

# Scintillation proximity assays for the real-time detection and quantification of the progress of reactions upon solid supports†

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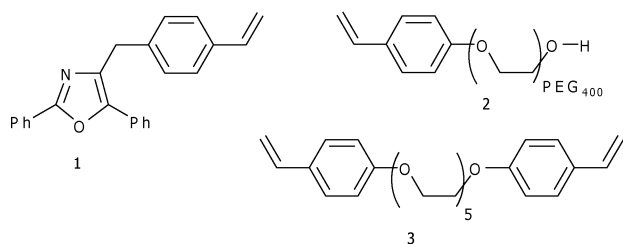
Received (in Cambridge, UK) 21st October 2003, Accepted 4th December 2003

First published as an Advance Article on the web 5th January 2004

A scintillation proximity assay (SPA) has been used successfully to detect and quantify, in real-time, the kinetic progress of hydrolysis of [<sup>3</sup>H]acetate esters from scintillant-containing styrenic and poly(ethylene glycol) (PEG)-based polymer supports in both organic and aqueous media.

The majority of combinatorial compound libraries have been synthesised upon chemically-functionalised insoluble polymer supports. Solid-phase synthesis frequently requires optimisation from the analogous solution-phase strategy and commonly the most unfavourable reactions are investigated in a separate study. Solid-phase optimisation studies often involve monitoring the conversion of substrate to product during the time course of the reaction. Typically, this is achieved by withdrawing a sample of polymer resin from the reaction mixture, which is washed exhaustively, dried to constant mass and then subjected to conventional analysis.<sup>1–7</sup> Despite the sensitivity of these analytical techniques they often necessitate the removal of a sample of solid support from the assay mixture, which necessarily perturbs the reaction equilibrium.

Within this communication we report the first example of an *in situ* SPA to monitor the progress of an organic reaction upon a solid support in real-time. Specifically, the terminal hydroxyl groups on two different scintillant-containing polymer supports, one styrenic and the other PEG-based, have been [<sup>3</sup>H]acetylated. The resultant [<sup>3</sup>H]acetate esters have been hydrolysed from each support by separate treatment with a solution of potassium hydroxide (KOH) dissolved in either benzene or water. The extent and the kinetic progress of each hydrolysis reaction were determined directly and in real-time, simply by monitoring each reaction mixture in a scintillation counter. In addition, the effect of the polymer matrix and reaction solvent upon the rate and extent of hydrolysis has been evaluated.



Prior to conducting this proof of concept SPA it was necessary to synthesise the scintillant-containing polymer supports. Previously, we reported the synthesis of scintillant monomer 4'-vinyl-4-benzyl-2,5-diphenyloxazole **1** and PEG-based monomers  $\alpha$ -styryl-poly(ethylene glycol)<sub>400</sub> **2** and  $\alpha,\omega$ -bis-styryl-penta(ethylene glycol) **3**.<sup>8–10</sup> Two monomer mixtures, one that contained **1**, **2**, and **3** and a second mixture containing **1**, styrene, chloromethylstyrene and divinylbenzene, were separately combined with the free radical initiator 2,2'-azobis-*iso*-butyronitrile (AIBN). Each monomer mixture was subjected to a thermally initiated, radical, polymerisation reaction.‡ Both polymerisation reactions proceeded smoothly to

give scintillant-containing, hydroxyl-functionalised, PEG-based polymer support **4** and scintillant-containing, chloromethyl-functionalised, styrene-based polymer resin **5** respectively.<sup>9,10</sup> Resin **5** was derivatised with 4-hydroxybenzyl alcohol to give scintillant-containing Wang resin **6**, with appropriate functionality for convenient acetylation.§

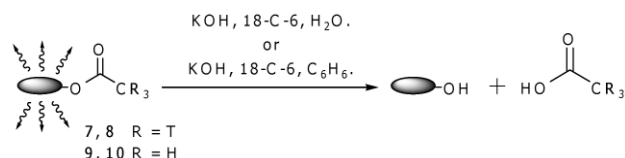
The hydroxyl loadings of polymer supports **4** and **6** were quantified experimentally using the well established Fmoc-coupling/cleavage assay.<sup>11</sup> The loading results calculated from the Fmoc-release assay of supports **4** and **6** were determined to be 0.81 and 0.65 mmol g<sup>-1</sup> respectively.

