Analysis of Surface Aldehyde Functions on Surfactant-Free Polystyrene/Polyacrolein Latex

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Received March 24, 1995; Revised Manuscript Received July 24, 1995[⊗]

ABSTRACT: The semicontinuous surfactant-free synthesis of a core/shell sytrene/acrolein latex and its analysis for surface aldehyde functions are described. A colorimetric assay based on reaction with dinitrophenylhydrazine is compared with reduction by tritiated sodium borohydride and integration of aldehyde peaks in the proton NMR spectrum of the dissolved polymer. X-ray photoelectron spectroscopy confirmed the surface location of the aldehyde functions and the analytical reaction products. The three assay procedures were in reasonable agreement, suggesting all the aldehyde functions were accessible to aqueous reagents.

Introduction

Latex particles bearing aldehyde functions on their surfaces are potentially useful in a variety of applications in which grafting or coupling reactions are required. Syntheses of pure polyacrolein,[1,2] latex consisting of acrolein/styrene copolymers,[3−5] and core/shell polystyrene/polyacrolein latexes[6] have been described, and a surfactant-free core/shell polystyrene/polyacrolein product is commercially available, described below. Aqueous suspensions of such latex have potential use as protein carriers since reductive amination reactions involving basic amino acids should be effective in covalent coupling of protein to latex. These have application in immunodiagnostic assays, for instance, in which antibody or antigen molecules are coupled to the latex surface in order to avoid displacement by competing protein adsorption in complex analyte mixtures.

In order to control the chemistry of such reactions, to study the stability of the surface functionality, and to provide some understanding of the nature of the latex surface, it is desirable to be able to determine the surface concentration of aldehyde functions. A colorimetric assay based on reaction with dinitrophenylhydrazine (DNPH) has been employed for a styrene/acrolein copolymer latex[3,5] but the assay was not validated with standards. Conductometric titration based on release of HCl following exposure of latex to hydroxylamine hydrochloride has also been used to determine aldehyde functions[4] as has nitrogen analysis of the latex following the same raction.[1] In none of the above studies were the results compared to those determined with a second method. Since a clear variation of reactivity due to varying accessibility of the aldehyde groups has been noted,[4] the question of how reactive all surface aldehyde functions are on the latex, and indeed how surface groups should be defined for the polymers involved, is of some concern.

In the present work, we describe a semicontinuous synthesis of a surfactant-free polystyrene latex to which a shell of acrolein was added by addition of acrolein monomer once most of the styrene monomer had been consumed. The DNPH reaction was utilized to optimize the time of acrolein addition. Synthesis of 3-phenylpropanal hydrazone provided a suitable standard with which to calibrate the DNPH reaction, allowing its use quantitatively. A second assay was developed based on reduction of the latex with sodium boro[^{3}H]hydride ($NaBT_4$) and scintillation counting of the product. The reaction was carried out at a series of ratios of $NaBT_4$:$NaBH_4$ and an extrapolation procedure applied to allow estimation of the total number of aldehyde groups present.

X-ray photoelectron spectroscopy (XPS) was applied to show the presence of aldehyde functions on the surface of the beads, to confirm the decrease in their number but not elimination following reaction with $NaBH_4$ to demonstrate the presence of the nitrogen peaks associated with the DNPH complex on the latex surface. Finally, the latex was dissolved in THF-d_8 and ^{1}H NMR performed. A peak in the region of $\delta = 8.9-9.4$ ppm was integrated to provide an estimate of the aldehyde concentration. It was found that the peak shape and location in this region could be interpreted to distinguish oligomers of pure acrolein from styrene/acrolein copolymers. On a representative latex, the DNPH, borohydride, and NMR assays gave close agreement on the concentration of aldehyde functions present. Since the NMR measurement does not distinguish surface groups from groups in the interior of the latex bead, the agreement demonstrates that in this sample all the aldehyde functions were available for reaction from the continuous phase and hence could be considered surface groups.

Experimental Methods

Latex Synthesis. Styrene monomer (ACS grade, Fisher) and acrolein (Aldrich) were distilled under reduced pressure and stored under nitrogen at 4 °C if not used immediately. Potassium persulfate ($K_2S_2O_8$, B.D.H.) and NaCl (Fisher) were used without further purification. All water was distilled and then deionized using a Millipore Milli-Q ion exchange apparatus. Polystyrene latex was synthesized in the absence of surfactant as described,[17] with the aim of producing a latex of diameter approximately 650 nm. The reaction was carried out in a 500 mL round-bottomed flask with four necks and an overhead stirrer, the whole thermostated in a water bath. Sodium chloride, 0.109 g dissolved in 132 mL of water, was added to 16.9 g of styrene in the flask with stirring at 350 rpm. Nitrogen was bubbled gently for 10 min, and an addition funnel was put in place with 0.130 g of persulfate initiator dissolved in 10 mL of water and left to sit 15 min while the flask equilibrated at 70 °C. The initiator was then added and the funnel washed with 20 mL of degassed water, and the reaction (180 mL total) was allowed to proceed for 24 h. In most experiments, at some time after initiation, acrolein monomer (1.0 g in 10 mL of degassed water) was added to the reaction vessel, rinsed in with an additional 5 mL of water,

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[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1995.

and the reaction allowed to continue. In batch 78, 2.0 g of acrolein was added 10 h after initiation. In two other cases, two-stage reactions were run, using batch 68 as a seed for addition of a shell of acrolein. In each case, nitrogen was bubbled through 50 g of 3.3% w/w latex plus 3 g of acrolein. The flask was equilibrated at 50 °C, 38 mg of persulfate was added in 8 mL of water, and the system was allowed to react for 12 h (batch 85). For batch 86, the seed latex was allowed to swell with 1 g of styrene for 15 min before addition of 3 g of acrolein and subsequent initiation as above.

