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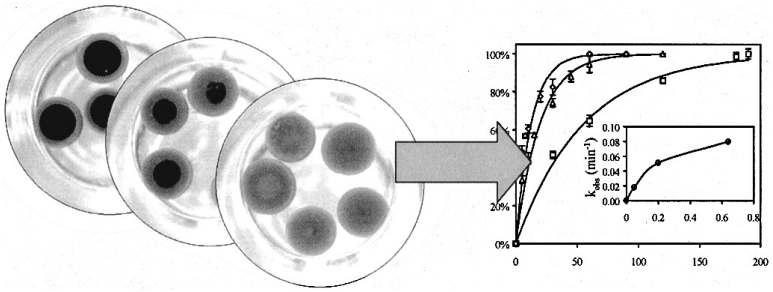

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Diffusion of Reagents in Macrobeads

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A simple and effective method of measuring diffusion rates of various acylating reagents in macro beads (in this work diameters greater than 570 μm) of amino-functionalized resins is presented. The extent of diffusion at various points of time was determined by treating beads with a staining reagent ("chloranil", 2,3,5,6-tetrachloro-1,4-benzoquinone) that colored the regions of the beads that had not yet been permeated by reagent (the central part of the beads). The volume of unstained resin (permeated part) was compared with the volume of the stained part, and diffusion rate constants were calculated. Factors influencing diffusion such as reagent concentration, solvent, temperature, resin type, and mechanical effects were investigated. The results showed that diffusion was promoted by increased temperature, good swelling of the resin, small reagents, and high concentrations but not by sonication or mechanical agitation.

Introduction

Polymer-supported synthesis has been implemented as an important new synthetic strategy in organic chemistry.[1,2] The development of solid-phase organic chemistry was stimulated by the need for the preparation of libraries of resin-bound compounds to be used in various screening protocols.[3,4] However, conversion from solution-phase chemistry to solid-phase chemistry is usually not straightforward in part because of lack of kinetic information on these reactions in a solid support. Characterization of basic resin properties and their influence on resin-supported reactions are required to understand and predict their outcome. Recently, reaction kinetics on solid phase have been measured by using on-bead IR spectroscopy,[5,6] NMR,[7] and fluorescence measurements[8] and by measuring cleaved Fmoc adducts.[9] In addition, determinations of compound diffusion rates in swollen resins have been performed by using various fluorescence-quenching techniques.[10,11]

Solid-phase combinatorial synthesis is typically performed on beads with diameters of 50−500 μm (swollen size) with a loading of 0.1−1.0 mmol/g corresponding to ∼30 pmol to 150 nmol of attached compound per bead. However, for some purposes it may be desired to have larger amounts of material anchored to each bead,[12] e.g., enough for NMR and even biological testing. A bead with a swollen diameter of 2−3 mm and loading in the vicinity of 1 mmol/g possesses about 2−8 μmol of substitution sites, corresponding to about 1−4 mg of compound of molecular weight 500 g/mol. When beads of this size are used, the time it takes a reagent to diffuse into the center of a bead becomes noteworthy and is consequently a reaction parameter that must be investigated.

When a reagent is added to a bead, two processes occur: diffusion and reaction. In a spherical bead submerged in a solvent containing a reactant A, the flow of A through the bead surface is proportional to the flux of A multiplied by the surface area. Since no reaction occurs until reactant A and a substitution site B come into contact, the total flow through the bead surface is constant and independent of bead radius.[13]

Direct measurements of diffusion rates are technically demanding,[10,11] and when diffusion is rapid, special equipment is required. The present work describes a simple and straightforward method that was used for measuring diffusion of reagents into amino-functionalized beads with diameters of 570 μm (swollen size) and larger using only simple reagents and a standard PC scanner.

Results and Discussion

The presented method was used to investigate the effects on reagent diffusion in macrobeads imposed by the following factors: reagent concentration, temperature, mechanical agitation, reagent, solvent, bead size, and resin type. For the diffusion investigations mainly $POEPOP_{1500}$[14] and $SPOCC_{1500}$[15] were used, which are two hydroxy-functionalized PEG-1500-based resins suitable for a broad range of conditions, including aqueous solvents.[11,16] Macrobeads of these two resins were prepared by the use of a recently published beading procedure,[17] and in order to obtain amino-functionalized resins, glycine was coupled to the resins. Resin macrobeads were treated with an acylating reagent and at various points of time; beads were retrieved and treated with acetaldehyde and chloranil,[18] which terminated the reaction and stained the remaining amino groups (Figure 1). High-resolution images of the beads suspended in acetonitrile were then recorded using a PC scanner (Figure 2), allowing calculation of the diffusion rate (vide infra). The diffusion rates measured in these experiments are relative to the bead radius because they are expressed as observed rate constants k_{obs} describing the percentage of bead penetrated by reagent as a function of time. Comparisons of diffusion rates of various reagents can only be performed between experiments

