Polymer-bound flavins: 2. Immobilization of linear flavin-containing polyelectrolytes by adsorption onto silica*

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The adsorption of quaternary ammonium derivatives of linear polystyrene, containing pendant flavin groups, from aqueous solution onto nonporous silica particles was studied. It is shown that interaction takes place between the quaternary ammonium groups in the polymer and ionized silanol groups on the silica surface. Since the maximum specific adsorption was not influenced by the content of quaternary ammonium groups, relatively few quaternary ammonium groups appear to be necessary to bind the copolymers to the silica surface, probably resulting in a large fraction of segments located in loops and tails. The reactivity of the bound flavin, as estimated from kinetics of aerobic oxidation of 1-benzyl-1,4-dihydronicotinamide (BNAH) in aqueous solution, is not influenced by the adsorption process. The immobilized reactive polymer can easily be re-used several times without loss of activity, indicating that desorption of the polymer can be neglected.

(Keywords: reactive polymer; polymer bound flavin; immobilization; adsorption; silica)

INTRODUCTION

The last ten to fifteen years have seen a fast growing interest in the study of insoluble reactive polymers[1,2]. The main reason for attaching chemically reactive species to polymeric supports is the simplified reaction work-up, because supported species are easily separated from soluble products and excess reagents by filtration. In addition, reagents or catalysts on insoluble supports can be used in flow systems, thus rendering the process amenable to automation. A considerable number of polymeric and inorganic materials have been investigated as support matrices. Inorganic oxides and glasses have been widely employed in large-scale applications, while crosslinked organic macromolecular resins have come to be used in more recent years. The disadvantages, however, of crosslinked polymeric resins, gel-type as well as macroporous or macroreticular, are the problems which arise with (spectroscopic) characterization, and with inaccesibility of functional groups leading to internal diffusional limitations[1,3,4]. It has been suggested that the grafting of linear polymers onto inorganic materials would lead to carriers that posses the superior mechanical properties of the inorganic support as well as all the advantages associated with soluble non-crosslinked macromolecules[1,4,5]. Indeed, promising results have been reported concerning solid state peptide synthesis[6], affinity chromatography[7], and immobilization of catalytic copper-complexes[8] or enzymes[9].

We now wish to present a support, akin to the carriers mentioned above, which was obtained by adsorption rather than grafting of linear polymers with pendant functional groups onto silica particles. Polymer adsorption phenomena play an essential role in a diversity of technical and practical applications. Fundamental research on the conformations of flexible polymer chains adsorbed on an interface have led to the nowadays widely accepted view that some portions of the polymer chain are in direct contact with the interface as trains, whereas remaining portions extend into the bulk solution as loops and tails[10]. It occurred to us that pendant reactive species localized in loops or tails might still behave like reactive species bound to a dissolved or terminally grafted macromolecule (see *Figure 1*). In our previous article we described the synthesis and chemical properties of a series of linear polystyrenes containing pendant quaternary ammonium and flavin groups[11]. Here we report on the adsorption of these polyelectrolytes from aqueous solution onto nonporous silica. We have studied the influence of adsorption on the catalytic activity of the flavin by using the aerobic oxidation of 1-benzyl-1,4-dihydronicotinamide (BNAH) as a test reaction (*Scheme 1*).

We have also compared the reactivity and re-usability of these physically bound copolymers with a copolymer chemically anchored to silica by graft polymerization.
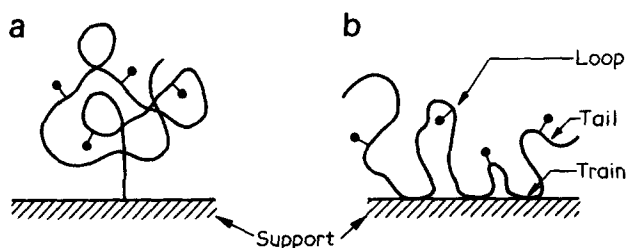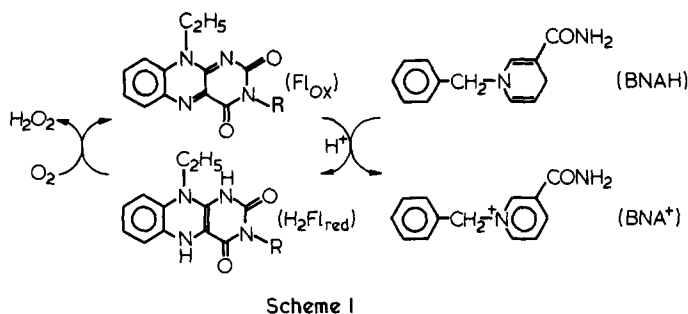

