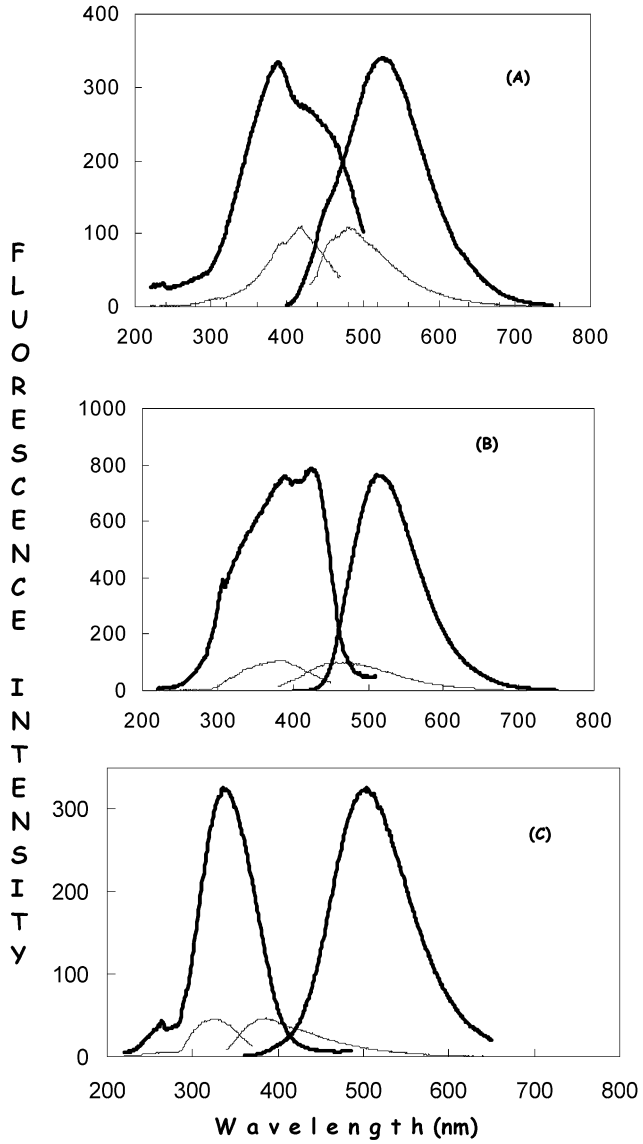


Figure 2. Fluorescence excitation and emission spectra of HO resins before (thin line) and after (thick line) reaction with dansyl chloride: (A) Argopore Wang, (B) PS Wang, and (C) Argogel Wang.

Table 1. Stokes Shifts, $\Delta\nu$ (in nm), and Peak Positions in the Excitation and Fluorescence Spectra of Dansyl Chloride in Different Microenvironments

dansyl environment	λ_{ext}	λ_{em}	Stokes shift
methanol	340	525	185
Argopore Wang	387	528	141
PS Wang	390	513	123
Argogel Wang	336	506	170

polymer branches, the spectral data in Table 1 seem to indicate that the fluorescence maximum of the immobilized dansyl label is not markedly sensitive to the microenvironment polarity. In contrast, the results support the idea that the molecular viscosity[18] of the polymer surroundings exerts influence on the relaxation processes of the excited dansyl molecules. This means that within the lifetime of the excited state of the dansyl probe, the local polymer microviscosity does not allow a sufficiently fast relaxation to a state of minimum free energy, as compared with a fluid homogeneous solution.[19]

According to the chemical structure of the polymer backbone of the resins used in this work, immobilized dansyl should be located in three distinctively different environments. The long chains or coils of poly(ethylene glycol) in the Argogel Wang resin, where the dansyl probe is incorporated, resulted in a more viscous microenvironment, as compared with that experienced by the probe in the "empty" channels and macroporous of the Argopore Wang solid phase. An intermediate situation would correspond to the PS Wang resin. It is interesting to observe that dansyl incorporated into such spaces could be used as a molecular probe of molecular microviscosity within polymers. Although direct measurement of solvatochromic parameters of solid polymers is of great interest, it is out of the scope of the present work.

