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Grafting of acrylamide onto Nylon-6 fabric by the electron beam preirradiation technique

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Summary

Nylon-6 knitted fabrics, preirradiated by electron beam accelerator, were grafted with acrylamide (AM) to form a graft-copolymer (NFgAM) gel-type hydrophilic porous matrix. Graft yields of up to 92% were attained within 10 min of grafting. At graft yields above ca 70% the matrix became a continuous, membrane-like barrier. The anchoring of TiO_2 onto the thus prepared support material was examined and the catalytic features of the grafted-support /semiconductor assemblies were characterized by test reactions. Anchoring of asparginaze onto the NFgAM support was also studied and the enzymatic activity evaluated.

Introduction

Hydrophilic polymeric matrices play an important role in synthetic membranes for separation processes [1-2], as well as in synthetic supports for immobilization of reagents [3-5]. In recent years, the expanding use of immobilized reagents affected an increase of the inquiry for new types of supporting matrices as well as new techniques for the anchoring of active reagents onto the supports. These needs have drawn attention to the possibility to use polymeric support-matrices in general, and the hydrophilic matrices in particular [5-11]. The need for improved supporting matrices is prominent regarding the anchoring of active reagents such as enzymes [5], catalysts [6], drugs [7], chelating agents [8], antibodies [9], immunoassays [10] and other bioactive species or living cells [11]. To this wide variety of applications, for each immobilized reagent a unique type of environment is required for optimal reactivity. The versatility of the polymeric support-matrices, which allow easy modification to attain almost any required adaptation, is very attractive both for R&D and for industrial processing and the research activity is expanding fast.

Another new, rather intriguing application for hydrophilic membranes, namely the encapsulation of transplanted organs inside the living body [11-13], was demonstrated few years ago. The membranes for that application were required to function as

a biocompatible and a permselective barrier, as well as to function as an encapsulating flexible (mechanical) support. The composite Teflon/pHEMA (polyhydroxyethylmethacrylate) membrane has fulfilled almost all expected characteristics (but accumulated deposits of calcification).

An applicable support characteristics should combine both the mechanical strength required for its use (e.g. separation processes) as well as the specific chemical nature required for anchoring of the reagents for each utilization process. The location of these anchoring sites can be limited to the surface of the support or may be spread throughout the matrix bulk. In the latter case, the matrix should be compatible with the solution of the reacting substrate-species to allow their accessibility to the matrix-anchored reagents at the innermost sites of the support. Hence, for the wide variety of reactions which take place in aqueous media, hydrophilic support matrices are superior to the hydrophobic ones.

In princip, hydrophilic support matrices can be fabricated either by the modification of the structure of a given polymer, which is already hydrophilic, or by modification of the chemical nature of (at least) the surface of the polymer, which has been already appropriately shaped to meet a specific ensemble of structural demands. The last attitude can perfectly be accomplished by the radiation induced grafting technique.

The radiolytic grafting of hydrophilic monomers onto nylon-6 film-matrices has been extensively studied [14-22], by both preirradiation and simultaneous irradiation methods. The radiation sources utilized were electron beam accelerators as well as Co^{60} γ-source. In previous communications [14-19] the grafting of acrylamide (AM) onto nylon-6 films, by the electron beam preirradiation technique, was studied in detail and the radiolytically grafted membranes were characterized. Recently, we have shown that TiO_2 can be anchored onto the nylon grafted with acrylamide (NYgAM) membranes, thus forming a membrane/semi conductor catalytic assembly.

In the present communication, the radiolytic grafting of AM onto a nylon knitted fabric-substrate, the anchoring of several reagents onto this grafted support and the consequent reactivity of the NFgAM supported reagents are discussed.

Experimental

Grafting

Nylon-6 knitted fabric was kindly supplied by Einat Textiles Ltd, Israel. It was washed with deionized water and dried at room temperature. The irradiation was performed with the 550kV, 25mA High-Voltage electron beam accelerator (EBA). Irradiation took place at room temperature and ambient atmosphere. The grafting step followed immediately after the irradiation and was performed in a double-compartment reactor system described in a previous communication [23]. The preirradiated fabric samples were placed in one arm of the reactor and the monomer solution in the other one. After purging with CO_2, the monomer solution was syphoned into the sample compartment and was kept stirred during the grafting period. The reaction was stopped at the desired time period by opening the reactor to air and washing the grafted fabric from

excess of monomer with running water. Homopolymer, when formed, was extracted with hot water. The grafting procedure is described in details in previous communications [14,23].