Figure 1 Schematic representation of reactive species (—●) bound to a grafted (a) or an adsorbed polymer chain (b)

Scheme I

EXPERIMENTAL

Materials

The synthesis of the water soluble copolymers **1a–d** (*Table 1*), prepared by coupling 10-ethylisoalloxazine to partially chloromethylated polystyrene and subsequent quaternization with triethylamine, has been described in detail in the previous paper[11].

A copolymer chemically anchored on the silica surface, sample **2**, was obtained via graft polymerization of vinylbenzylchloride (*m/p*-isomers, a gift of DOW Chemicals) initiated by a radical initiator bound to Aerosil 200 V, according to procedures described
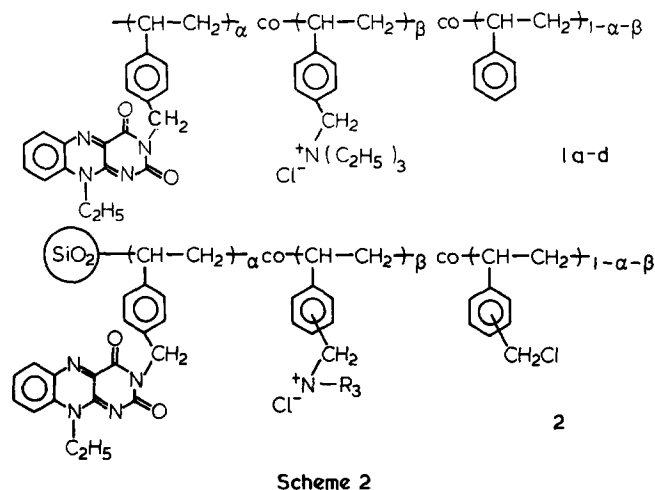

Scheme 2

Table 1 Composition of flavin-containing polymers

Sample	α^a	β^a
1a	0.004	0.19
1b	0.017	0.54
1c	0.013	0.76
1d	0.014	0.95
2^b	0.008	0.6

^{a} Mole fractions
^{b} weight fraction copolymer on silica $\theta = 0.09$

elsewhere[12]. The further functionalization procedure of the grafted polymer was the same as for the soluble polymers[11]. The flavin-content of this polymer was estimated from its u.v. absorption at 440 nm. Although this is a heterogeneous system, a fairly good spectrum could be recorded using the special turbid sample holder of the Pye-Unicam SP8-200 spectrophotometer. Data on the composition of the samples are gathered in *Table 1*.

The nonporous silica samples Aerosil 200 V, 0 X 50 and R972 were used for adsorption experiments as received (from Degussa AG, FRG). The hydrophobicity of the surface of R972 was further enhanced (R972-S) by silanization with trimethylchlorosilane as described elsewhere[13].

Adsorption of copolymers onto silica

Stable dispersions of hydrophilic silica in water, were made up with sonification in order to degrade the larger aggregates of primary silica particles. The hydrophobic silicas, R972 and R972-S had to be dispersed in some propan-2-ol before adding water. The silica dispersions were mixed with known volumes of copolymer solutions of known concentration in a stoppered centrifuge tube, and the mixture was stirred with a magnetic stirring bar at constant temperature (25°C) for 20 h. The dispersions were centrifuged at 3000 rpm for 10 min to sediment the silica, and the supernatant was carefully removed. The equilibrium concentration of the flavin-containing copolymer, C_p, in the supernatant solvent was calculated from the absorption of the flavin group at $\lambda = 440$ nm. The adsorbed amount of copolymer, A, was calculated from the decrease in polymer concentration and expressed in milligram adsorbed copolymer per square metre of silica surface (mg.m^{-2}). The aqueous media were buffered with tris(hydroxymethyl)aminomethane (pH $\geqslant 8$), phosphate (KH_2PO_4) (pH 6–8), or phthalate ($KHC_8O_4H_4$) (pH < 6) buffers respectively. The pH's of the media were determined with a WTW 610-E universal-digital metre and an Ingold 3M KCl electrode. Ionic strength of the buffers was kept around 0.05 M by addition of KCl.