Dansyl Binding. The coupling of dansyl chloride was investigated in solution employing benzyl alcohol in order to evaluate its applicability to the solid supports used in this work (*p*-hydroxybenzyl alcohol polymer-bound-type resins). Chromatography of the crudes deriving from the reaction allowed the isolation of the sulfonated compound **3a** (75%), which was characterized by ^{1}H NMR, IR, and EIS-MS, and the data indicated that the reaction was run successfully in solution. This fact prompted us to explore the scope and limitations of the dansyl labeling reaction on the different resins.

Resins were loaded with variying equivalents of the fluorescent probe, and the excitation and emission spectra were recorded. Because all the solids presented a similar behavior, only the spectra of dansyl-Argopore loaded resin is presented (Figure 3a). As observed, the spectral maximums were independent of the probe concentration, and peak intensity increased continuously with concentration of incorporated dansyl. In Figure 3b, the fluorescence intensity versus the dansyl/resin mmol/weight ratio for the Argopore Wang and the PS Wang systems is presented. The dansyl/resin mmol/weight ratio is a useful tool for estimating the dansyl fractional occupancy in the resin. From the data in Figure 3a,b, we can conclude that fluorescence intensity exhibits a linear dependence on the amount of fluorophore attached to the resins, even for high loadings of the dye, and no self-quenching or reabsorption effects were observed. Because only the attached label is fluorescent (beads were subjected to repeated washes), the intensity of fluorescence will be directly proportional to the amount of reaction that occurs. At a given dansyl/resin mmol/weight ratio, fluorescence intensities reached a limiting value, indicating dye saturation at reactive sites. The method is analogous to a fluorometric titration that allows the determination of the resin loading at the breakthrough point (fluorescence remains constant). By extrapolation of the linear segments of each curve, the intersection point would be a measure of the corresponding resin-bound hydroxyl groups. So, the values 1.14, 0.36, and 0.60 mmol g^{-1} resin were found for PS Wang, Argogel, and Argopore Wang, respectively. These values are in good agreement (accuracity within 1−5%) with the loading values reported by the manufacturer.[20] These results are consistent with the fact that all binding sites are accessible to the dansyl label.

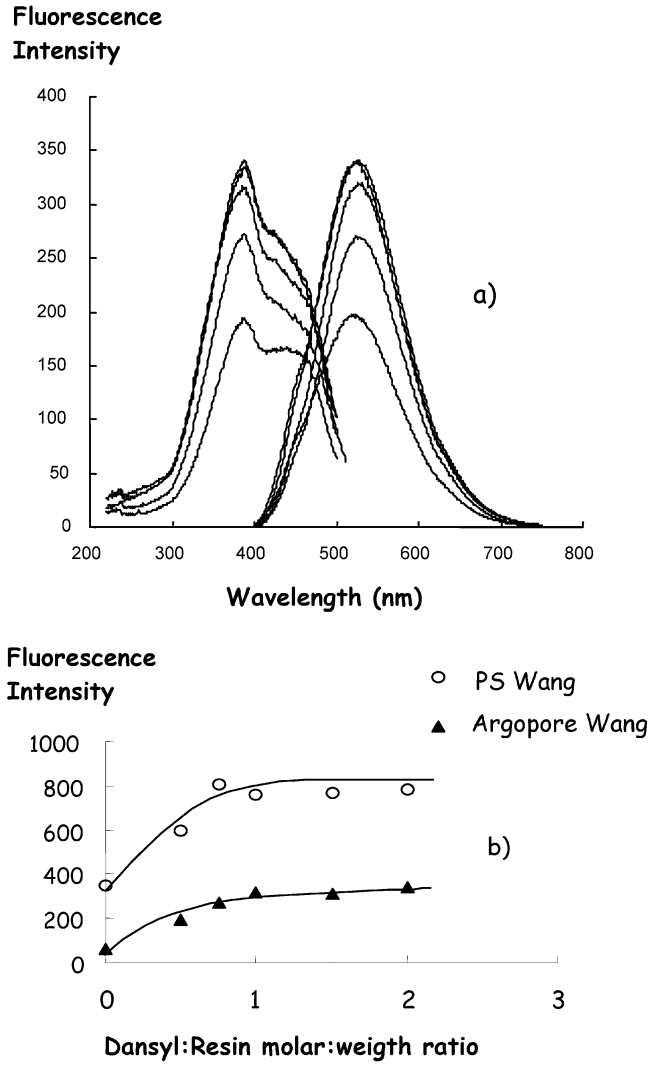


Figure 3. (a) Fluorescence spectra of labeled Argopore Wang resin obtained at different concentrations of dansyl chloride (λ_{exc} = 387 nm, λ_{em} = 528 nm). (b) Dependence of the fluorescence intensity with dansyl/resin molar/weight ratio. Argopore Wang (λ_{em}= 528 nm) and PS Wang (λ_{em}= 511 nm).