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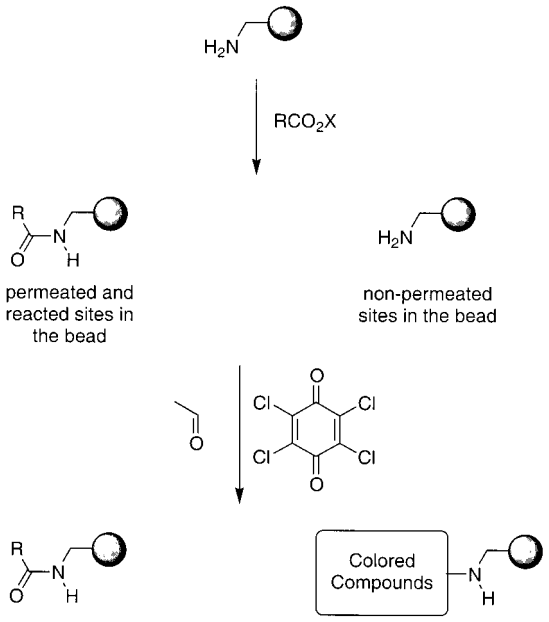

Figure 1. Partial acylation of an amino-functionalized resin and successive staining with, of the unreacted part of the bead, acetaldehyde and chloranil. RCO_2X = Fmoc-Phe-OPfp, Fmoc-Asn-OPfp, 3-hydroxybutyric acid−TBTU complex, or Ac_2O.

conducted on beads of uniform size. Consequently, the calculated rates are only referred to as *apparent diffusion rates*, although they provide a good representation of the actual rate of diffusion and thus are valuable for comparison of different acylating reagents and conditions.

Monitoring Method. Five beads or more were placed in each well of a microtiter plate, and a solution of the selected acylating reagent dissolved in the appropriate solvent was added (Table 1). At various points in time a DMF solution of 1% acetaldehyde and 1% chloranil was added. The beads were washed, each well was filled with acetonitrile, the microtiter plate was placed on a PC scanner, and an image of the plate was recorded. Each well was then individually printed on a piece of paper as large as possible for accurate measurement, and bead diameter and stained diameter were measured. For converting the measured diameters into a normalized scale, the well diameter was used as reference.

Data Fitting. The measured diameters were used to calculate the percentage of bead volumes that were acylated, and the average and standard deviation of the five or more beads used in each well were calculated. In a few instances, a bead contained a crack or a cavity and was therefore omitted in the calculation. The average percentages of reacted resin were plotted as a function of time, and the data points were in all cases found to fit well to a pseudo-first-order curve, which is expressed by

$$y = a_0(1 - e^{-k_{obs}t}) \quad (1)$$

where y is the measured percentage of reacted sites, a_0 the reaction percentage when the reaction is complete, k_{obs} the observed rate constant, and t the time. In the present case, a_0 was always equal to 100%. Fitting the data to a pseudo-first-order curve was performed in order to allow facile calculation of the observed apparent diffusion rate constant k_{obs}. The rate constant was used to compare the various experiments. The observed apparent diffusion rates k_{obs} obtained by these analyses are summarized in Table 1.

Control Experiments. The observation of the migration zone is highly dependent on the difference in refractive index between the bead and the submersion solvent, and this difference should preferably be zero so that the image is not distorted. To investigate this, several beads that had reacted for various lengths of time with Fmoc-Phe-OPfp and subsequently quenched/stained with acetaldehyde−chloranil were cut through their center. The two halves of each bead were placed in a microtiter plate in acetonitrile, one with the flat side up, the other with the round side up. The beads were scanned, and the observed stained diameters of each half of each bead were compared. No difference in the observed stained diameter was found, and hence, it was concluded that when swollen and submerged in acetonitrile, the bead does not behave as a magnifying glass. Therefore, the stained diameters observed are equal to the actual stained diameters within the beads. However, this was not the case when the beads were swollen but not submerged in the solvent, where the difference in refractive index resulted in a magnifying effect.

The presented method measures a combination of diffusion rate and reaction rate; however, it was assumed that diffusion was the rate-limiting step. This was substantiated by initial experiments that showed that the border between stained and unstained bead was sharp. In the eventuality of the opposite, the border between stained and unstained parts of a resin bead would be diffuse, and consequently, the determination of the diffusion rate would be complicated. The technique presented here also involves diffusion of the staining reagents into the beads; however, acetaldehyde diffuses into the beads *very* rapidly under the applied conditions because of its small molecular size. Again, this was verified by the sharp border between stained and unstained bead regions. Furthermore, an experiment was conducted where two portions of beads

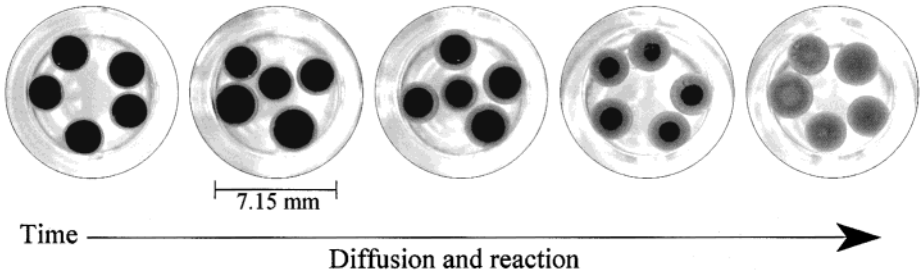

Figure 2. Scanned images of microtiter plate wells each holding five beads submitted to acylation for various amounts of time followed by staining with acetaldehyde and chloranil.