Prior to conducting the [<sup>3</sup>H]acetate ester hydrolysis experiment it was necessary to esterify polymer supports **4** and **6** with [<sup>3</sup>H]acetic anhydride and [<sup>1</sup>H]acetic anhydride, which gave [<sup>3</sup>H]acetylated polymers **7** and **8** and [<sup>1</sup>H]acetylated polymers **9** and **10** respectively.<sup>12</sup> The acetylated polymer supports **7–10** were evaluated for the ability to scintillate spontaneously.¶ As expected, the blank controls **4** (49 cpm mg<sup>-1</sup>) and **6** (15 cpm mg<sup>-1</sup>) and the negative controls **9** (26 cpm mg<sup>-1</sup>) and **10** (4 cpm mg<sup>-1</sup>), gave no appreciable scintillation counts. However, polymers **7** (39550 cpm mg<sup>-1</sup>) and **8** (12358 cpm mg<sup>-1</sup>) scintillated spontaneously. Commercial scintillation fluid was then added to each assay mixture. A second scintillation counting procedure then gave the total number of counts present in each assay system and enabled the scintillation efficiencies of polymers **7** and **8** to be calculated. Polymer supports **7** and **8** both scintillate with relatively high efficiencies of 59% and 71% respectively.||

Following the successful synthesis and evaluation of [<sup>3</sup>H]acetylated polymer supports **7** and **8** a sample of each was subjected to a hydrolysis reaction by separate treatment with KOH in either water or benzene with a catalytic amount of 18-crown-6 (18-C-6) (Scheme 1).\*\*

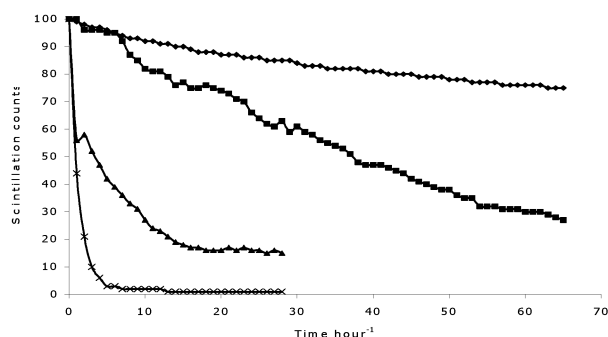
Each of the four reactions was monitored in a scintillation counter with the detected counts per minute (cpm) recorded as a function of time.† Subsequently, the number of counts at specific time intervals was converted into a percentage of the initial number of counts detected (*t* = 0 h). Figure 1 shows the percentage values obtained in this manner plotted *versus* time for each of the four hydrolysis reactions. Each plot in Fig. 1 shows a decrease in the number of scintillation counts detected with time as a direct consequence of the progressive cleavage of [<sup>3</sup>H]acetate groups from the polymer support.

The kinetics of each hydrolysis reaction is first order with respect to both polymer supported [<sup>3</sup>H]acetate ester and KOH.<sup>1</sup> However, previous work has demonstrated that support loading level is inconsequential to initial reaction rate and that on-support reactions may be rendered pseudo first order with respect to the substrate on the polymer support by using an excess of a soluble reagent(s).<sup>13</sup> Accordingly, the vast excess of KOH employed in the hydrolysis reaction renders the reaction kinetically pseudo-first-order. Each



Scheme 1 Hydrolysis of polymer supports **7–10**.

† Electronic supplementary information (ESI) available: cpm data, pseudo-first-order plots. See <http://www.rsc.org/suppdata/cc/b3/b313223d/>



**Fig. 1** Percentage scintillation counts *versus* time profiles for assay mixtures **8**/KOH/18-C-6/H<sub>2</sub>O (◆), **7**/KOH/18-C-6/H<sub>2</sub>O (■), **7**/KOH/18-C-6/C<sub>6</sub>H<sub>6</sub> (▲) and **8**/KOH/18-C-6/C<sub>6</sub>H<sub>6</sub> (x).

set of scintillation counting data, represented in Fig. 1, fits well with a pseudo-first-order rate equation. For each assay mixture a graph of natural logarithm of (initial percentage scintillation counts/percentage scintillation counts) *versus* time gives a linear plot, with the gradient of each plot being equal to the pseudo-first-order rate constant ( $k'$ ).<sup>†</sup> This value quantifies the rate of hydrolysis of [<sup>3</sup>H]acetate from the polymer support. In addition, the percentage scintillation counts value at a specific time quantifies the extent of the hydrolysis reaction at that time (Fig. 1).