Recently, Bradley et al.[13] reported that for high fluorophore loadings (above 0.1%) the resin beads become optically thick, and a reduction in fluorescence intensity is caused by reabsorption of the dyed bead. This effect is much more significant at the core than at the bead edges due to the longer light path from the bead center. These findings were obtained by confocal fluorescence microscopy for probing single beads using rhodamine as the fluorescent probe. Under our measurement conditions, high dye loading was not quite so problematic as Figure 3a,b reveals. We could rationalize this fact considering that only the fluorescence of those molecules located at the outer surface of the beads is being effectively emitted. Taking into account the extinction coefficient of dansyl ϵ = 3900 M^{-1} cm^{-1} at 372 nm,[21] a light path of 1 μm, and a dye concentration of 0.3 M (as calculated by Bradley for TentaGel,[13] a resin with a nominal loading similar to that of Argopore), an absorbance of A = 0.12 would be calculated. At 5 μm, A = 0.6, and at 50 μm, A = 6.

It is worthy of attention that when light penetrates into a solid support with a rough surface, it can be reflected, refracted, or diffracted at particle boundaries and interfaces

(diffuse reflectance). In addition, it can be absorbed (it is significant in volume reflectance). Consequently, light can penetrate the bead core by different pathways, and application of the Beer−Lambert Law may be limited by several factors (scattering of light, fluorescence of the sample, stray light, etc.). A simple approach to describe the interaction of light with a diffusing (opaque) sample was proposed by Kubelka and Munk.[21] In this model, the reemission function $F(R)$ for an ideal diffuse scatterer which is *optically thick* at the wavelength of choice and for a homogeneous distribution of ground state molecules (absorbers) throughout the sample is given. The intensity of fluorescence of a dye in an opaque medium is related to the concentration of the dye through the reflectance that can be experimentally evaluated at the excitation wavelength.[22]

On the other hand, the process of reabsorption of emitted fluorescence depends not only on the overlapping between absorption and emission bands, but also on the fluorescence quantum yield of the dye, Φ. The quantum yield for n reabsorption processes is Φ^n. With $\Phi \ll 1$ the quantum yield approaches 0 with increasing n and the fluorescence emission disappears in the high frequency fraction of the emitted radiation. The quantum efficiency of dansyl and rhodamine dyes is affected by experimental conditions, such as solvents; however, their high fluorescence quantum yield exceeding 0.7 in nonaqueous media[23,24] is a particular advantage in minimizing reabsorption processes. Therefore, for the dansyl probe, there is a combination of desirable properties for use as a solid-phase probe, including an extinction coefficient typically $<5000\ M^{-1}cm^{-1}$, high fluorescence quantum yield, chemical stability of the conjugates, and very good resistance to photobleaching.

It is well-known that resin composition and solvents affect reaction kinetics on solid supports.[25,26] Some recent reports deal with the use of fluorescent dyes to study functional site distribution in resin beads using a virtual optical slicing technique.[27,28] The results have demonstrated that distribution of functional sites depends on the nature of the resin under study: for some resins, the dye would be confined predominantly to the outer shell of the beads, whereas for others, the binding sites are uniformly functionalized throughout the entire bead. In addition, the physicochemical properties of the fluorescent label, such as the spectral characteristics, tendency to aggregate,[29,30] hydrogen bonds with polymer chains and other nonspecific interactions with the solid support, and so forth, are of paramount importance to monitor solid-phase reactions and should be taken into account. At present, the conclusion must be that the results presented in our work are for these particular resin/solvent/dye selections only and not representative of other systems.