Anchoring of Reagents

The anchoring of crystallites of TiO_2 onto the grafted support was attained as follows. A sample of the grafted matrix was soaked with an aqueous solution of the precursor of TiO_2: $Ti(OR)_2OAc$ (R = $-CH(CH_3)_2$; the solution is prepared by dissolving $Ti(OR)_4$ in glacial acetic acid followed by gradual dilution in water). After wiping off the ecxess of reagent solution, the TiO_2 crystallites were produced **in situ** at 65°C; 100% RH (48h). This procedure is described in detail in a previous communication [24]. The anchoring of L-asparginase [9] was attained by reacting the amide groups of the support with glutaraldehyde followed by anchoring of the enzyme via its $-NH_2$ groups. These reactions are described in detail elsewhere [25-26].

Results and Discussion

Table 1: Graft Yields of Acrylamide onto Nylon Fabrics

No.	AM Conc. (%)	bisAM Conc. (%)	Radiation Dose (Mrads)	Grafting Temp. (°C)	Grafting Period (min.)	Graft Yield (%)	Remarks
1	9.5	0.5	12	30	4	13.2	
2	9.5	0.5	12	30	8	30.5	
3	9.5	0.5	12	30	12	51.0	H.P.
4	9.5	0.5	12	30	18	90.9	H.P.
5	9.5	0.5	12	30	24	91.8	H.P.
6	4.85	0.15	4.8	50	4	9.0	
7	4.85	0.15	4.8	50	12	44.3	
8	4.85	0.15	4.8	50	24	49.4	
9	6.86	0.14	7.2	50	6	40.0	
10	6.86	0.14	7.2	50	8	42.4	
11	6.86	0.14	7.2	50	12	50.9	
12	6.86	0.14	7.2	50	16	78.8	H.P.
13	9.5	0.5	12	30	10	20.2	
14	10	---	12	30	10	69.6	NMAM
15	9.5	0.5	12	0	10	0.5	
16	9.5	0.5	12	0	15	0.9	
17	24	1.0	12	0	10	2.8	
18	24	1.0	12	0	15	3.6	

H.P. - denotes homopolymerization and gelation of the monomer solution.

NMAM - the monomer N-methylolacrylamide used instead of AM.

Grafting onto the Nylon-Fabric Substrate

Table 1 presents typical results of graft yields of mixtures of AM and the crosslinking agent methylene-bisacrylamide (**bisAM**), attained onto the nylon fabrics under various grafting conditions.

Fig. 1 shows the graft yield vs. the period of grafting under the various experimental conditions studied.

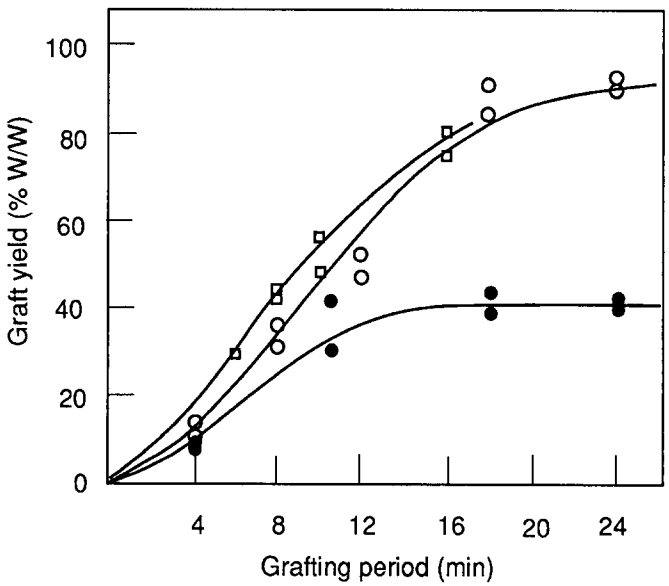

Fig. 1: Graft yields of AM onto the preirradiated knitted nylon fabric, vs. the grafting period, under various experimental conditions. (o) 12 Mrads, 9.5% AM, 0.5% bisAM, Temp.= 30°C.
(•) 4.8 Mrads, 4.85% AM, 0.15% bisAM, Temp.= 50°C.
(□) 7.2 Mrads, 6.86% AM, 0.14% bisAM, Temp.= 50°C.