Both the rate and extent of the hydrolysis reaction are dependent upon the composition of the polymer support, linker group and solvent in which the assay was performed.<sup>13</sup> The greater the swelling and mass-solvent uptake by a polymer when brought into contact with a solvent, the greater is the accessibility of this solvent into the matrix of the polymer. Previous swelling and mass-solvent uptake studies with non-polar solvents demonstrated that styrene-based polymer supports swell far more and imbibe a greater mass of solvent than PEG-based supports when brought into contact with the same solvent.<sup>9,10</sup> This observation suggests that a solution of KOH/18-C-6/benzene will permeate into styrene-based matrices more rapidly than PEG-based matrices. This supposition fits exactly with the observation that hydrolysis of [<sup>3</sup>H]acetate ester from styrene-based polymer **8** ( $k' = 0.693 \text{ h}^{-1}$ , 99% @ 28 h) initially proceeds seven times faster and more fully to completion compared with that observed for PEG-based support **7** ( $k' = 0.095 \text{ h}^{-1}$ , 85% @ 28 h).

In contrast, PEG-based supports in contact with water exhibit greater swelling and mass-water uptake compared with styrene-based supports.<sup>9,10</sup> Again this observation was found to be in excellent agreement with the greater initial rate and extent of hydrolysis in water of PEG-based support **7** ( $k' = 0.021 \text{ h}^{-1}$ , 37% @ 28 h) compared with styrene-based support **8** ( $k' = 0.004 \text{ h}^{-1}$ , 15% @ 28 h).

The greater initial rate and extent of hydrolysis of [<sup>3</sup>H]acetate from polymer **8** by treatment with a solution of KOH/18-C-6/benzene compared with KOH/18-C-6/water are fully consistent with previous solvation studies where the styrene-based matrix is better solvated by toluene than water.<sup>9</sup> However, when the same comparison is made for polymer **7** the reverse of what is predicted from swelling and mass uptake data is observed. The hydrolysis of [<sup>3</sup>H]acetate from polymer **7** proceeds more slowly in water than benzene. This anomalous finding most likely results from the decreased nucleophilicity of the hydroxide anion in water compared with benzene. In benzene the hydroxide anion is poorly solvated and as such will be strongly nucleophilic, whereas in water, the hydroxide anion will be solvated by the water molecules through the formation of hydrogen bonds and will thus be a weaker nucleophile.

In conclusion, scintillant-containing polymer supports have, for the first time, been used successfully in a proof of concept SPA to detect and quantify, in real-time, the kinetic progress of an organic

reaction upon a solid support. Chemically-functionalised, scintillant-containing, polymer supports of the type described herein may be of considerable utility for use in SPAs to monitor, *in situ* and in real-time, the progress of chemical reactions upon solid supports.

## Notes and references

‡ **1** (3.5 mg, 0.011 mmol), **2** (84.1 mg, 0.163 mmol), **3** (1.6 mg, 0.004 mmol) and AIBN (0.3 mg, 0.002 mmol) were combined and degassed in an ultra-sound bath (2 min). N<sub>2</sub> gas was bubbled through the monomer mixture (5 min). The mixture was then placed in an oven (72 °C) overnight. The resultant monolithic polymer was broken up into small irregular pieces and washed with THF, MeOH–THF 1 : 1 and MeOH (3 × 30 mL). The polymer was dried to constant mass to give **4** (48 mg, 54%).

§ Styrene (63.0 mL, 549 mmol), 4-vinylbenzyl chloride (9.9 mL, 70.5 mmol), divinylbenzene (2.3 mL, 12.9 mmol), **1** (3.36 g, 9.96 mmol) and AIBN (0.70 g, 4.27 mmol) were combined and stirred (25 min). This monomer mixture was added to a stirred aqueous solution of a 1% polyvinyl alcohol (87–89% hydrolysed, Mw 85000–146000) (700 mL) under a N<sub>2</sub> atmosphere and the resultant suspension stirred (600 rpm, 30 min). The temperature was increased (72 °C) and stirring continued for a further 4 h. The mixture was cooled to room temperature and poured over a sieve (38 µm). The beaded polymeric product was washed sequentially with water, distilled water, MeOH, MeOH–THF 1 : 1, THF, MeOH–THF 1 : 1 and MeOH (500 mL). The polymer was extracted with dioxane overnight in a Soxhlet apparatus, washed with MeOH–THF 1 : 1, MeOH (500 mL), dried to constant mass (80 °C, 4 h) and separated into size ranges to give **5**: [150–75 µm, 20.05 g], [75–38 µm, 5.49 g], [ $< 38 \mu\text{m}$ , 0.89 g].