Kinetics

The initial reaction rates of the aerobic oxidation of BNAH by dissolved or adsorbed polymer-bound flavin were measured by monitoring the concentration of dissolved oxygen in the reaction medium with a YSI 5331 Oxygen probe[14] back-diffusion of oxygen during the reaction is negligible, since the piston, in which the Clark-type electrode is mounted, tightly fits into the thermostatically controlled reaction vessels. Reaction was started by injection of an aliquot of BNAH dissolved in propan-2-ol, via a narrow cylindrical channel in the electrode piston. In all measurements the reaction volume was 5 ml, buffered at pH 8.0 (Tris/HCl) with ionic strength $I = 0.05$ M (KCl), and contained 1 or 5% (v/v) propan-2-ol. The glass reaction vessels could be centrifuged, thus simplifying isolation of immobilized polymer and minimizing work-up losses when re-using this material.

RESULTS AND DISCUSSION

It has been reported that the catalytic activity of copper complexes, with silica grafted copolymers of styrene and 4-vinylpyridine as macromolecular ligands, can decrease owing to the partial adsorption of the grafts onto the

surface of the silica to which it was anchored[13]. Adsorption of unbound polystyrene or copolymers containing styrene units from solution onto silica has also been observed by several other authors[15-17]. To find out whether adsorption phenomena could influence the reactivity of the flavin-containing polystyrene derivatives shown in *Table 1*, differing amounts of nonporous silica were added to aqueous reaction mixtures containing a constant amount of copolymer. After 20 min an aliquot of BNAH solution was injected and reaction rates were recorded. Afterwards the extent of adsorption of the copolymers was determined. The results of these orientating experiments are shown in *Figure 2*. To our surprise, the reaction rates r_0 were not affected although all of the polymers adsorbed onto the added silica, independent of copolymer composition. This finding urged us to study the adsorption behaviour of these copolymers in more detail.

Adsorption behaviour

The time dependence of the adsorption process was followed for one copolymer under representative conditions. Although maximum adsorption was achieved within one hour, values reported in the following are for 20 h adsorption time. We assumed this period to be sufficient for all systems to reach the equilibrium values. Examples of adsorption isotherms for copolymer **1b** from water onto several silicas are given in *Figure 3*. For adsorption on A200V the same adsorption isotherms
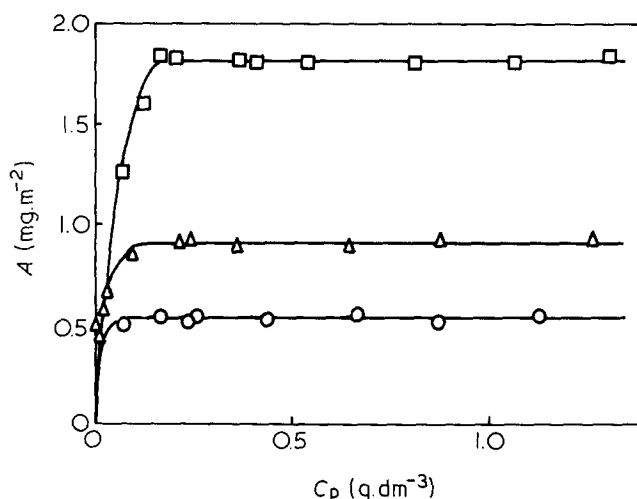
Figure 3 Adsorption isotherms for polymer **1b** from aqueous solution onto OX50 (□), 200V (△) and R972 (○) at pH 8.0, 25°C. ((□) and (△) in water, (○) in 5% (v/v) 2-propanol in water; [silica] = 2 g.dm^{-3})

Table 2 Adsorption of polymer **1b** onto different silicas

Silica	Average particle[a] diameter (nm)	Specific surface[a] area (m^2.g^{-1})	SiOH.nm^{-2}	Adsorbed amount[b] of **1b** (mg.m^{-2})
OX 50	40	50	unknown	1.8
200 V	12	200	2.6	0.9
R972	16	120	1.2	0.5
R972-S[c]	16	–	<1.2	0.15