Kinetics of a Chlorination Reaction on Solid-Phase. The chlorination reaction was carried out as described in the Experimental Section. The reaction was stopped at different times as specified, and the fluorescence spectra of the chlorinated resins were recorded. It was observed that the fluorescence intensity of the unlabeled Argopore Wang and Argogel Wang ("blank" resins) remained substantially constant (≤0.5% decrease) while the chlorination reaction is taking place. However, the native fluorescence intensity of unlabeled PS Wang resin decreased continuously as the OH− groups were substituted by chlorine. This fact could be ascribed to the heavy-atom effect promoted by chlorine on the intrinsic fluorophors present in the resin backbone and may be related to the higher capacity of PS Wang resin, as compared to the others, which, in turn, leads to a highly effective concentration of the heavy-atom substituent in the vicinity of the native resin fluorescent moieties. The magnitude of the fluorescence decrease (≤2%) does not affect dansyl fluorescence monitoring.

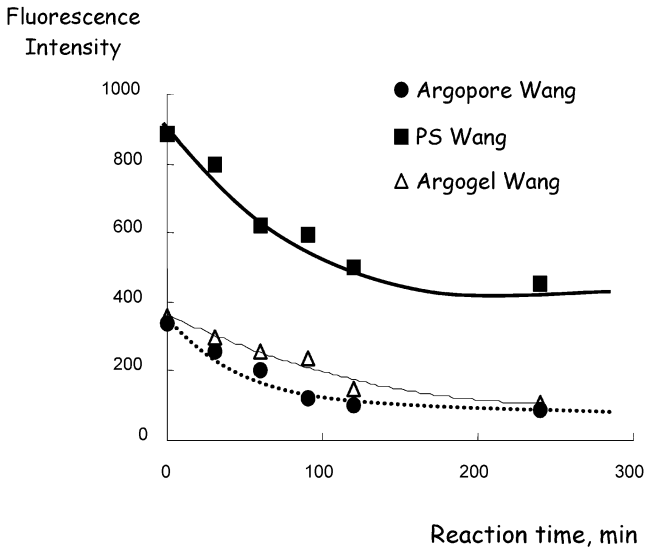


Figure 4. Fluorescence intensity vs reaction time for the conversion of hydroxyl resins to chlorinated ones. Argopore Wang ($\lambda_{em} = 528$ nm), PS Wang ($\lambda_{em} = 511$ nm), and Argogel Wang ($\lambda_{em} = 506$ nm).

Table 2. Kinetic Parameters Resulting from the Chlorination of HO Resins

solid phase	$K \times 10^5\ s^{-1}$	$t_{1/2}$, h
PS Wang	5.06	4
Argogel Wang	7.36	2.5
Argopore Wang	11.6	2

The beads removed from the chlorination reaction vessel at specified times after the initiation of the reaction were dansylated (blocking the unreacted hydroxyl groups) in order to assess the effectiveness of the dansyl probe as a tool to monitor the chlorination reaction on solid phase. The fluorescence intensity of the dansylated beads reflected the progression of the chlorination reaction, and in this manner, real-time monitoring of the dansyl fluorescence provides valuable kinetic information. Figure 4 shows that the conversion of hydroxylated resins to chlorinated ones seemed to proceed slowly. Kinetics of the chlorination reaction on the different solid supports (see Appendix I and Appendix II) showed that the time course of the reaction fitted to a pseudo-first-order reaction rate equation. Kinetic data are collected in Table 2. Similar results were obtained when the fluorescence of the supernatant in the resin mixture was used to follow the kinetics.

It is known that more than 99% of the reactive binding sites on resin beads are located in the pores of the resin,[25] and reactions at these sites are diffusion-controlled.[25,31] As can be seen in Table 2, we have found that the chlorination

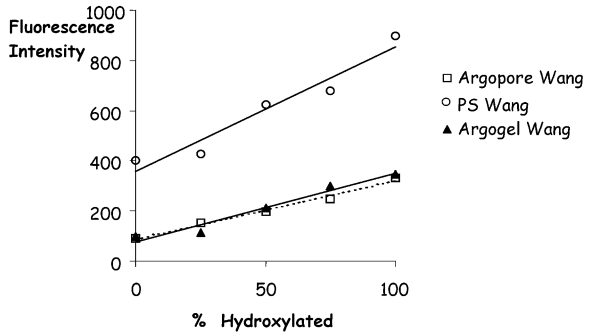


Figure 5. Calibration graphs for binary mixtures of hydroxyl/chlorinated resins obtained by fluorescence labeling of the HO resin portion.