Table 1. Parameters and Apparent Rates of Diffusion of Various Acylation Reagents under Various Conditions in Selected Amino-Functionalized Resins

entry	resin[a]	swelling (mL/g)	solvent	reagent	concn (M)	temp (°C)	av bead size (mm)	k_{obs} (min^{-1})	$t_{1/2}$ [b] (min)
1	POEPOP-1	4.4	DMF	Fmoc-Phe-OPfp	0.05	20	2.42 ± 0.19	0.018	38.5
2	POEPOP-1	4.4	DMF	Fmoc-Phe-OPfp	0.20	20	2.38 ± 0.13	0.051	13.6
3	POEPOP-1	4.4	DMF	Fmoc-Phe-OPfp	0.64	20	2.40 ± 0.16	0.080	8.7
4	POEPOP-1	4.4	DMF	Fmoc-Phe-OPfp	0.20	5	2.38 ± 0.17	0.035	19.8
5	POEPOP-1	4.4	DMF	Fmoc-Phe-OPfp	0.20	68−70	2.37 ± 0.19	0.100	6.9
6	POEPOP-1	4.4	DMF	Fmoc-Phe-OPfp	0.20	20−47, sonicated[c]	2.39 ± 0.15	0.060	11.6
7	POEPOP-1	4.4	DMF	Fmoc-Phe-OPfp	0.20	20, shaken[d]	2.43 ± 0.19	0.047	14.7
8	POEPOP-1	4.4	DMF	Fmoc-Asn-OPfp	0.20	20	2.29 ± 0.16	0.040	17.3
9	POEPOP-1	4.4	DMF	3-hydroxybutyric acid−TBTU	0.20	20	2.37 ± 0.14	0.130	5.3
10	POEPOP-1	4.4	DMF	Ac_2O	0.20	20	2.29 ± 0.16	0.513	1.4
11	POEPOP-1	7.1	CH_2Cl_2	Ac_2O	0.20	20	2.37 ± 0.15	1.050	0.66
12	POEPOP-1	5.1	MeCN	Ac_2O	0.20	20	2.41 ± 0.18	0.550	1.3
13	POEPOP-1	3.8	$PhCH_3$	Ac_2O	0.20	20	2.17 ± 0.18	0.600	1.2
14	POEPOP-2	8.0	DMF	Fmoc-Phe-OPfp	0.20	20	0.72 ± 0.16	1.200	0.58
15	POEPOP-2	8.0	DMF	Fmoc-Phe-OPfp	0.20	20	1.14 ± 0.19	0.500	1.4
16	POEPOP-2	8.0	DMF	Fmoc-Phe-OPfp	0.20	20	2.30 ± 0.27	0.140	5.0
17	SPOCC	9.3	DMF	Fmoc-Phe-OPfp	0.20	20	0.78 ± 0.11	1.100	0.63
18	TentaGel	3.4	DMF	Fmoc-Phe-OPfp	0.20	20	0.57 ± 0.03	3.200	0.22
19	PLAMS	3.2	DMF	Fmoc-Phe-OPfp	0.20	20	0.57 ± 0.05	0.380	1.8

[a] POEPOP-1: more cross-linked. POEPOP-2: less cross-linked. [b] $t_{1/2} = \ln 2/k_{obs}$. [c] Initial temperature of 20 °C, rose to 47 °C during the experiment. [d] Shaken on a shaker table at 500 rpm.

after treatment with Fmoc-Phe-OPfp for a limited period of time were drained. One portion was stained as usual, and the beads of the other portion were sliced through the equator and then stained. The stained volumes of each portion were then compared and were found to be identical, thus proving that the diffusion of acetaldehyde into the beads is very rapid.

To support the presented optical method of measurement, it was chosen to verify a set of measurements by comparison with other methods. Beads that were not at all, partly, and fully permeated by Fmoc-Phe-OPfp and successively submitted to the chloranil test conditions were, after being washed, submitted to alkaline cleavage (0.1 M NaOH, 1 h), and the supernatants were analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC). However, HPLC traces pertaining to the partly and fully chloranil stained beads did not exhibit the presence of discrete compounds and therefore could not be used for the assigned purpose. The chloranil test has been known for many years,[18,19] and the identity of the colored chloranil adduct has been reported to be a resin-bound 2-(2-amino-vinyl)-3,5,6-trichloro-1,4-benzoquinone. Although this compound undoubtedly forms, other colored quinones most likely also form because it is known that primary and secondary amines may react with chloranil in the absence of acetaldehyde.[20] It should be considered that the imine, which is formed initially during the chloranil test, is in equilibrium with the amine, and thus, both species are present and may react with chloranil. Furthermore, it is highly possible that some quinones react with more than one site on the resin. Thus, the reason for the magnitude of HPLC peaks may be a combination of two factors: (1) presumably multiple quinones are formed; (2) the compounds may be base-labile.