The data in Table 1 shows that attaining high graft yields onto this type of nylon substrate is somewhat problematic. Unlike the nylon films, which could easily be grafted to graft yields of up to 1300%, the present substrate could not be grafted to (true) graft yields exceeding ca 90%, when a fast levelling off of the graft yield vs. grafting period occurs. This observation is inconsistent with the expected much higher accessibility of the nylon subssrate to the penetration of the monomer, due to the fact that the knitted nylon fabric has a much higher surface area than the nylon films employed in former investigations. One could expect, therefore, that the substrate with the higher surface area would exhibit higher grafting rates. However, a second prominent observation is that the levelling off of the graft yield vs. time is accompanied with the gelation of the monomer solution which occurs much earlier than in the experiments of grafting onto preirradiated films of nylon.

The simultaneous occurence of the graft yield levelling off and the homopolymerization and fast gelation of the monomer solution most probably manifests chain transfer reactions from the polymeric matrix to the monomer solution. These reactions are eccelerated by the very large surface area of the polymeric substrate. The consequent fast depletion of the population of radicals in the polymeric matrix of the substrate is the slow down of the grafting rate and a fast levelling off. The observation that even the initial grafting rate onto the nylon fabric is lower than onto the nylon film can be rationalized by concluding that the decrease of the grafting rate, following the fast depletion of radicals, is not compensated by the enhanced monomer penetration through the high surface area. This is reasonable, since the nylon fibers in the knitted fabric are extremely thin and the monomer penetration into the nylon matrix, is most probably not the rate determining step of the grafting process. This rationale is consistent with previous observations [15], where the grafting reaction of AM onto preirradiated nylon films was found to become diffusion-free at a very initial stage.

Several lower preirradiation doses, as well as lower monomer and crosslinking-agent concentrations were employed to try and optimize the grafting process, attempting mainly to try and overcome the obstacle of homopolymerization. As shown in Table 1 and in Fig 1, a decrease of the preirradiation dose and monomer concentrations delays the formation of the homopolymer, even though the temperature was raised by 20°C. However, this was attained at a penalty of slowing down the grafting reaction almost proportionally to the dose, despite the elevation of the temperature and the decrease of the ratio of the crosslinking-agent to monomer. The lowering of the temperature during the grafting from 50°C and 30° to 0°C eliminates most of the homopolymerization, with the penalty of an extreme slow-down of the reaction. However, the grafting at lower temperatures was employed also in attempt to try to achieve a thin, surface-mounted grafted layer on the fibers of the substrate, as will be discussed in the following.

The change of monomer from AM+bisAM to NMAM manifests in an increase in the graft yield by a factor of more than 3 (cf. #13 and #14 in Table 1). This finding may reflect either a higher propagation rate of the latter monomer, or a lower rate of chain transfer from its propagating chains to the monomer in the solution, a question which remains to be solved by further experiments. Another interesting observation is the lower graft yield in #13 in Table 1 as compared with #2, although both were grafted at similar conditions and #13 was grafted for a longer period than #2. This differenc manifests the fact that in experiment #13 the weight-ratio of substrate to monomer is much higher than in #2. Thus, there occured faster depletion of the monomer solution followed by a cease of the grafting process.

The above described findings lead to a conclusion that the nylon fabric substrate has a severe limitation for uses where very high graft yields are required. However, the salient feature of the knitted fabric substrate is its elasticity, which may be extremely benefitial for some practical uses (e.g. for encapsulation of transplanted organs [11-13]). This elasticity is being retained only if the graft yield of AM is

limited to few tens of percents. Furthermore, at high graft yields care must be taken to wash out all the homopolymer which is occluded in the fabric (e.g. with hot water), in order to assure the anchoring of the desired reagents to the true graft in order to prevent their loss in the following eperiments.