reaction on PS Wang resin was slower than on Argogel Wang and Argopore Wang. These findings could be rationalized taking into account that the reaction inside and on the surface of the more rigid Argopore Wang beads should progress at the same rate, whereas on the Argogel Wang resin, due to its "solution-like" structure, there was no restriction to the diffusion of the reactants into the beads. Finally, the low fluidity of the PS Wang beads could afford restriction of diffusion, this being the rate-limiting step. The influence of the polymer matrix on the reaction rate should be similar to the influence of the solvent on a homogeneous solution reaction rate.[32]

The above experiments demonstrated that the reaction rate of the chlorination reaction was not improved when carried out on solid supports, compared with the reaction in a homogeneous solution.[14] Although this is a general trend for solid-supported reactions, there are examples for which the reaction rate is similar to or faster than in solution,[33] suggesting that the nature of the support, the affinity of reagents for it, and the nature of the reaction involved may play an important role in the reaction kinetics.

Quantitative Determination of Resin-Bound Hydroxyl Substituted Groups. To calibrate the resin-bound hydroxyl groups substituted by chlorine, control mixtures of chlorinated/hydroxylated commercial resins were prepared. The mixtures were then reacted with dansyl chloride in order to get the corresponding chlorinated/dansylated mixtures. The decrease in the fluorescence intensity of the dansyl probe (measured at the maximum emission wavelength for each solid, according to Table 1) with the increase of the ratio chlorinated/dansylated (*p*/*p*) was taken as a measure of the extent of the chlorination reaction. Figure 5 shows the calibration plots for the three systems under study. The plots exhibit linearity at a chlorination percent in the range between the detection limit and 100% for the three resins assayed. Again, it is noteworthy that for high dye loading, no self-quenching or reabsorption effects are observed. The detection limits expressed as chlorine concentration were determined as defined by IUPAC.[34] The values were calculated with the equation

$$\mathrm{DL} = k\sigma_B/m$$

where σ_B is the standard deviation of the blanks (dansylated resins), $k = 3$ for a confidence level of 99.86%, and m is the analytical sensitivity (slope of the calibration graph). The values currently achieved by us are 1.5×10^{-5}, 7.5×10^{-6}, and 6×10^{-6} mol Cl for PS Wang, Argogel Wang, and Argopore Wang resin, respectively. These results demonstrate that fluorescence is a highly sensitive technique suitable for continuously monitoring solid-phase organic synthesis. Validation of the results was assessed by the volumetric Volhard method.[35]

Conclusions

The results obtained in this work demonstrate that the use of a fluorescent tracer for monitoring the progression of organic reactions on resin supports is a highly sensitive and rapid procedure, as compared to the traditional ones in which the cleavage and subsequent analysis of the released products are necessary.

The method has been demonstrated to be a valuable tool from a basic point of view for the quantitative determination of resin-bound hydroxyl groups for continuously monitoring the progress of the chlorination reaction on solid phase and to study the reaction kinetics.

Potential applications can be envisaged by extending the approach to monitor a variety of reactions on solid supports. As other commonly used spectrophotometric approaches (e.g., the ninhydrin test for primary amine, the Ellman test for thiol, or the dinitrophenylhydrazine test for carbonyl functionality), the technique described in this paper is intrusive in the sense that chemical derivatization of unreacted functionalities present in the resin (such as in the studies reported here) is necessary to give species that can be detected fluorometrically. However, this limitation could be turned off by developing a different monitoring scheme. For example, a fluorescent molecule sensitive to a physicochemical property (e.g., mobility, viscosity, polarity, or any other), permanently attached to the solid support, could be used as an intrinsic sensor of the support microenvironment during the progress of the reaction under study. In this sense, dansyl fluorescence is sensitive to changes in the probe environment and has no tendency to aggregate either in solution or on solid supports. Its convenient spectral characteristics make dansyl a fluorescence probe of choice for solid-phase monitoring. The use of a flow-through cell system for fluorescence measurement allows a flexible configuration to study basic parameters, such as the influence of different solvents on the dye/resin system or its chemical and mechanical stability, with an important reduction in time and in the use of solvents. Work in this direction is currently in progress in our lab. Improving the methodology by measuring the process in a real-time in-situ basis with the aid of optical fiber devices will open new fields of applications.