[a] Information from Degussa AG
[b] $C_p = 1$ g.dm^{-3}, pH 8.0, $I = 0.05$M at 25°C
[c] Further hydrophobized by silanization

were found for the other copolymers. The values of the adsorbed amount A show an initial steep rise with increasing concentration and then reach a plateau region. This indicates that the adsorption isotherms are of the high-affinity type. Note that for the different silicas varying plateau values are observed. The values of A measured at $c_p = 1$ g.dm^{-3} are summarized in *Table 2*, together with some data on the silicas used. These data indicate that there may be a correlation between the silanol group content on the surface and the amount of adsorbed polymer. Interaction between the quaternary ammonium groups in the polymer and (ionized) silanol groups could be responsible for adsorption[18]. In water the silica particles behave as weak polymer acids and the degree of dissociation of the silanol groups will be dependent on the pH of the medium. Therefore we investigated the influence of the medium pH on the adsorption in order to clarify the type of interaction. In

$$ \text{(SiO}_2\text{)}-OH \rightleftharpoons \text{(SiO}_2\text{)}-O^- + H^+ $$
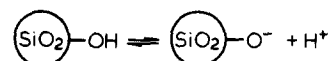
Scheme 3

Figure 4 the adsorbed amount from a solution containing a large excess of copolymer is shown at different pH values (0 symbols). In acid media there is almost no adsorption observed, but upon increase of the pH the adsorbed amount increases strongly. In the same Figure desorption of the polymer from a sample prepared at pH 8 is shown. The observed hysteresis between adsorption and
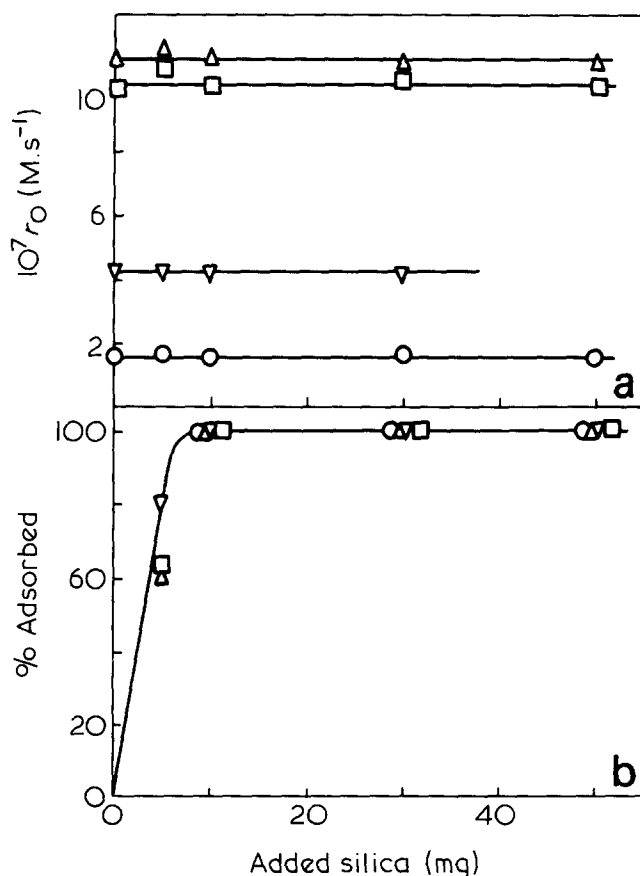
Figure 2 Influence of addition of silica (A200V) on BNAH oxidation rates r_0 by polymer-bound flavins (a), and extent of copolymer adsorption (b) for **1a** (○); **1b** (△); **1c** (△) and **1d** (□). Conditions [1a-d] = 0.225 g.dm^{-3}; [BNAH]$_0$ = 3.7 × 10^{-4} M in 1% (v/v) 2-propanol in water; pH 8.0; I = 0.05 M at 25°C; reaction volume 0.005 dm^3
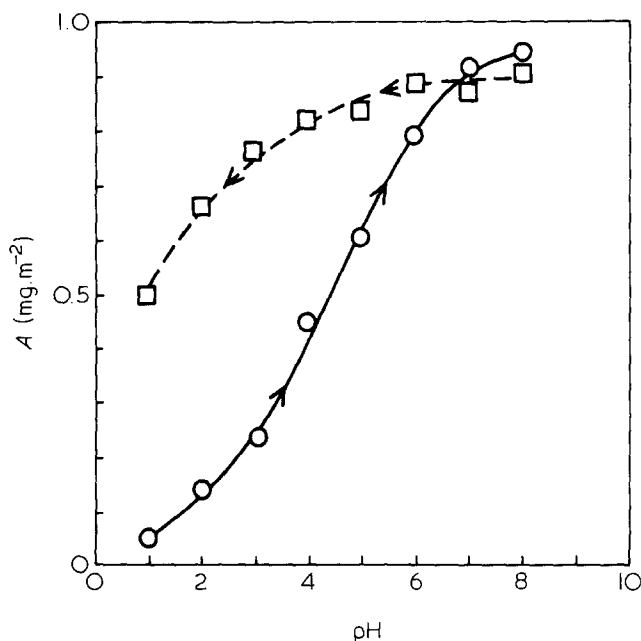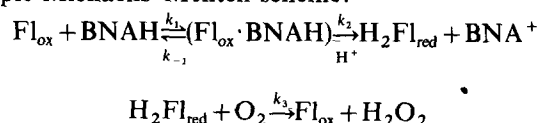
Figure 4 Adsorption (○) and desorption (□) of polymer **1b** on A200V as a function of increasing and decreasing medium pH, respectively