In all cases, upon completion the preparation was cooled and filtered through glass wool to remove any large aggregates. The latex was then dialyzed against 12 L of water changed every 24 h for a week followed by washing three times in water by centrifugation, and the dry weight of an aliquot was determined by drying to constant weight at 65 °C. Latex was stored at 4% w/w in 50 mL polypropylene tubes at 4 °C until used.

In several runs in which no acrolein was added, the reaction was monitored from 0.5 mL samples taken every hour, diluted 1:12 in methanol, the sample centrifuged to pellet the latex, the supernatant absorbance at 247 nm read (in some cases following dilution), and the soluble styrene concentration calculated from $\epsilon_{247} = 15\,290$ L/(mol cm), determined from dilutions of a standard.

Size Determination. Freeze-dried latex samples were sputter coated on graphite disks with 60:40 Au:Pd and examined at 15 000 magnification in a Hitachi S-2300 scanning electron microscope. Size distributions were determined manually from SEM images utilizing the internal magnification calibration of the instrument.

DNPH Analysis. Dinitrophenylhydrazine (Aldrich), ethanol, methanol, and sulfuric acid (ACS grade, Fisher) and tetrahydrofuran (THF) (BDH, spectroscopic grade) were used as supplied. A standard for the DNPH reaction, 3-propanal hydrazone, was synthesized as follows: 0.25 g of DNPH was dissolved in 5 mL of methanol, and 0.5 mL of sulfuric acid was added carefully. The solution was filtered, 0.2 g of 3-phenylpropanal was added, and the solution was filtered again and washed with methanol before crystallization from ethanol.[19] The measured extinction coefficient in THF was $\epsilon_{260} = 26\,760$ L/(mol cm), comparable to the literature value.[20] It has been reported[20] that the extinction coefficient of hydrazone complexes does not vary much among different types of aldehydes.

For the assay, 10 mL of a saturated solution of DNPH in ethanol was added to a 2 mL sample of 1% w/w latex suspension in a 15 mL polyethylene tube and mixed at room temperature for 24 h; the accompanying latex swelling facilitates subsequent dissolution but may change access to the reactive groups. The latex was washed in ethanol by centrifugation until the supernatant showed no UV absorbance above the blank and then dried under reduced pressure. A known amount was dissolved in THF, the absorbance measured at 360 nm, and the concentration of hydrazone (and hence aldehyde) calculated using the above extinction coefficient. In a few experiments, methanol was substituted for ethanol or the ratio of water:ethanol was made 1:1 or 3:1.

Radiolabeled Sodium Borohydride Assay. Sodium boro[^{3}H]hydride ($NaBT_4$) was purchased from Amersham Canada Ltd., Oakville, ON; batch 183 was 1 mg of solid with specific activity of 220 GBq mmol^{-1}. Atomlight scintillation cocktail was purchased from Dupont, Boston, MA. A Philips PW 4700 liquid scintillation counter was used for all scintillation counting.

The reactions were performed in a 1.5 mL Eppendorf centrifuge tubes; $NaBH_4$ and $NaBT_4$ in various ratios were mixed with latex in 200 μL of 0.1 M NaOH (to limit hydrolysis[21]) and 200 μL of ethanol added to swell the latex somewhat and perhaps improve access to the reactive groups. The tubes were mixed for 24 h at room temperature. The suspension was centrifuged, the supernatant removed, and the latex resuspended in 0.8 mL of water plus 0.1 mL of 0.1 M HCl to terminate the reaction. The latex was washed three times and then dissolved in 1.0 mL of THF, 10 mL of scintillation cocktail was added, and activity was counted on the β counter.

The data were analyzed by considering the following reaction:

$$BH_4^- + RCHO + H^+ + 3H_2O \rightleftharpoons RCH_2OH + H_3BO_3 + 3H_2$$

Let the concentrations of the above species at equilibrium be defined as follows: $B_T = [BT_4^-]$ (tritiated), $C_O = [RCHO]$, $C_{OH} = [RCTHOH]$ (labeled with tritium), $H = [H_3O^+]$, $B_O = [H_3BO_3]$, $A = (C_O + C_{OH})$ = total aldehyde concentration before reaction. The measured quantity is C_{OH}, the concentration of the reduction product which is tritiated during the reaction as shown. Assuming only tritiated borohydride is used, at equilibrium the equilibrium constant, K, is given by

$$K = \frac{C_{OH}B_O}{B_T C_O H} = \frac{C_{OH}B_O}{(A - C_{OH})B_T H}$$

Rearranging gives

$$\frac{C_{OH}}{B_T} = -(KH/B_O)C_{OH} + (KH/B_O)A \qquad (1)$$

Hence, a plot of (C_{OH}/B_T) vs C_{OH} allows a linear extrapolation to $(C_{OH}/B_T) = 0$, and the intercept on the C_{OH} axis gives A, the desired quantity. When mixtures of tritiated and cold borohydride are used to form B_T, if there is a significant isotope effect, it would be reflected in the value of K, potentially introducing some curvature into the plot. The extrapolated estimate for A should no be affected significantly, however. Also, strictly speaking, the concentration of borohydride referred to, B_T, is the concentration in equilibrium with the latex, not the amount added initially. However, under the reaction conditions employed, such a small amount of borohydride in fact reacted so that the added borohydride concentration is used with no significant error. If tritium is taken up by the latex by nonspecific adsorption, the plots will also appear curved but the initial slope, representing the strongest reaction mechanism, still can be extrapolated to obtain an estimate of the specifically reacting species, A.

