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The Use of Graft Copolymers as Enzyme Supports
The Preparation and Use of Polyethylene-Co-Acrylic Acid Supports

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Summary

A study has been made of the radiation-induced grafting of acrylic acid onto polyethylene. Grafting was found to be steady state in character in the early stages, when carried out in a suitable medium. Evidence is presented showing that, in the later stages, autoacceleration becomes a feature of the grafting process. Thermal analysisprocedures were used in an attempt to show the occurrence of grafting.

Immobilization using N-ethoxycarbonyl-2-ethoxy,-1,2-dihydroquinoline (EEDQ) as the coupling agent was attempted. This met with various levels of success. The copolymer-EEDQ-enzyme system was used to couple bovine serum albumin (65 mg protein/g of substrate) and phenyl propylamine (95 mg phenylpropylamine/g of substrate).

Introduction

Various supports have been used in attempts at enzyme immobilization. Details relating to the use of graft copolymers as supports have been reported (BEDDOWS et al, 1979 and ABDEL-HAY et al,1979). These workers, coupled various enzymes onto nylon-co-acrylamide graft copolymers. Two coupling procedures were examined, namely the glutaraldehyde route and the azide coupling procedure. ABDEL-HAY et al (1980) used the nylon-co-acrylonitrile system in enzyme immobilization. In such coupling,

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water soluble carbodi-imides were used as the coupling agent. In general, satisfactory yields of coupling are achieved using graft copolymers as supports, though the level of resultant activity could be significantly improved.

In the present work poly(acrylic acid) was grafted directly onto polyethylene and the resultant copolymer was used to couple with bovine serum albumin(BSA) and phenyl propylamine. This study is an attempt at obtaining a greater understanding of the nature of the copolymer and the copolymer-enzyme composite. Our interest, in this instance, lies in the provision of a large number of hydrophilic surface grafts on a hydrophobic substrate. In an aqueous coupling medium, the hydrophilic branches are then readily available for the coupling reaction.

Experimental

Reagents: Acrylic acid was obtained from B.D.H. Ltd., Poole, Dorset, U.K., and was purified by standard means. The polyethylene powder (Telcon Plastics Ltd..U.K.) was Soxhlet extracted with ethanol for 6 hours before drying to constant mass under vacuum at 313K. Bovine serum albumin and N-ethoxycarbonyl-2-ethoxy,-1, 2-dihydroquinoline (EEDQ), were obtained from the Sigma Chemical Company, St. Louis, U.S.A.

Grafting Procedure: The grafting procedure was as follows:

The sample of polyethylene used in the present work was of low density (ρ = 0.935 g cm^{-3}). For this reason, the solvent system for the monomer was designed to ensure complete immersion of the polyethylene powder. Experiments were undertaken to find a mixed solvent system (methanol/water) which would guarantee complete immersion of the polyethylene and act as a good solvent for both the acrylic acid and any poly(acrylic acid) homopolymer. The most satisfactory combination was methanol/water in the proportion 77/23.

The purified polyethylene (2g) was immersed in solutions of acrylic acid in methanol/water (77/23), (30 cm^3). The mixture was then irradiated at 170 rad min^{-1} using a Co(60) source located in the Department of Physical Chemistry, the University of Leeds. The irradiation was carried out at 298K.

The concentrations of acrylic acid used were 3%, 5%, 8% and 10% (w/v), (0.417 to 1.389 mol l^{-1} of bulk system). After irradiation in the presence of air, the bulk solutions and grafted polyethylene powder were transferred to distilled water, filtered and the solid washed thoroughly with distilled water. The poly(acrylic acid) homopolymer was removed by extraction with distilled water and then the grafted products were dried in vacuo at 313K.

Samples of poly(acrylic acid) homopolymer, polyethylene and the polyethylene-co-acrylic acid graft polymer were subjected to thermal analysis in a Du Pont 990 Differential Thermal Analyser located in the Wolfson Unit of the Department of Colour Chemistry, the University of Leeds, U.K. Identical samples were examined for their coupling efficiency with regard to bovine serum albumin and phenyl propylamine.

Coupling: The coupling sequence began by activating the copolymer with EEDQ in benzene/ethanol (4:1). The activated copolymer was then coupled to the protein, BSA, and to phenyl propylamine. The details are as follows:

Coupling to BSA: The copolymer (100 mg) was shaken with EEDQ (100 mg) in benzene/ethanol (4:1), (10 cm^3), for 2 hours at 308K. The solid was filtered, washed with benzene;ethanol (4:1) and then water before being added to a solution of BSA in distilled water (10cm^3) (4 mg/cm^3) and stirred overnight at room temperature. The products were filtered. The filtrates and washings were made up to 100 cm^3 with distilled water before being assayed for their protein content by the method of LOWRY et al (1951).