Anchoring of Active Reagents onto the Grafted Supports and Characterization of Their Reactivity

Typical results of anchoring of TiO_2 onto the NFgAM and other support matrices are presented in Table 2

Table 2: TiO_2 Anchored on Grafted Nylon-Fabric and other Supports

No.	Support Substrate	Monomer		Graft Yield (%)	TiO_2 Load
1.	Nylon Fabric	AM bisAM	(9.5%) (0.5%)	20	8.5
2.	Nylon Fabric	NMAM	(10%)	70	5.3
3.	Nylon Film	AM	(10%)	256	2.2
4.	PVA Film	--	--	--	9.8

The highest load of TiO_2 onto grafted nylon substrates is attained onto the NFgAM one, although grafted only to 20%. Similar load of this semiconductor is attained onto a support made of PVA (poly-vinylalcohol) commercial film (cf. #4 in Table 2), which exhibits much inferior mechanical properties than the NFgAM one. However, it was soon found out that it is the site and the size of the anchored semiconductor crystallites, rather than the high capacity of loaded TiO_2, that determines its reactivity characteristics in test reactions such as photocatalytic generation of hydrogen from water [24].

Inspection of the TiO_2 crystallites on the highly loaded supports, by electron microscopy techniques, reveals that they have been grown mostly external to the outer surface of the frafted fabric and are attached to it but not surrounded by the grafted matrix. Furthermore, these semiconductor crystallites grew to a very large size - ca. 10μm, as compared with the <1μm crystallites formed within the grafted membranes [26]. The latter exhibited a very high specific catalytic activity, combined with stability of the ensembles of support/catalyst during prolonged experiments. However, the practical use of these film-anchored catalyst systems was limited due to the very slow fluxes of permeants through these matrices [17], a draw back which is overcome by introducing the knitted nylon fabric as the substrate for the grafted supports.

In light of all these observations, the lower reactivity

of the NFgAM-supported TiO_2, as compared with the NYgAM film-supported TiO_2 can be attributed to two factors: First, the larger size of the crystallites which manifests in lower surface area and hence a lower catalytic reactivity. Secondly, the fact that the major part of the catalytically active surface is bare. Thus, it can not share the environmental advantages [5] of the support, namely, the grafted polyacrylamide (PAM) chains [6], such as the concentration of reactants in the close vicinity of the catalyst. Furthermore, the surface-attached TiO_2 crystallites were found to be somewhat mechanically eroded following extensive use.

The practical conclusion of these findings is that for optimized catalytic reactivity, one should have grown the semiconductor crystsllites **inside** the polymeric grid, as it was performed in the case of the grafted NYgAM films [6,24]. To attain this goal, the grafting process should be optimized to produce a thicker and less crosslinked grafted layer of PAM, which will allow internal growth of TiO_2 crystallites. Yet, this layer must be confined to the surface of the substrate in order to avoid diffusion limiting of the catalytic processes. Hence, the feedback for modifying the parameters of grafting procedure should most probably be as follows:

a. to use a more concentrated AM grafting solution (e.g. 25%), and much smaller concentrations of bisAM. These changes will most probably effect occlusion of homopolymer onto the fabric. Its removal requires an its extraction with hot water.

b. from the data in Table 1 it seems that grafting at a higher dose, combined with a shorter period of grafting might be preferable for the above described purpose.

A totally different behaveour of the anchored reagent is observed in the case of the L-Asparginase immobilized onto NFgAM of 7.5% and 13.2% graft yield [25]. Its reactivity was tested at pH 7.35 using 0.1M L-Aspargine [26]. It was found that the lower the graft yield, the higher the specific activity of the anchored enzyme is, inspite the fact that the amounts of anchored enzyme were proportional to the graft yield. This observation is consistent with the findings of an earlier investigation where L-Asparginase was anchored onto film support of polypropylene grafted with polyacrylicacid. It is not reasonable to attribute the loss of activity of the enzyme in the depth of the water-swollen PAM matrix to lack of acessibility. Therefore, these findings manifest most probably the fact that the PAM environment is **not favorable** for the activity of this enzyme and only species which are pendent from the grafted polymer are fully active

The salient conclusion derived from these observations is that for an optimal reactivity of a specific enzyme immobilized onto a grafted support, its reaction to the synergistic effect of the environment of the grafted polymer and the feed solution must be carefully evaluated. Thus, for the system asparginase immobilized on NFgAM a very low graft yield of AM, which is highly crosslinked, is preferred to attain particularly surface pendent anchoring of the enzyme molecules. To attain this goal, the grafting parametrs should be modified, e.g. by using a grafting solution with low AM and high bisAM concentrations (e.g. 4.5% AM + 0.5% bisAM), and by prforming the grafting step for a very short period, and at a low temperature.

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