X-ray Photoelectron Spectroscopy. Latex suspensions were freeze-dried, coated on one side of a double-sided adhesive, and mounted on an aluminum sample holder. The samples were introduced under continuous vacuum (2×10^{-10} mbar base pressure) into the analysis chamber of a Leybold MAX200 spectrometer. An unmonochromatized Al Kα (1486.6 eV) excitation source was operated at 10 kV and an X-ray current of 20 mA. The emitted photoelectrons were collected from a 2×4 mm^2 area.

Nuclear Magnetic Resonance. Tetrahydrofuran-d_8, 99.5 atom % D, was obtained from Aldrich. Freeze-dried latex was dissolved in THF-d_8 and the ^{1}H NMR spectra recorded on a Bruker WH 400 spectrometer at a proton frequency of 400.13 MHz.

Results

Latex Synthesis. Figure 1 shows the time dependence of the concentration of methanol-soluble styrene and styrene oligomers through the course of latex synthesis. Figure 2 shows the effect of adding acrolein either at the same time as styrene or after the times from initiation indicated, as measured by the aldehyde incorporation reported by the DNPH assay.

Size Distribution. Size distributions were only determined for the polystyrene latex made with no acrolein addition, batch 68, and the product obtained upon addition of the higher amount of acrolein 10 h after initiation, batch 78. Electron micrographs of the preparations represented in Figure 2 were taken and examined but not subjected to detailed size analysis. The estimates were for batch 68 diameter = (717 ± 153) nm, $n = 73$, and for batch 78 diameter = (706 ± 23) nm, $n = 59$.

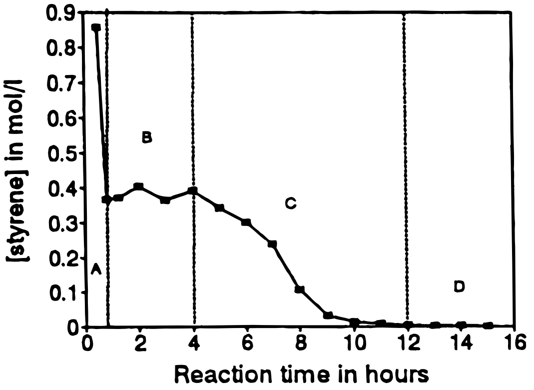

Figure 1. Time dependence of the amount of methanol-soluble styrene and styrene oligomers in the reaction mixture during the synthesis of surfactant-free polystyrene latex.

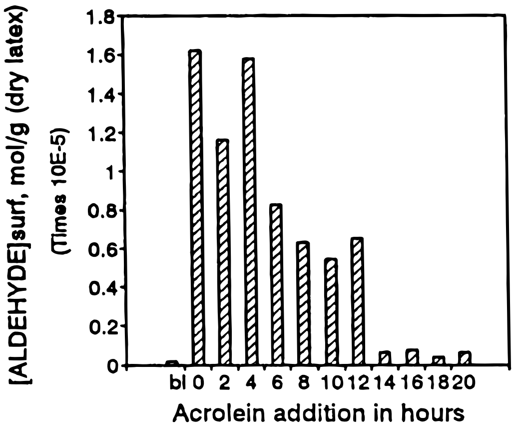

Figure 2. Amount of acrolein associated with the latex following addition of acrolein monomer at the times indicated; initiator added at time 0; acrolein incorporation determined from DNPH assay.

DNPH Assay. Aside from its use in monitoring the effects of acrolein addition, the DNPH assay was used to estimate the concentration of aldehyde on batches 78, 85, and 86. While the blank, batch 68, produced no detectable signal, batch 78 gave 2.2 × 10^{-5} mol/g of dry latex. This represents 1% of the added acrolein. The limit of sensitivity of the assay appeared to be near 2 × 10^{-7} mol/g of dry latex (data not shown). A commercial sample of polystyrene latex from Interfacial Dynamics Corp. (batch 2-286-97, diameter 604 ± 21 nm; designated IDC) bearing a surface shell containing acrolein was also examined. The DNPH assay gave 9.0 × 10^{-5} mol/g of dry latex for the IDC material. Using methanol as the DNPH solvent or varying the ratio of water to ethanol had no effect on the results.