desorption at different pH values again is an indication that the binding constants for adsorption from aqueous solution (pH 8) must be high. Therefore, we can conclude that the interaction between the quaternary polystyrene derivatives and silica is electrostatic, the number of salt bonds formed between quaternary nitrogen and ionized silanol groups increasing with increasing pH-values. A similar result has been reported for the interaction of poly-N-ethyl-4-vinylpyridinium bromide with silica sols[19]. Now it can also be understood why all four polymers showed the same maximum specific adsorption values, independent of the ratio quaternary ammonium-/styrene-units. The ammonium–silanol interaction is so strong that it overwhelms the phenyl–silica interactions, and only a few salt bridges are sufficient to bind the copolymer to the silica surface. Little change in specific adsorption over a wide range of copolymer compositions was found for the adsorption of styrene–methyl-methacrylate copolymers, and even the number of bound carbonyl groups was reported to remain constant[17]. In our case, it is not possible to estimate the fraction of bound segments from changes in the i.r.-spectrum of the silanol groups, as performed by others[15-17], since our medium is water, which disturbs the relevant part of the i.r. spectrum.

Kinetics

In our previous article[11], we reported on the kinetics of the oxidation of 1-benzyl-1,4-dihydronicotinamde (BNAH) catalysed by the polymer-bound flavins. It appeared that the model reaction can be described by a simple Michaelis–Menten scheme:

$$Fl_{ox} + BNAH \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (Fl_{ox} \cdot BNAH) \overset{k_2}{\underset{H^+}{\rightarrow}} H_2Fl_{red} + BNA^+$$

$$H_2Fl_{red} + O_2 \overset{k_3}{\rightarrow} Fl_{ox} + H_2O_2$$

Under aerobic conditions the reoxidation of the reduced flavin is extremely rapid[20], so the following rate equation can be derived:

$$r_0 = \frac{k_2[Fl_{ox}][BNAH]_0}{K_m + [BNAH]_0}$$

In this expression r_0 is the initial velocity, $[BNAH]_0$ is the initial substrate concentration and $K_m = (k_2 + k_{-1})/k_1$, the Michaelis constant. Values for k_2 and K_m can be derived by plotting the data of substrate concentration-dependent measurements in a double-reciprocal Lineweaver–Burk plot. All previously reported kinetic parameters were derived in this way from data obtained by monitoring the reaction in a u.v./visible spectrophotometer, but since the present system is heterogeneous this technique could not be applied. So, reaction rates were determined as dioxygen consumption rates (see Experimental section). The agreement with results found spectrophotometrically for the homogeneous systems was very good.