Experimental Section

Materials. All resins used in this work were purchased from Argonaut Technologies Inc. PS Wang and PS Wang-Cl resins (polystyrene backbone lightly cross-linked with divinylbenzene) have loadings between 1.21 and 1.61 mmol/g, Argopore Wang and Argopore Wang-Cl resin (polystyrene polymer highly cross linked with divinylbenzene) have loadings of 0.6 mmol/g, and Argogel Wang and Argogel

Wang-Cl resin (polystyrene backbone lightly (1−2%) cross-linked with divinylbenzene grafted with poly(ethylene glycol)) have loadings between 0.38 and 0.39 mmol/g. All other reagents, if not specified, were purchased from Aldrich.

(3a) Benzyl (1-*N*,*N*-dimethyl-5-naphthalenesulfonate. Dansyl chloride (0.6 g, 2.2 mmol) in DCM (5 mL) was added to a solution of benzyl alcohol (0.2 g, 1.9 mmol) in DCM (5 mL) and TEA (4 drops). The reaction was stirred in an ultrasonic bath for 30 min at 60° C. The mixture was washed with a solution of $NaHCO_3$ (5%) and, finally, with a solution of NaCl (saturated). The organic phase was separated, and the excess of solvent was removed at reduced pressure. A yellow compound was isolated from the crudes by chromatography (0.47 g, 75%). IR(KBr): 3026, 2933, 1606, 1510, 1453, 1376, 1236, 1023 cm^{-1}. ^1H RMN($CDCl_3$): δ 8.62 (d, 1H, J = 8.6 Hz, Naph-H), 8.41 (d, 1H, J = 8.9 Hz, Naph-H), 8.24 (d 1H, J_1 = 1.2, J_2 = 7.8 Hz, Naph-H), 7.62 (dd, 1H, J_1 = 7.8, J_2 = 8.6 H_z, Naph-H), 7.43 (dd, 1H, J_1 = 7.8, J_2 = 8.6 H_z, Naph-H), 7.37−7.34 (m, 5H, Ar-H), 7.18 (d, 1H, J_1 = 1, J_2 = 7.8 Hz, Naph-H), 4.58 (s, 2H, Ar-CH_2), 2.80 (s, 6H, CH_3-N) ppm. EIS-MS(*m*/*e*): 364 (M^+ + Na), 284 ($M^+ - Me_2N{=}CH)^+$.

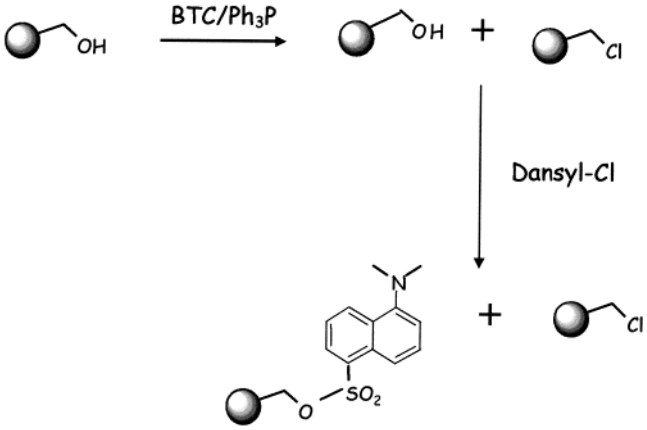
Conversion of Hydroxyl Resins to Dansyl Resins. An aliquot of the different resin beads (20 mg) was placed in the reaction vessel and suspended in 2 mL of DCM and 0.2 mL of TEA. A 2-mL portion of dansyl chloride (different molar ratio relative to the loading on resin) in DCM was added to the mixture. Each reaction vessel was placed in an ultrasonic bath for 45 min at room temperature. The resins were then filtered and washed with DCM (4 × 1 mL) and methanol (4 × 1 mL). The resins were dried in a vacuum and protected from light.

Fluorescence Spectroscopy. Fluorescence spectra were recorded on a Shimadzu (RF-5301PC) spectrofluorophotometer. Beads were washed free of any starting materials, and solvent and was placed into a Hellma model 176.52 flow-through cell (25 μL). Measurements of fluorescence intensity were obtained directly from the dry resin beads.