Sodium Borohydride Reduction. A series of 20 mg samples of batch 78 were reduced with increasing concentrations of unlabeled $NaBH_4$, scaled up to 5 mL, following which the DNPH assay was performed; the results are shown in Figure 3. Since this figure shows evidence that reduction did occur, the tritiated borohydride reaction was carried out on 1 mg latex samples containing a constant amount of $NaBT_4$ (1 × 10^{-7} mol/g of latex) and varying concentrations of $NaBH_4$ ((0−1) × 10^{-2} mol/g of latex). The tritium incorporation was analyzed and the data plotted according to eq 1 (Figure 4). The same reaction was carried out on batch 68, in which significant low-affinity, nonspecific uptake of tritium by the beads was measured (data not shown). This is presumably the source of the nonlinearity seen in Figure 4, the lower slope at higher incorporation indicating a more weakly associating component. Extrapolation of the initial slope in Figure 4 gave an estimate for total aldehyde of 2.3 × 10^{-5} mol/g of latex.

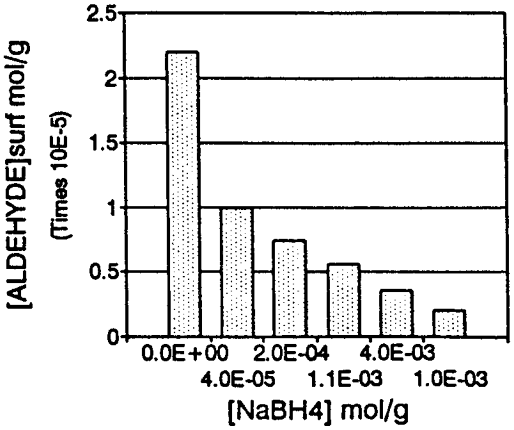

Figure 3. Aldehyde concentration associated with sample 78 following reduction with $NaBH_4$ of the amounts indicated (mol/g of latex); aldehyde assayed by the DNPH reaction.

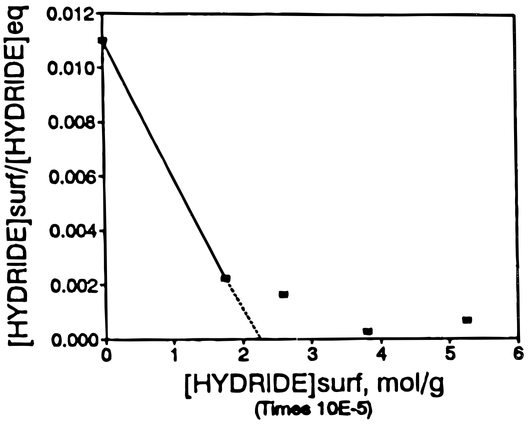

Figure 4. Analysis of the reduction of batch 78 with various ratios of tritiated to nontritiated $NaBH_4$ according to eq 1. Vertical axis: C_{OH}/B_T; horizontal axis: C_{OH}.

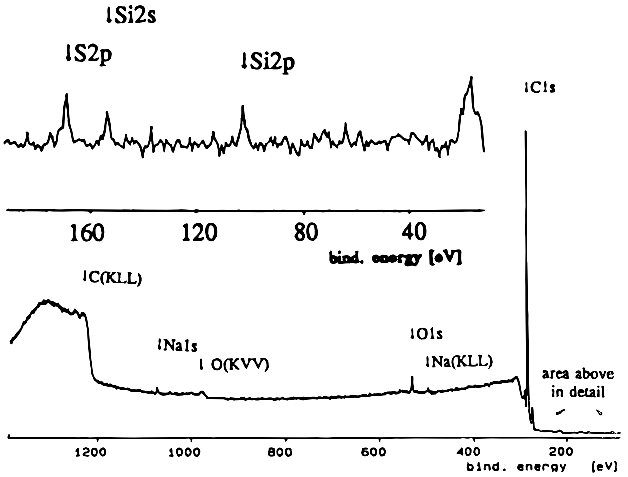

Figure 5. XPS spectrum of batch 68 polystyrene latex with no acrolein.

X-ray Photoelectron Spectroscopy. The XPS spectrum of batch 68, containing no acrolein, is shown in Figure 5. Carbon is the major detectable element, the C1s peak having the greatest intensity. The O1s, S2s, and S2p peaks derive from the sulfate group originating from the initiator. The Na1s likely comes from Na^+ counterions to the surface sulfate groups as NaCl is present in the synthesis recipe. The Si2s and

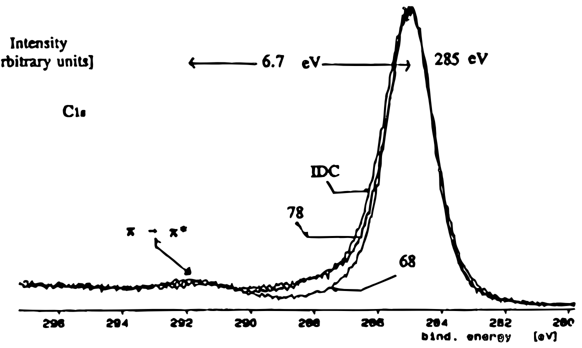

Figure 6. XPS spectrum of the C1s region for samples 68, 78, and IDC. The $\pi \rightarrow \pi^*$ shake-up peak is indicated.