Therefore, direct single-bead spectroscopic analysis of the resin-bound compounds was attempted by high-resolution magic angle spinning (HR MAS) NMR. The ^1H spectra of partly and fully chloranil−acetaldehyde stained beads indicated the presence of multiple compounds, and consequently, HR MAS NMR could not be used to determine the nature of the color reaction and progress of diffusion in a bead.

Because the chloranil test is sensitive and can be used to detect very low percentages of amine, a control experiment was conducted in order to verify that the region of a bead that was stained was not permeated by reagent, even to a minor extent. This consisted of derivatization with fluorescent group, namely, Abz. Beads were treated with TBTU-activated Boc-Abz-OH, and before the reagent had reached the center of the beads, quenching was performed in two ways: (1) with 1% chloranil and 1% acetaldehyde in DMF and (2) with 1% acetaldehyde in DMF. The beads were then washed, the Boc group was removed, and slices of the beads were inspected under a fluorescence microscope. It was found that the central region of the bead that was stained by chloranil corresponded perfectly to the central region of the bead that did not fluoresce. Lack of fluorescence from the stained region of the beads was not caused by the attachment of chloranil; the beads that were only quenched with acetaldehyde did not fluoresce in the center either. The conclusion of the control experiments is that when an intact bead submerged in acetonitril is observed, the observed diameter of the stained bead region is identical to the diameter of the actual stained region within the bead. Furthermore, the stained region is not permeated by reagent even to a slight extent, and consequently, the observed stained diameters provide an accurate measurement of the diffusion progress.

Effect of Concentration. The rate of diffusion is ideally proportional to the concentration gradient of the diffusing reagent. Increasing the concentration of reagent in solution therefore causes the flux of reagent into a bead to increase, thus affording a higher k_{obs}.[13] This effect was evident from the experiments (entries 1−3 of Table 1, Figure 3), which showed that increasing the concentration of Fmoc-Phe-OPfp from 0.05 to 0.64 M caused a more than 4-fold increase in the apparent diffusion rate k_{obs}. It also showed that the

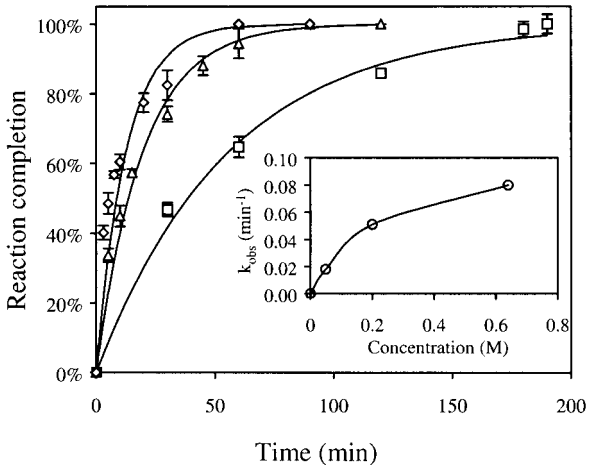

Figure 3. Reaction completion curves for Fmoc-Phe-OPfp acylations (in DMF at 20 °C) of POEPOP-1 at various reagent concentrations: 0.05 M (squares), 0.20 M (triangles), and 0.64 M (diamonds). Inset: Diffusion rate constant k_{obs} as a function of concentration.

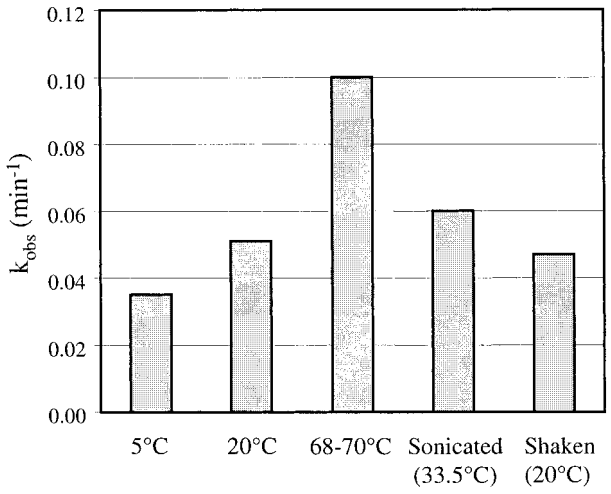

Figure 4. Observed diffusion rates of Fmoc-Phe-OPfp in POEPOP-1 in DMF under various conditions.

diffusion did not occur under adiabatic conditions, leading to a nonlinear correlation between k_{obs} and the concentration.