Coupling to phenyl propylamine: After activation with EEDQ at 308K for 2 hours, 50 mg of the copolymer was added to 11.5 mg of phenyl propylamine in 5 cm^3 of benzene/ ethanol (4:1) and the mixture shaken for 18 hours at room temperature. The solids were filtered and washed. The filtrate and washings were made up to 50 cm^3 with distilled water. A representative sample (15 mg) of copolymer-phenyl propylamine was heated with 2cm^3 of 2M NaOH on a boiling water bath for 1 hour. After filtering, washing and acidifying, the filtrate was made up to 10cm^3 with distilled water. The optical density of the solution at λ_{260} was obtained and the concentration of originally bound phenyl propylamine was determined from a calibration curve assembled via standard solutions of phenyl propylamine in water.

In all these experiments a control was used. This involved treating the copolymer with the protein or amine solution, in the absence of coupling agent (EEDQ), under identical conditions to those described above.

Results and Discussion

In coupling reactions between the polyethylene-co-acrylic acid and phenyl propylamine or bovine serum albumin, the graft copolymer used was that containing 13.6% by weight of acrylic acid branches. The amount of acrylic acid grafted is perhaps best expressed, for kinetic purposes, as the number of mol of acrylic acid grafted per l of bulk system. This may be calculated from a knowledge of the mass and density of polyethylene and the volume of all other components. Such an approach assumes that the volumes of all species present can be handled additively. Though an oversimplification, it is thought that any errors ensuing are likely to be minor in the current context. The concentration of acrylic acid in the bulk medium was limited to 0.42 to 1.39 mol l^{-1} in a range of grafting experiments. Thus, problems arising from involuntary gelation during irradiation, were avoided, at least in the early stages.

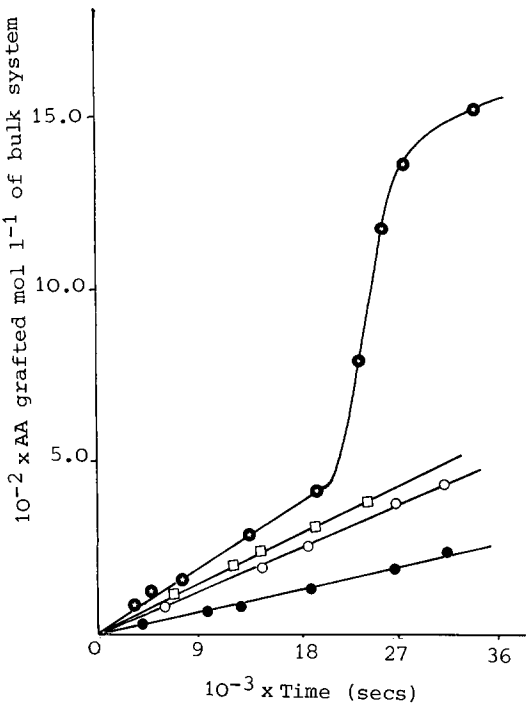

Figure 1. Variation in the extent of grafting of acrylic acid to polyethylene powder, with time of irradiation for different bulk monomer concentrations. (●(3%); O (5%); □ (8%); ⊕ (10%)) in bulk medium

Figure 1 shows the variation in grafting levels with increase in the irradiation time for various bulk monomer concentrations. For each monomer concentration, linear relationships were obtained for the increase in grafting levels with increase in total dose or irradiation time. The initial rates of grafting for bulk monomer concentrations of 0.42, 0.69, 1.11 and 1.39 mol l^{-1} of bulk solution were 0.75, 1.40, 1.60 and 2.20 (x 10^{-6}) mol l^{-1} s^{-1} respectively. The corresponding log-log treatment of these data shows the dependence of the grafting rate on the bulk monomer concentration to be near unity in the early stages. One is tempted to conclude that the grafting process occurs under steady-state conditions.

Such conclusions should be drawn with caution. That this is so can be seen from Figure 1. At a bulk monomer concentration of 1.39 mol l^{-1} of the bulk medium, apparent autoacceleration effects are observed when the irradiation time exceeds 2×10^4 seconds, the dose rate being 2.9 rad sec^{-1}. Such autoacceleration becomes important if it leads to markedly changed structural characteristics in the resultant graft copolymer, especially when the copolymer is to be used in immobilization studies.

An attempt at partial characterisation of the polyethylene-co-acrylic acid polymer (13.6% graft), used in the enzyme immobilization studies, was attempted via differential scanning calorimetry/differential thermal analysis procedures.