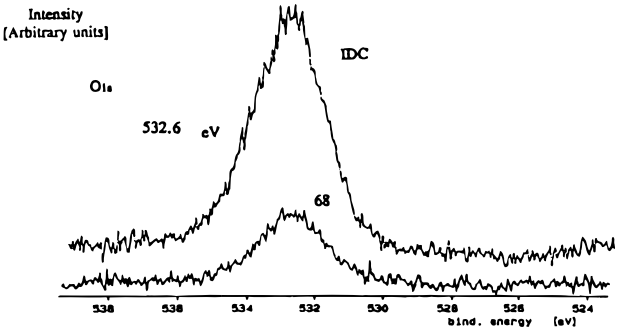

Figure 7. XPS spectra of samples 68 and IDC in the O1s region.

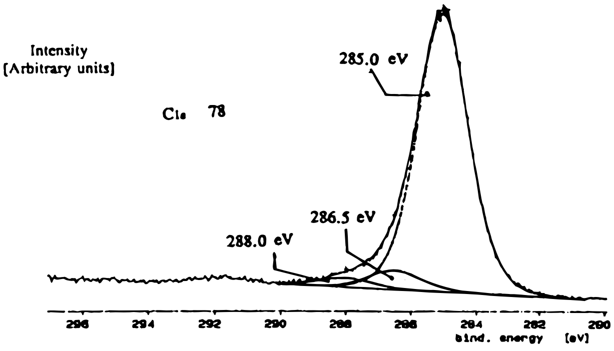

Figure 8. Decomposition of the C1s peak for sample 78 into three components.

Si2p peaks are likely impurities on the surface associated with handling and storage of samples under vacuum in glass containers.

Figure 6 shows the C1s region for batch 68, batch 78, and the IDC sample at high resolution. The peak at 292 eV is typical of an aromatic $\pi \rightarrow \pi^*$ shake up.[7] The carbons in the polystyrene chain all share a similar environment and produce a symmetrical C1s peak. However, addition of the carbon−oxygen double bond in the aldehyde shifts the bindng energy by 3 eV[8] to 288 eV as is apparent for samples 78 and IDC but not 68, in agreement with the observations of others.[3] In the oxygen region, the O1s peak is significantly increased for 78 and IDC compared at 68 due to the aldehyde oxygen (Figure 7). Figure 8 shows a decomposition of 78 into three curves, which gave the best fit. The major peak centered at 285.0 eV is due to the polystyrene, the 286.5 eV peak is due to C−O, probably associated with $C{-}OSO_3^-$ in this case, and the peak at 288 eV is due to C=O.

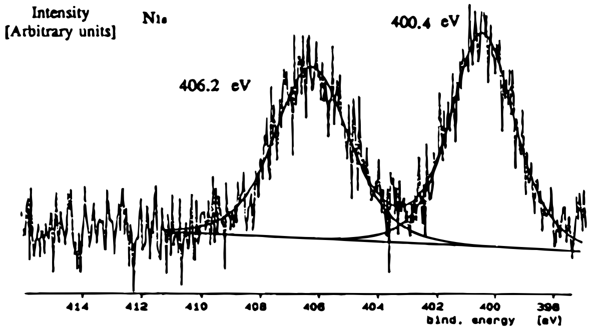

Figure 9. N1s region of the XPS spectrum of the IDC latex following reaction with DNPH.

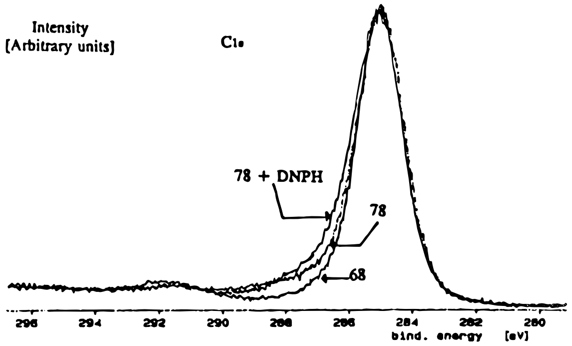

Figure 10. C1s region of the XPS spectrum of latex 78 following reaction with DNPH.

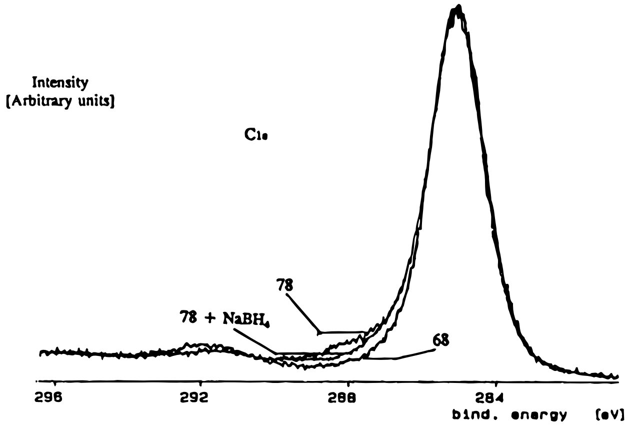

Figure 11. C1s region of the XPS spectrum of latex 78 following reaction with $NaBH_4$ (1×10^{-2} mol/g of latex).