Effect of Temperature. Since molecular translocation is influenced by temperature, an increase in diffusion rate was expected as a result of increased temperature. The diffusion of Fmoc-Phe-OPfp was investigated at 5, 20, and 68−70 °C (entries 4, 2, and 5, respectively, and Figures 4 and 5). At 5 °C, the rate was reduced to 69% of the rate at 20 °C, and at 68−70 °C the rate was almost the double the rate at 20 °C. The inset in Figure 5 shows that in this case there seemed to be an almost linear relationship between k_{obs} and the temperature ($\Delta k_{obs} = 0.010$ min^{-1} °C^{-1}). This is in contrast to *reaction* kinetics, where the rate increases exponentially with the temperature.

Effect of Mechanical Agitation. Solid-phase reactions are often conducted with shaking of the resin, bubbling with an inert gas, or sonication under the assumption that the rate of conversion increases. The effect of various means of resin agitation has previously been investigated[21] and showed a pronounced difference in reaction progress for agitated and nonagitated resins. Here, the effect of sonication and shaking

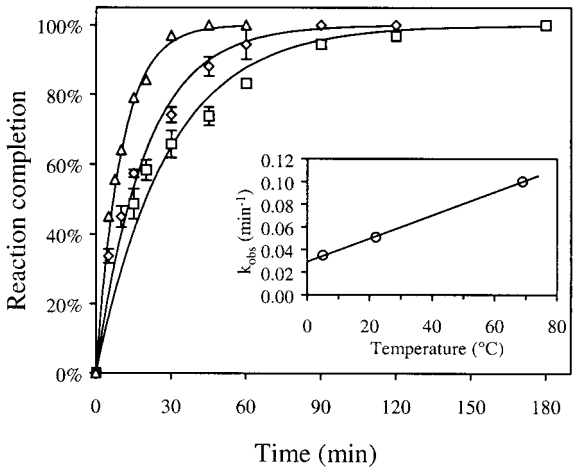

Figure 5. Reaction completion curves for Fmoc-Phe-OPfp acylations (0.2 M in DMF) of POEPOP-1 at various temperatures; 5 °C (squares), 20 °C (diamonds), and 68−70 °C (triangles). Inset: Diffusion rate constant k_{obs} as a function of temperature.

of resin during a reaction was investigated. Beads treated with Fmoc-Phe-OPfp while shaken on a mechanical shaker at 500 rpm did not present any increase in apparent diffusion rate (entry 7 of Table 1, Figure 4); on the contrary, the observed rate was slightly lower than that from the same experiment with no shaking (entry 2 of Table 1). This is presumably an effect of using the slightly larger beads in the former experiment compared to those in the latter (vide infra). While sonication in many reactions has been demonstrated to have a positive effect on the rate of heterogeneous reactions,[22] the same was not evident for the rate of diffusion.

A sonicated reaction was performed at a $T_{average}$ of 33.5 °C. During the reaction, heat generated by the sonication was deposited in the reaction mixture, increasing T from 20 to 47 °C. The diffusion at 33.5 °C without sonication is $k_{obs} = 0.060$ min^{-1} (Figure 5), which is identical with the value obtained with sonication. Thus, it may be concluded that neither mechanical agitation nor sonication promotes diffusion in the polymer network and when diffusion-controlled reactions are investigated, development of heat due to the agitation process must be considered. It should be noted that the previous investigation[21] of the effect of sonication focused on the reaction progress while this investigation has been directed toward the rate of diffusion.

Effect of Solvent. The rate of diffusion has been shown to increase with increasing swelling volume.[10] The more swollen a polymer matrix is, the more open the matrix becomes, thus allowing faster diffusion. Therefore, it was expected that altering the resin swelling would alter the apparent diffusion rate. This value was determined for acetic anhydride in four different solvents; dimethylformamide, dichloromethane, acetonitrile, and toluene (entries 10−13 of Table 1, Figure 7). The highest rate constant was found for dichloromethane (entry 11), and this may be explained by this solvent's ability to swell the resin considerably. The other unpolar solvent investigated, toluene (entry 13), did not cause the resin to swell nearly as much as dichloromethane, and for this solvent a 57% lower k_{obs} was measured. The swelling volumes of POEPOP in DMF, acetonitrile, and toluene were

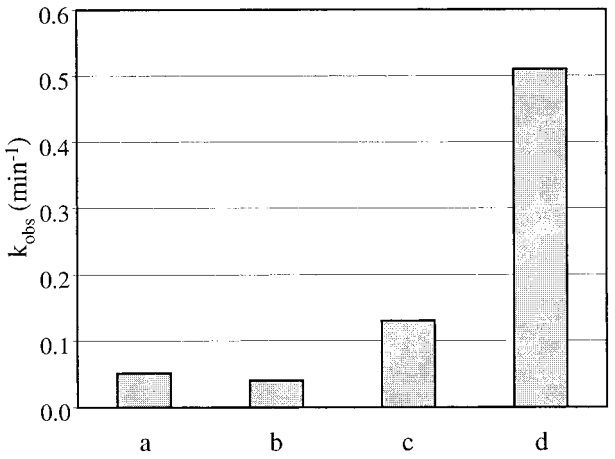

Figure 6. Observed diffusion rates of reagents of different polarity and size, all in POEPOP-1 at 20 °C and with 0.2 M reagent in DMF: (a) Fmoc-Phe-OPfp; (b) Fmoc-Asn-OPfp; (c) 3-hydroxybutyric acid−TBTU complex; (d) Ac_2O.