Chlorination Method. Conversion of hydroxyl resins to chlorinated ones was carried out according to the chlorination method described elsewhere.[14] We applied some modification to the original procedure as follows: Triphosgene (BTC) (1.46 g) was added with constant stirring to a solution of triphenylphosphine (3.472 g) dissolved in DCM at 0 °C (1:2.7 stoichiometric molar ratio), then the mixture was stirred for 20 min at 20°C, after which the solvent was removed in a vacuum. The crude white solid obtained was dissolved in 20 mL of DCM to obtain the chlorination reagent solution. PS Wang, Argopore Wang and Argogel Wang resins (150 mg), placed in parallel reactors, were swollen in DCM (5 mL) and agitated for at least 30 min, then the vessels were drained, and the chlorination reagent solution (1 mL = 1 equiv chlorination solution) was added to the resins. The reaction was allowed to proceed for 0, 30, 60, 120, 180, and 240 min. Parallel chemistry was carried out in a Quest Argonaut reactor, model SLN-210.

Operation parameters in the equipment were as follows. Agitation parameters: mix event, 4.0 s; up stroke, 3.0 s; % upward, 80%. Temperature, 20 °C; volume, 5 mL; automated solvent wash, DCM (3 × 5 mL), methanol (3 × 5 mL), and finally, DCM (3 × 5 mL). Agitation, 5 min; and drain time, 25 s. The resins were removed from the corresponding vessels and were dried under high vacuum for 18 h. Quantitative recoveries of the resins were obtained as white solids.

Scheme 2. Reaction Sequence for the Derivatization of Unreacted HO Resins after the Chlorination Reaction



Compounds 3b. Argopore Wang resin-(1-*N*,*N*-dimethyl-5-naphthalenesulfonated): IR(KBr) 1376 cm^{-1} (S═O). **Compound 3c.** PS Wang resin-(1-*N*,*N*-dimethyl-5-naphthalenesulfonated): IR (KBr) 1380 cm^{-1} (S═O). **Compound 3d**. Argogel Wang resin-**(**1-*N*,*N*-dimethyl-5-naphthalenesulfonated): IR (KBr) 1376 cm^{-1} (S═O). MS (*m*/*e*) = 251 (M^+) (2%). Fluorescence spectra of resin beads were obtained as described above.

Appendix I

For the irreversible chlorination reaction B-OH + X → B-X + OH, the rate equation can be written as

$$d[B\text{-}X]/dt = k'[B\text{-}OH][X] \quad (1)$$

where k' is the rate constant. Here, it has been assumed that X, the chlorination reagent, was present in excess over B-OH, the hydroxyl resin, so that the X concentration remained constant in time. After replacing [B-OH] with $[B\text{-}OH]_0$ − [B-X], the following expression

$$d[B\text{-}X]/dt = k(1 - [B\text{-}X]/[B\text{-}OH]_0) = k(1 - X_t) \quad (2)$$

can be obtained, in which $X_t = [B\text{-}X]/[B\text{-}OH]_0$ is the fraction of reacted hydroxylated beads, and k reflects the rate constant. This expression is similar to that proposed by Bing Yan et al.[33] for the kinetics of a solid-phase reaction. Integration of this equation yielded a relationship between $[B\text{-}X]_t$, the concentration of B-X at any time t, and the initial concentration of reactive sites, $[B\text{-}OH]_0$.

$$[B\text{-}X]_t = [B\text{-}OH]_0(1 - e^{-kt}) \quad (3)$$

Appendix II

After the remaining unreacted hydroxyl groups on the resins were labeled with dansyl, it was possible to quantify k, the rate constant, by monitoring the fluorescence of the system (see Scheme 2), taking into account that at any time,

the observed fluorescence intensity of a given sample was equal to the sum of the fluorescence intensities of the species present in it. B-X and B-D (chlorinated and dansylated resin, respectively) were the only fluorescent material because free dansyl was removed by washings; then, under the experimental conditions,

$$I_0 = a[\text{B-OH}]_0 \quad \text{at } t = 0 \tag{4}$$

$$I_t = b[\text{B-X}]_t + c[\text{B-D}] \quad \text{at any time } t \tag{5}$$

In these expressions a, b, and c are functions of the concentrations of B-OH, B-X, and B-D resins, respectively. The conservation equation, expressed in terms of total concentration of dansylated beads can be written as

$$[\text{B-OH}]_0 = [\text{B-X}] + [\text{B-D}] \tag{6}$$

Taking into account that $c \gg b$, eqs 4, 5, and 6 may be combined with eq 3 into the form

$$\log(I_t/I_0) = \log c/a - kt/2303 \tag{7}$$

Plots of $\log(I_t/I_0)$ versus t resulted in straight lines from which the k values were obtained for the solid phases under study.

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