The products of the aldehyde reagents could also readily be detected by XPS. DNPH reaction with the IDC sample produced the N1s peaks at 406.2 and 400.4 eV seen in Figure 9 due to the two nitrogens in the hydrazone complex, the former from the nitro group and the latter from the C=N bond. Batch 68 showed no nitrogen peaks (not shown). DNPH also broadens the C1s peak, particularly due to the $C{-}NO_2$ carbon, which produces a shift to 286.8 eV[8] (Figure 10). The partial reduction by $NaBH_4$ (1×10^{-2} mol/g of latex; Figure 3) is also seen by XPS as a decrease in the peak at 288 eV in the C1s spectrum, as shown in Figure 11. There was no evidence of acid groups in the C1s peak, which would have been expected at 289.5 eV.[8] Since OH groups would produce a C1s shift indistinguishable from the putative $C{-}OSO_3^-$ feature, it is not possible to assess their presence unequivocally on the surface.

Nuclear Magnetic Resonance. Figures 12 and 13 show the relevant portions of the proton NMR spectra of samples 78 and IDC. Broad peaks in the aliphatic region $\delta = 1{-}2$ ppm were present due to the aliphatic backbone of the polymer;[9] this region was not used in the present analysis. In this region, additional peaks due to THF ($\delta = 0.75$ and 3.6 ppm) and water trapped

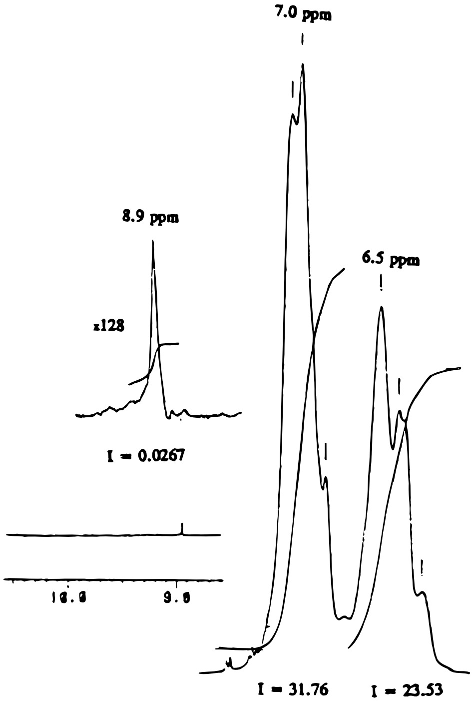

Figure 12. Aromatic and aldehyde portions of the ^{1}H NMR spectrum of sample 78 in THF-d_8 with the values of the integrals of the peaks indicated.

in the sample were also found. Two further groups of peaks in the region from 6.0 to 7.5 ppm were due to the five aromatic protons, the multiple peaks being those usually observed.[10] The aldehyde peak appears between 8.9 and 9.4 ppm, the exact value probably depending on the type of structure in which the acrolein occurs. An additional peak at 10.8 ppm was also present associated with the presence of water in the sample, possibly due to an interaction between THF and water.

The concentration of aldehyde groups per gram of latex was determined as follows. The mole ratio, R, of aldehyde to styrene is given by the ratio of the integral of the aldehyde peak to 1/5 of the integral of the aromatic region. Assuming the latex mass to be due essentially to styrene, the number of moles of styrene per gram of latex is 1/MW of styrene $= 9.6 \times 10^{-3}$ mol/g of latex. Hence, the aldehyde concentration per gram of latex is (R/MW of styrene) $= R/104$ g/mol. This approach gave for sample 78 an aldehyde concentration of 2.3×10^{-5} mol/g of latex and for the IDC sample 5.4 $\times\ 10^{-5}$ mol/g of latex.

The peak shape and location in the aldedhyde region varied depending on how the latex was synthesized. The semicontinuous reaction that produced batch 78 gave a reasonably narrow aldehyde peak at $\delta = 8.9$ ppm (Figure 12). The IDC sample showed a broader peak at $\delta = 9.0$ ppm. The NMR spectrum of batch 85 in which the acrolein shell was added in the absence of styrene monomer gave a reasonably sharp aldehyde peak at $\delta = 9.4$ ppm (Figure 14). When the identical synthesis was carried out except with latex preswollen with styrene before the acrolein shell was added (batch 86), two nonoverlapping aldehyde peaks were observed, one relatively sharp at 9.4 ppm and a broader peak of complex shape from 9.0 to 9.25 ppm (Figure 14). Finally, NMR of a sample of styrene/acrolein copolymer (3:1 feed ratio) showed a relatively broad peak from 9.0 to 9.3 ppm (not shown).

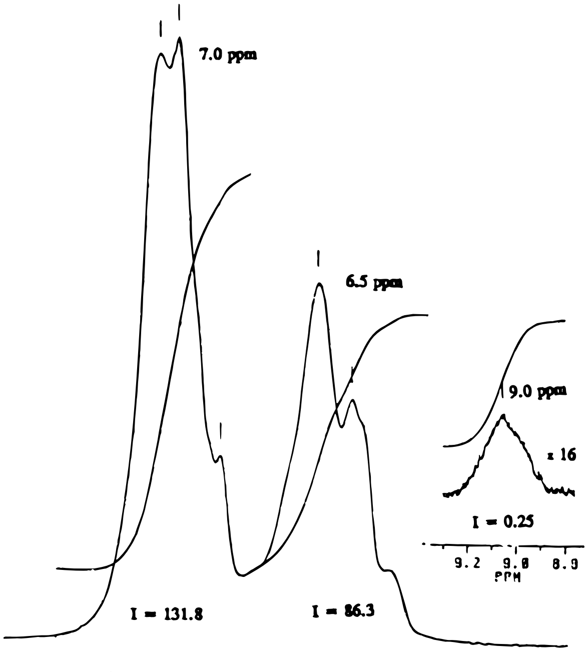

Figure 13. Aromatic and aldehyde portions of the ^{1}H NMR spectrum of the IDC sample in THF-d_8 with the values of the integrals of the peaks indicated.