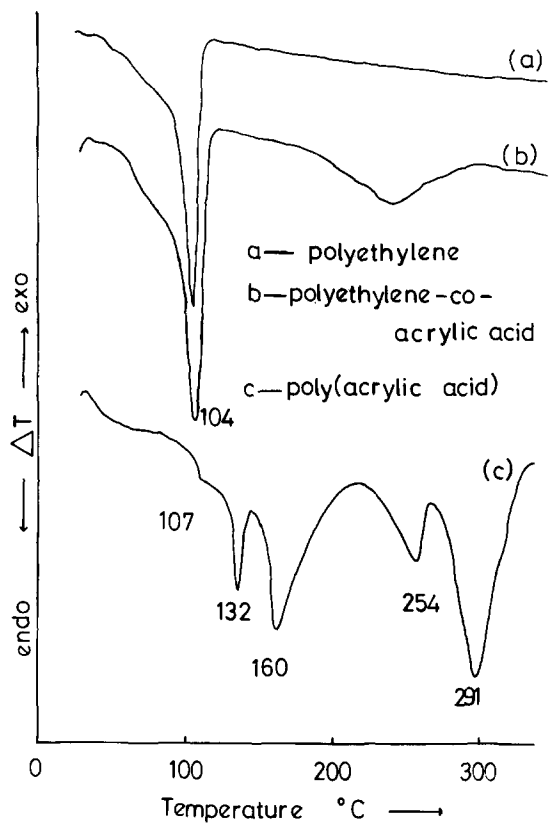

Figure 2. Thermal analysis of homopolymeric species ((a) and (c)) and the polyethylene-co-acrylic acid polymer (b).

Figure 2 gives the thermograms of polyethylene (a), poly (acrylic acid) (c) and the polyethylene-co-acrylic acid composite (13.6% graft) (b). In polyethylene (a) an endotherm is seen at 104°C (377K). This is typical of low density polyethylene (AGGARWAL (1975)).

The thermogram of the poly(acrylic acid) (c) is more complex. The shift in base line at 107°C (380K) is indicative of the glass transition temperature (HUGHES and FORDYCE (1956)). It is known that, when poly(acrylic acid) is heated in an inert atmosphere, the major change up to 160°C (433K), involves dehydration and the formation of anhydride groups and bridges (FRANK and WUENSCHER (1966); GAUGH and KOTTLE (1967)).

Between 160°C and 295°C (433 - 568K) the anhydride concentration is thought to decrease and unsaturation as well as cyclic ketone formation begins to occur. At 310°C (583K) major crosslinking, resulting in further ketone formation, is observed, while at 380-400°C (653-673K) CO_2 and unidentified polymer fragments have been detected. The endotherms, shown in Figure 2 (c), at 132°C (405K), 160°C (433K), 254°C (527K) and 291°C (564K) could all be interpreted along such lines. However, the main interest in this thermogram of poly(acrylic acid) lies in the possibility that it might provide an insight into the nature of the polyethylene-co-acrylic acid polymer (b).

Thermogram (b) carries more of the details of change shown at the lower temperatures in thermogram (c). In thermogram (b), the glass transition temperature of poly(acrylic acid) is not seen. However, the copolymer possesses a similar melting endotherm to that shown by polyethylene homopolymer. In addition, a broad endotherm commences at 150°C (423K) and minimises at 243°C (516K). This latter endotherm indicates that changes are taking place which do not occur in homopolymeric polyethylene. The magnitude of the endotherm at 243°C (516K) increased with increase in the extent of grafting, indicating that its presence is a consequence of the grafting process.

Such information can be taken as circumstantial evidence that grafting has in fact occurred and is thus in support of the gravimetric data. However, further work is required before grafting can be unambiguously proven to have taken place

Protein and Amine Coupling

When BSA was coupled to the graft copolymer using EEDQ as the coupling agent, it was found that 65mg of protein were coupled per g of copolymer, while 34mg of protein were adsorbed per g. The adsorption of the BSA to the copolymeric substrate might arise from an ion exchange effect. These results may be compared with those of BARTLING et al (1974), in their work with EEDQ. When 1,4-divinylbenzene-crosslinked-poly(4-methacryloxybenzoic acid) was activated with EEDQ and then coupled to aldolase, α-amylase, peroxidase and lysozyme, the activities obtained were 7.6, 1.9, 1.6 and 3.3mg of active enzyme per g of substrate.

When phenyl propylamine was coupled to the graft copolymer using EEDQ as the coupling agent, a yield of 95mg of phenyl propylamine per g of copolymer was obtained. Hence, it appears that this activated copolymer system provides a useful supporting medium for both BSA and amines. Coupling of amines to copolymers of this type may be an efficient method of producing selective affinity chromatography systems.

Conclusions

From the information provided by the grafting reaction, one can speculate as to the nature of the 13.6% grafted sample. The possibility of grafting onto the poly(acrylic acid) grafted branches arises. Hence, the grafts themselves would be branched in character. Such an occurrence would markedly influence the coupling behaviour of the copolymer-coupling agent-enzyme system. This possibility is to be examined further by carrying out graft copolymerization reactions under truly homogeneous solutions. Such work will be reported in due course.

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