¶ **5** (150–75 µm, 2.0 g, 0.96 mmol Cl g<sup>-1</sup>), hydroxybenzyl alcohol (0.73 g, 5.76 mmol) and sodium methoxide (0.32 g, 5.63 mmol) were stirred in DMF (40 mL) overnight (50 °C). The reaction mixture was cooled to room temperature and the polymer resin separated from the reaction mixture by filtration. The resin was then washed sequentially with dioxane, dioxane–water 3 : 1, dioxane and MeOH (50 mL) before being dried to constant mass to give **6** (1.78 g, 82%).

¶¶ **4**, **6** and **7–10** (1–2 mg) were weighed accurately, in triplicate, into glass scintillation vials. These polymer samples were monitored in a scintillation counter (1 min). Commercial scintillation fluid (5 mL) was added to each sample which was subsequently re-monitored in the scintillation counter (1 min).

|| All values have been adjusted to accommodate the fact that a fluor based solely on 2,5-diphenyloxazole gives 70% of the cpm obtained with a multi-fluor scintillation fluid.<sup>14</sup>

\*\* An aqueous solution of KOH (10 mL, 0.18 M, 18-C-6, 1.0 mg) and a benzene solution of KOH (10 mL, 0.18 M, 18-C-6, 1.0 mg) were separately added to **7** (2.9 mg), (1.2 mg) and **8** (3.1 mg), (3.0 mg) respectively to give four separate reaction mixtures. Each mixture was monitored in a scintillation counter (15 min h<sup>-1</sup> for up to 74 h).

- W. Li and B. Yan, *J. Org. Chem.*, 1998, **63**, 4092.
- B. Yan, Q. Sun, J. R. Wareing and C. F. Jewell, *J. Org. Chem.*, 1996, **61**, 8765.
- B. Yan, G. Kumaravel, H. Anjaria, A. Wu, R. C. Petter, C. F. Jewell and J. R. Wareing, *J. Org. Chem.*, 1995, **60**, 5736.
- B. D. Larsen, D. H. Christensen, A. Holm, R. Zillmer and O. F. Nielsen, *J. Am. Chem. Soc.*, 1993, **115**, 6247.
- B. Schneider, D. Doskocilova and J. Dybal, *Polymer*, 1985, **26**, 253.
- W. L. Fitch, G. Detre, C. P. Holmes, J. N. Shoolery and P. A. Keifer, *J. Org. Chem.*, 1994, **59**, 7955.
- S. L. Manatt, C. F. Amsden, C. A. Bettison, W. T. Frazer, J. T. Gudman, B. E. Lenk, J. F. Lubetich, E. A. McNelly, S. C. Smith, D. J. Templeton and R. P. Pinnell, *Tetrahedron Lett.*, 1980, **21**, 1397.
- B. Clapham and A. J. Sutherland, *J. Org. Chem.*, 2001, **66**, 9033.
- M. C. McCairn, S. R. Tonge and A. J. Sutherland, *J. Org. Chem.*, 2002, **67**, 4847.
- M. C. McCairn, A. V. Hine and A. J. Sutherland, *J. Mater. Chem.*, 2003, **13**, 225.
- NovaBiochem Catalog and Peptide Synthesis Handbook*, NovaBiochem, La Jolla, CA, 1998, p. S37.
- B. Clapham and A. J. Sutherland, *Tetrahedron Lett.*, 2000, **41**, 2253.
- D. P. Walsh, C. Pang, P. B. Parikh, Y. Kim and Y. Chang, *J. Comb. Chem.*, 2002, **4**, 204; D. Walsh, W. Wu and Y. Chang, *Curr. Opin. Chem. Biol.*, 2003, **7**, 353.
- B. Clapham, A. J. Richards, M. L. Wood and A. J. Sutherland, *Tetrahedron Lett.*, 1997, **38**, 9061.