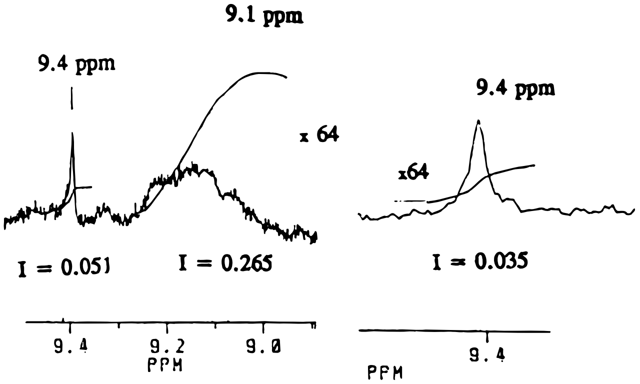

Figure 14. Aldehyde peak regions of the ^{1}H NMR spectra of samples 85 and 86 with the values of the integrals of the peaks indicated.

Discussion

One goal of this study was to develop a latex particle which had a useful concentration of aldehyde groups accessible from the aqueous phase and that could be quantitated to allow subsequent coupling and grafting reactions to be analyzed. Too copious a layer would likely leave many sites inaccessible and unreactive; too dilute a coating would render the particle less useful for subsequent reactions. We examined semicontinuous synthesis as a method by which to control the surface properties of the copolymer latex.

Latex Synthesis. The time course shown in Figure 1 is consistent with the general features of the contemporary theory of latex formation in surfactant-free systems.[11-13] Initially, oligomeric free radicals form and remain in solution until they undergo homogeneous nucleation to form unstable precursor particles which grow by coagulation and propagation until the aggregates attain sufficient charge density to become colloidally stable. Presumably, the sharp decrease in concentration of methanol-soluble material seen within the first hour represents the formation of the stable suspension.

For the next 3 h, the total concentration of styrene-containing material which is extracted into methanol and which does not centrifuge out of suspension remains essentially constant. This represents unreacted sytrene both in aqueous solution and dissolved in the growing polymer latexes as well as extractable styrene oligomers. While the monomer concentration must be falling, the oligomer concentration is increasing, apparently such that the sum of the extractable material remains essentially constant. As the reaction continues, monomer is exhausted and extractable oligomer concentrations decrease as their molecular weights increase until after 12 h virtually no styrene-containing material is extracted.

Addition of acrolein during the first 4 h produced poor products, either strongly aggregated (addition of acrolein with styrene) or with a very heterogeneous size distribution (4 h). Addition at later times produced lower incorporation of acrolein (Figure 2) but gave more monodisperse preparations. Even with addition at 12 h, where styrene monomer has almost all been used up, significant incorporation was achieved.

At this late stage in the reaction, the number of radicals per latex particle is believed to be higher than earlier in the process, due to increased viscosity and a lower diffusion rate for the higher molecular weight polymers, thus lowering their termination rate.[14] The higher number of radicals presumably initiates copolymerization with acrolein when it is added, a reaction which will then tend to continue at the interface due to the polarity of the aldehyde group. Since the acrolein monomer is water soluble, it will stay largely in the aqueous phase and react predominantly at the interface, producing surface-associated aldehyde functions. The copolymerization reactivity ratios ($r_{\mathrm{sty}} = 0.034$; $r_{\mathrm{acro}} = 0.32$[15]) are such that the fraction of styrene available must be less than 5% of the total monomer concentration to produce blocks of polyacrolein. If only the interfacial region is considered, this condition will be unlikely to occur until styrene monomer, concentrated within the drop due to its partition coefficient, has been almost totally exhausted. Hence, a copolymer might be expected to form at the interface as the predominant species.

Termination will occur in some cases by extinction by terminal styrene radicals to form copolymers and in other instances by combination with a second acrolein radical to form at least a pair of acroleins and possibly a region of homooligomer. It was decided on the basis of this study to add acrolein after 10 h of polymerization to optimize surface localization of the acrolein without approaching too closely the time at which no incorporation would be possible.

Aldehyde Concentration Estimates. One qualitative and three quantitative methods were examined in this work for their suitability in assaying functions in or on the latex. A summary of the measurements is given in Table 1. It is seen that for sample 78, made by semicontinuous synthesis with no addition of initiator after the beginning of the reaction, the three methods are in remarkable agreement at 2.3×10^{-5} mol/g of latex. The DNPH assay evidently works well for levels of aldehyde corresponding to 2×10^{-7} to 2×10^{-5} mol/g, as its background level was just below $2 \times 10^{-}17$ mol/g. At higher levels, the agreement with the NMR-derived values is less good, the colorimetric assay underestimating that determined by integrating the proton NMR peak in two of three cases (Table 1). Whether or not the NMR value is underestimated seems to depend on the environment of the aldehyde groups as reported by the location and shape of the aldehyde peak.