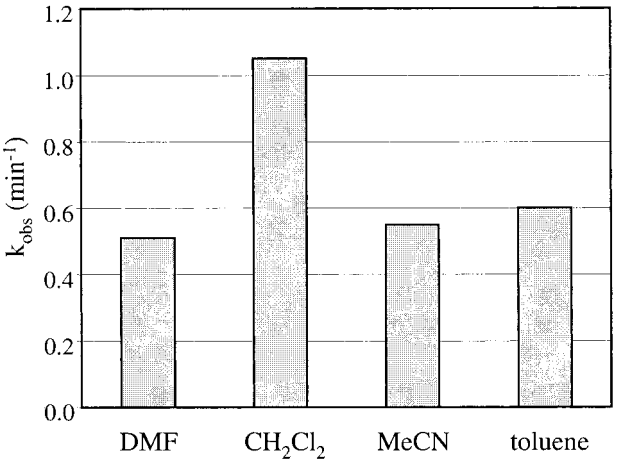

Figure 7. Observed diffusion rates of 0.2 M Ac_2O in various solvents at 20 °C in POEPOP-1.

comparable, which was reflected in the observed diffusion rates that were identical within ±11% (entries 10, 12, and 13 of Table 1, respectively). Nevertheless, it is noteworthy that toluene, causing the lowest swelling, had the highest k_{obs} of these three experiments. This indicates that diffusion is faster in unpolar solvents of low viscosity, although the effect is not dramatic.

It is also worth noting the pronounced difference in the diffusion rates of Fmoc-Phe-OPfp and acetic anhydride in DMF (entries 2 and 10, respectively). The molecular tumbling and Brownian motions of the reagent influence the diffusion rate, and consequently, large molecules diffuse more slowly than smaller molecules.[23]

Effect of Bead Size. The flux of reagent into the bead is independent of bead radius (vide supra); however, this is not the case for the apparent diffusion rates described here. The effect of bead size was investigated by measuring k_{obs} for Fmoc-Phe-OPfp in POEPOP resin of average diameters of 0.72, 1.14, and 2.30 mm (entries 14−16 of Table 1). As was expected, the smaller the bead size, the greater the apparent diffusion rate. As a consequence of the flow of reagent into a bead being proportional to the surface area, the volume of polymer matrix in a small bead that has been

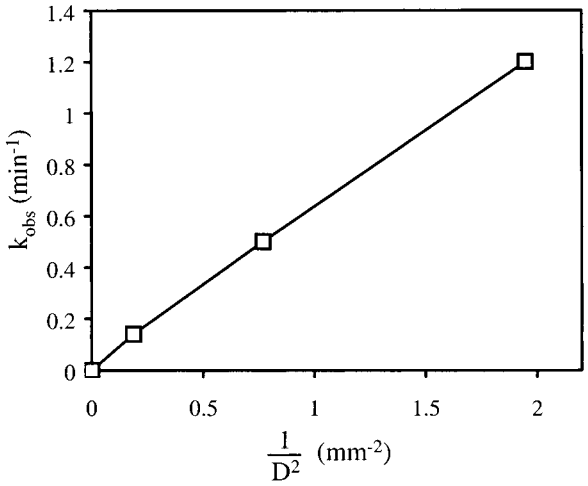

Figure 8. k_{obs} for diffusion of Fmoc-Phe-OPfp (0.2 M, DMF, 20 °C) in POEPOP-1 as a function of D^{-2}, where D is the average diameter of the beads.

permeated by reagent at a given time is not necessarily large. Correspondingly, in a large bead the volume of matrix that has been permeated by reagent is not necessarily small, even though the rate constant is. Therefore, to compensate for this aspect, plotting the rate constants as a function of bead diameter (D) over bead volume was performed. The bead volume equals $(4/3)\pi r^3$; hence, bead diameter over bead volume is proportional to $1/D^2$. Figure 8 shows the apparent diffusion rates plotted against $1/D^2$, affording a straight line with an intercept at 0. An intercept at 0 is valid because this corresponds to an infinitely large bead into the center of which a reagent can never diffuse, and hence, it will have a k_{obs} of 0 min^{-1}.

Effect of Resin. The effect of the degree of cross-linking has been thoroughly investigated,[24] showing that for highly cross-linked polystyrene, resin diffusion can become rate-limiting. Also, this study showed that the choice of solvent is important and that a solvent that causes a higher degree of swelling results in a higher diffusion rate. In the present work, this is evident by comparing entries 2 and 16 of Table 1, in which the same type of resin with different degrees of cross-linking was used. POEPOP-1 contained a higher degree of cross-linking than POEPOP-2 (see Experimental Section), and it was therefore expected that the latter resin would have a more open matrix, resulting in higher swelling and faster diffusion of reagents. In fact, the diffusion rate was found to be 174% faster in POEPOP-2.