In order to study the influence of adsorption phenomena on kinetic behaviour in more detail, reaction rates at different substrate concentrations were determined for silica samples with a maximum amount of adsorbed copolymer. These were obtained by isolating the silica after 20 h from a solution containing an excess of flavin polymer. We found that reaction rates and also k_2 and K_m^{-1} values were, within experimental error, identical to those of the original dissolved polymers. Kinetic parameters are gathered in *Table 3*, whereas in *Figure 5* some examples of Lineweaver–Burk plots are given. As in the case of the adsorption experiments (see above), we can explain these results by assuming that large parts of the polymer chain exist as loops and tails where the character of the chain should not be substantially altered by the adsorption process. Each chain carries only a few pendant flavin groups which do not bind to the silica surface, and these will therefore be located in the tails or loops where their microenvironment will be similar to flavins bound to a dissolved polymer coil.

Another indication for protrusion of a greater part of the macromolecular chain into the solution was obtained from the (visible) spectrum of the bound flavin. We were able to record the spectrum of immobilized flavin against a reference containing an equal amount of silica-adsorbed flavin-free copolymer, by using the special turbid-sample holder of our spectrophotometer. The characteristic absorption maximum of the flavin around 440 nm has identical shape and intensity for both dissolved and silica-adsorbed copolymer, as shown in *Figure 6*.

Table 3 also comprises some data on a flavin-containing

Table 3 Kinetic parameters for BNAH oxidation by dissolved and silica adsorbed (grafted) flavin-containing polymers[a]

Sample	Adsorbed amount (mg.m^{-2})	$k_2(s^{-1})$	$K_m^{-1}(M^{-1})$	$k(M^{-1}.s^{-1})$
1d	dissolved	0.52	525	327
1d/200V	0.9	0.60	550	331
1b	dissolved	0.20	1100	220
1b/OX50	1.8	0.19	1000	190
1b/200V	0.9	0.20	1100	220
1b[b]	dissolved	0.18	1000	180
1b/R972[b]	0.5	0.19	950	180
2	grafted[c]	0.09	1850	160

[a] Reaction medium: 1% (v/v) 2-propanol in water, pH 8.0, I = 0.05M at 25°C
[b] Medium contained 5% (v/v) 2-propanol
[c] About 0.4 mg copolymer m^2 silica

polymer (**2**) chemically anchored on the silica surface. Although the silica-grafted homopolymer of vinylbenzyl-chloride was allowed to react with triethylamine over a prolonged time, only about 60% of the CH_2Cl groups reacted, as was calculated from the nitrogen content. Probably the polymer already adsorbed during the quaternization reaction, thus hindering further reaction. The kinetic results are in agreement with this suggestion. We found values for k_2 and K_m^{-1}, which were consistent, for a polymer with 50% rather than 100% quaternized monomer units[11]. In addition, no significant desorption of adsorbed polymer was observed (*vide infra*), so the laborious synthesis of terminally anchored chains offers no special advantages in this case.
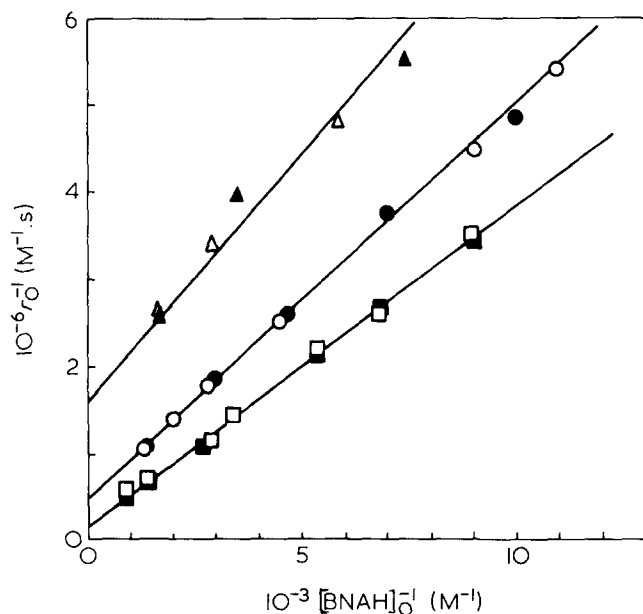