The employed batches of POEPOP-2 and SPOCC are comparable resins in terms of chemical structure,[11] bead size, degree of cross-linking (see Experimental Section), and swelling (entries 14 and 17 of Table 1), and the diffusion rates of Fmoc-Phe-OPfp in these two resins are also comparable.

The smallest beads employed in this work were of TentaGel and PLAMS (entries 18 and 19) and are comparable because of their equal sizes and similar swellings in DMF. Interestingly, the apparent diffusion rate of Fmoc-Phe-OPfp in TentaGel was found to be more than 8-fold greater than that of PLAMS, and there is no obvious explanation for this.

A third commercially available resin, PEGA (Polymer Laboratories, 300−500 μm, 0.2 mmol/g), was also investigated; however, in this case a well-defined border between stained and unstained parts of the resin was not visible. This suggests that the diffusion rate of Fmoc-Phe-OPfp in beads of the specified size of this resin is too high to be measured by this technique. Diffusion of a protic acid in PEGA swollen in DMF has been shown to be slower than that in, for example, PLAMS,[11] and a possible explanation for this may be the presence of amide bonds in the PEGA matrix that may become protonated and thus retard diffusion.

Conclusions

A novel, simple, and reliable method for rapid assessment of diffusion of reagents in beaded polymers has been presented. Acylation reactions on beads of swollen diameter size 2.0−2.5 mm require considerable time because of the relatively long time necessary for reagent to diffuse to the center of these beads. It has been demonstrated here that the rate of diffusion may be increased by increasing the reagent concentration or reaction temperature. The rate of diffusion may also be increased by choosing a solvent causing increased resin-swelling volume, and furthermore, use of unpolar solvents was also found to result in slightly increased diffusion rates. It was demonstrated that agitation or sonication has no influence on reagent diffusion within a resin bead of the gel type. The presented method for comparing the diffusion rates did not rely on sophisticated equipment or techniques and was found to be very practical for determination of diffusion in several hundred beads simultaneously. The presented technique was applied to beads of a size down to 570 μm but should be applicable on smaller beads provided that the scanning resolution is higher than 1450 dpi and that the diffusion is sufficiently slow to be monitored by this optical technique. Slow diffusion rates observed in macrobeads should not be seen as a limitation to working with these. Smaller beads may be studied by considering the ratio between reaction rate and diffusion rate. The diffusion rate will increase depending on the ratio between bead surface and migration pathway as well as on the increase in concentration gradient. The reaction rate may therefore soon be the overall rate-limiting factor with small bead sizes.

Experimental Section

General Procedures. Resin loadings were determined by Fmoc[25] cleavage off a sample of resin, optical density measurements of the eluate at 290 nm, and calculation by employing a calibration curve.[26] Prior to this, hydroxy-functionalized resins (POEPOP and SPOCC) were esterified with Fmoc-Gly-OH in DCM using MSNT,[27] and amino-functionalized resins (TentaGel and PLAMS) were coupled with Fmoc-Gly-OPfp in DMF until the reaction was complete according to the Kaiser test.[28] To calculate the OH loading of the resin from the measured Fmoc loading, the following equation was employed:

$$\text{loading}_{\text{OH}} = (\text{loading}_{\text{Fmoc}}{}^{-1} - \Delta M)^{-1}$$

where ΔM is the difference in mass in g/mmol, i.e., $M_{\text{Fmoc-Gly}} = 0.2793$ g/mmol. Resin swellings were determined by the syringe method.[29]

Scanning of beads was performed on a Hewlett-Packard DeskScan 4c scanner, and images were recorded at 1450 dpi with HP DeskScan II version 2.4 software.

Resins. (a) POEPOP-1. The macromonomer for this resin was prepared as previously described,[11] yielding a product with 100% epoxide incorporation. The macromonomer was polymerized by suspension polymerization,[17] the dried resin was sieved, and beads of diameter >1000 μm were used. Loading was 0.73 mmol of Fmoc-Gly/g of resin corresponding to 0.92 mmol of OH/g of unfunctionalized resin.

(b) POEPOP-2. The macromonomer for this resin had a 74% epoxide incorporation. The dried suspension-polymerized resin was sieved through a series of sieves and the fractions between 300 and 500 μm, between 500 and 1000 μm, and above 1000 μm were used. Corrected loading was 0.54 mmol/g.

(c) SPOCC. The macromonomer for this resin was prepared as previously described[15] and had a 76% oxirane incorporation. The macromonomer was suspension-polymerized,[17] and the dried resin was sieved through a series of sieves. The fraction between 500 and 300 μm was used. Corrected loading was 0.68 mmol/g.

(d) TentaGel. The sample was from Rapp Polymer Macro Beads MB-300-02, and the loading was 0.25 mmol/g. The measured average diameter size (swollen in DMF) was 570 μm.

(e) PLAMS. The sample was a Polymer Laboratories aminomethylated macroporous polystyrene, batch number AMS 011. The loading was 0.9 mmol/g, and the measured average diameter size (swollen in DMF) was 570 μm.