Figure 5 Lineweaver–Burk plots of BNAH oxidation by flavin-polymers in solution ($\triangle$, $\bigcirc$, $\square$) and adsorbed on A200V silica ($A=0.9$ mg m^{-2}) ($\blacktriangle$, $\bullet$, $\blacksquare$) for **1a** ($\triangle$, $\blacktriangle$; [flavin] = 1.5 × 10^{-5} M); **1b** ($\bigcirc$, $\bullet$; [flavin] = 1.04 × 10^{-5} M) and **1d** ($\square$, $\blacksquare$; [flavin] = 0.83 × 10^{-5} M) in aqueous medium, pH 8.0, $I=0.05$ M at 25°C
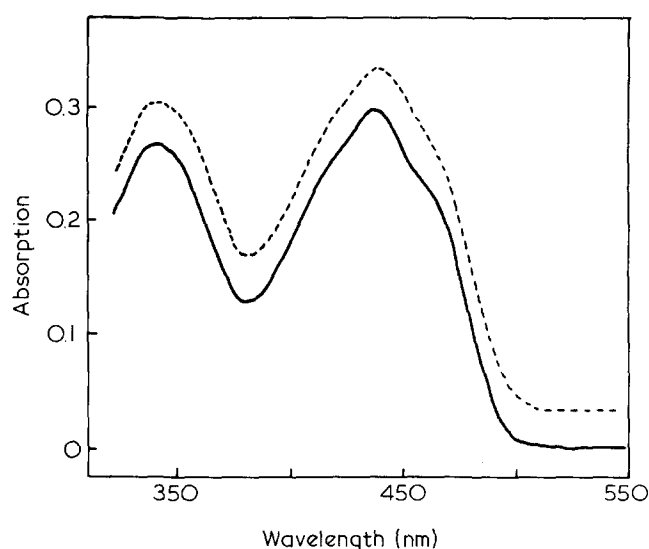

Figure 6 Visible spectra of flavin-polymer **1b** in homogeneous solution (——) and immobilized by adsorption onto A200V (0.9 mg.m^{-2}) (---; for clarity the spectrum is shifted upwards) ([**1b**] = 0.5 g.dm^{-3} in 5% (v/v) 2-propanol in water, pH 8.0)
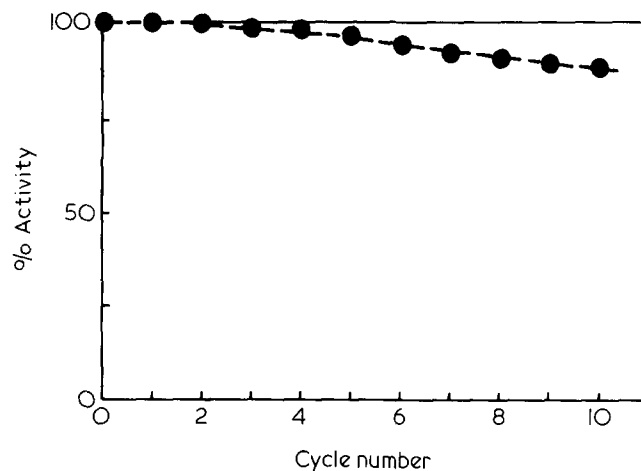

Figure 7 Activity of immobilized copolymer **1b** (0.9 mg.g^{-2} on A200V) as a function of the recycling. The apparent loss of activity is not caused by lowering of intrinsic flavin activity, but is due to work-up losses (see text)

Re-use of silica-adsorbed reactive polymer

One of the first targets of immobilization of linear reactive polymers is re-use of the material by simple procedures. The present support can be separated from the solvent quickly by low-speed centrifugation, owing to the fairly high specific density of silica*. After centrifugation and decantation of the solvent, the sedimented support is dispersed again and the reaction rate is measured. The results of ten subsequent cycles are presented in *Figure 7*. After ten runs about 90% of the original activity was still preserved. However, when the material was washed and dried, it was found that about 9% of the silica-based catalyst had been lost during the reactions cycles. This corresponds with the loss of catalyst activity. It was also found that when the material was dried after each reaction cycle, its reactivity was preserved. Clearly, there is no detectable desorption of adsorbed copolymer under the reaction conditions employed.

CONCLUSIONS

A promising new immobilized homogeneous catalyst is obtained by adsorption of linear flavin-containing poly-electrolytes onto silica:

— the reactivity of the bound flavin is not influenced by the adsorption process;

— the material can easily be re-used many times without loss of activity;

— spectrophotometric characterization of the immobilized flavin remains possible.

Adsorption of such previously functionalized and characterized macromolecules onto silica supports may provide new materials for catalytic application and in related fields.

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