H-Gly Resins. POEPOP-1 (3.1 g, D > 1000 μm, 0.92 mmol/g) was treated with Fmoc-Gly-OH (2.54 g, 3 equiv), MSNT (2.53 g, 3 equiv), and *N*-ethylimidazole (1.36 mL, 4 equiv) in CH_2Cl_2 (40 mL) (coupled twice, 18 and 4 h). The resin was washed with DCM (3 × 3 min) and DMF (3 × 3 min), treated twice with 20% piperidine in DMF (3 min, then 60 min), and washed with DMF until all piperidine was removed (determined by a chloranil test of the eluent). The beads were then sieved through two stainless steel sieves (hole diameters of 2500 and 2000 μm, respectively) while being sprinkled with DMF. Beads in the range 2500−2000 μm were examined visually, and those with cracks or internal air bubbles were discarded.

For POEPOP-2 and SPOCC, each size fraction of these resins was esterified as described for POEPOP-1.

For TentaGel and PLAMS, each of these resins were treated with Fmoc-Gly-OPfp in DMF until a negative Kaiser test[28] was observed. The resins were washed and treated with piperidine as described for POEPOP-1.

Diffusion Studies. (a) Acylation and Quenching/Staining. Five beads swollen in the appropriate solvent were placed in a 2 mL round-bottomed Eppendorf tube. The appropriate solvent containing the acylating reagent was added to the beads which were submitted to conditions presented in Table 1. 3-Hydroxybutyric acid (0.21 M), NEM (0.21 M), and TBTU[30] (0.20 M) in DMF were coupled after

10 min of preactivation. In all cases between 4 and 5 equiv of reagent was added (ca. 200 μL). At various points in time, 1 mL of freshly prepared solution of 1% acetaldehyde and 1% chloranil in DMF was added and the staining was allowed to proceed for 30−60 min. The resins were washed with DMF (3 ×), left in DMF overnight, washed with MeCN (3 ×), left in MeCN overnight, transferred to a 96-well microtiter plate, and covered with MeCN.

(b) Measurement of Bead Diameter and Stained Volume. The microtiter plate was placed on the PC scanner and scanned. The resulting image was printed on paper (two wells per sheet), after which the diameter of the bead and the diameter of the stained part of the beads could be measured precisely, using the diameter of the microtiter well as a scale. The actual diameter of the slightly conical microtiter plate wells was 7.15 mm in the bottom. The volume of reacted resin $V_{reacted}$ is given by $V_{reacted} = V_{bead} - V_{stained} = (^4/_3)\pi(r_{bead}^3 - r_{stained}^3)$.

Control Experiment A. Two Eppendorf vials were each loaded with 10 beads of POEPOP-1 and were treated with a 0.20 M solution of Fmoc-Phe-OPfp in DMF (500 μL) for 40 min, after which the beads were drained. The beads from one vial were sliced into halves at the equator, and immediately after, a freshly prepared solution of 1% acetaldehyde and 1% chloranil in DMF (1 mL) was added to both vials. After 2 h, both portions were washed with DMF (2 × 10 min) and MeCN (2 × 10 min, then overnight) and analyzed on the PC scanner. For the sliced beads a k_{obs} value of 0.77 ± 0.02 min^{-1} was determined, and for the whole beads a k_{obs} value of 0.77 ± 0.06 min^{-1} was determined.

Control Experiment B. Two Eppendorf vials were each loaded with five beads of POEPOP-1, and a preincubated (10 min) mixture of Boc-Abz-OH (0.21 M), NEM (0.21 M), and TBTU (0.20 M) in DMF (200 μL) was added. After 30 min, 1% acetaldehyde in DMF (1 mL) was added to one vial, and a freshly prepared solution of 1% acetaldehyde and 1% chloranil in DMF (1 mL) was added to the other. After 2 h, both portions were washed with DMF (2 × 10 min), MeCN (2 × 10 min), 10% TFA in DCM (2 h), DMF + 0.1% TEA in DMF (3 d), and DMF (3 × 1 h). Slices of the central part of the beads were prepared with a scalpel and investigated under a fluorescence microscope. The nonfluorescent central region of the bead slices corresponded perfectly to the stained central region of the bead slices.

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Abbreviations

Abz: 2-aminobenzoic acid
Boc: *tert*-butyloxycarbonyl
dpi: dots per inch
k_{obs}: observed apparent diffusion rate
MSNT: 2,4,6-mesitylenesulfonyl-3-nitro-1,2,4-triazolide
NEM: *N*-ethylmorpholine
PEG: poly(ethylene glycol)
PEGA: poly(ethylene glycol)−poly(acrylamide) copolymer
Pfp: pentafluorphenyl
PLAMS: Polymer Laboratories aminomethylated polystyrene
POEPOP: polyoxyethylene−polyoxypropylene copolymer
SPOCC: polyoxyethylene−polyoxetane copolymer
TBTU: *N*-[(1*H*-benzotriazol-1-yl)-(dimethylamino)-methylene]-*N*-methylmethanaminium tetrafluoroborate *N*-oxide

Three-letter codes are used for amino acids according to IUPAC recommendations; see http://www.chem.qmw.ac.uk/iupac/AminoAcid/.

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