Table 1. Summary of Aldehyde Concentrations (mol/g of Dry Latex) Determined by DNPH, NaBT$_4$, and NMR

sample	DNPH	NaBT$_4$	NMR
68	1.8×10^{-7}[a]	0	0
78	2.2×10^{-5}	2.3×10^{-5}	$\delta = 8.9$: 2.3×10^{-5}
85	8.8×10^{-6}	nd[b]	$\delta = 9.4$: 3.0×10^{-5}
86	1.1×10^{-5}	nd	$\delta = 9.1$: 9.8×10^{-5} $\delta = 94$: 1.9×10^{-5}
IDC	9.0×10^{-5}	nd	$\delta = 9.0$: 5.4×10^{-5}

[a] Background signal. [b] Not determined.

The aldehyde peak generally was found either at $\delta = 8.9-9.2$ ppm or at 9.4 ppm. The latter case was observed when the acrolein was added in a second polymerization reaction run on dialyzed latex which must have had a very low if not zero styrene monomer concentration (sample 85; Figure 14). Hence, the acrolein was likely added as an adsorbed shell of homopolyacrolein, producing the peak at 9.4 ppm. This peak was also seen in the preparation in which the latex was preswollen with styrene monomer before acrolein and initiator were added (sample 86), but it was accompanied by a second peak at 9.1−9.2 ppm (Figure 14). The latter peak was similar to that observed with a styrene/acrolein copolymer synthesized under conditions to give an approximately alternating monomer sequence. Hence, we assign the peak at 9.4 ppm to a homopolymer environment dominated by acrolein−acrolein interactions and those seen below 9.3 ppm to aldehyde in a copolymer environment with frequent nearest-neighbor interactions with styrene.

This assignment implies that sample 78, synthesized semicontinuously, carries predominantly a copolymer on its surface, a conclusion consistent with the above discussion on its synthesis. It also implies that the IDC latex likewise carries a copolymer on its surface, although we have no specific information on the means by which it was synthesized.

Based on the above interpretation, it would seem that the DNPH assay is most successful with low to medium levels of aldehyde in the copolymer environment. It seriously underestimated the homopolymer aldehyde concentration of sample 85, for instance, probably because of crowding and the difficulty of reacting the relatively bulky DNPH group with each successive acrolein monomer in a homopolymer region. The disagreement between NMR and DNPH for the IDC sample suggests that the agreement among the three methods for sample 78 was to some degree fortuitous. For aldehydes in a copolymer environment, the DNPH assay would appear to be reliable to better than a factor of 2 but there seem to be details of the reaction which are still not understood. Light scattering from incompletely dissolved material appears to be a problem in some cases, for instance.

If the aldehyde concentration is sufficiently high, the NMR analysis is the most revealing and quantitative. However, its sensitivity is limited. A sample which gave a value of 1.8×10^{-6} mol/g in the DNPH assay produced no measurable aldehyde peak in the NMR spectrum, for instance.

The tritiated borohydride reaction was surprisingly inefficient and difficult to apply. In principle, it is worth pursuing because of the extreme sensitivity of the technique. In the present case, even when present in huge excess, the reduction did not go to completion. It therefore was necessary to utilize an extrapolation procedure, which was successful. However, the presence of nonspecific uptake by the latex in the absence of acrolein complicates the interpretation and reduces confidence in the answer. The method does have the advantage that it gives some idea, from the degree of linearity of the plot illustrated in Figure 4, of the spectrum of reactivities of the aldehyde functions since different effective equilibrium constants would produce different contributions to the slope.

The overall inefficiency of the reaction makes the assay quite expensive to use, since high intital specific activities are needed to produce a measurable signal. This inefficiency is likely due to electrostatic interactions between the negatively charged latex and the anionic borohydride. Particularly under the low ionic strength conditions employed to maximize colloidal stability, the surface potential, $\psi(0)$, would be expected to be large and negative. Hence the borohydride concentration at the surface should be much lower than the bulk concentration. The effect can be estimated from[16]

$$[BH_4^-]_{surf} = [BH_4^-]_{bulk} \exp(e\psi(0)/kT) \qquad (2)$$

Since $\psi(0) \ll 0$ for persulfate-initiated latex, the decrement at the surface would be expected to be considerable. We did not titrate the surface sulfate groups but a very similar recipe to that used has been reported to give a surface charge density of about 0.03 C/m^2.[17] The corresponding surface potential was calculated[16] at the highest ionic strength used here (50 mM). The value of the exponential factor in (2) calculated for these conditions was found to be

$$[BH_4^-]_{surf}/[BH_4^-]_{bulk} = 9.6 \times 10^{-5}$$

Hence, it is perhaps not surprising that the surface reduction did not proceed to completion.

Chemical Nature of the Latex Surface. The XPS results outlined above are completely consistent with expectation based on the chemistry performed. With the exception of Si peaks presumably derived from glass contact, there are no peaks evident that do not correspond to expected surface groups. The obvious presence of the aldehyde peak, the hydrazone components, and the partial reduction of the aldehyde all agree with expectation based on the results determined from the three quantitative assays discussed above. The percentage of the C1s signal that comes from aldehyde for sample 78 is 3.3%. The XPS signal originates from the outermost 30 Å or so in solid polymers[7] so the carbons being sampled include some material deeper than the most superficial layer. Hence, the above percentage would be expected to underestimate the true surface concentration.

Another estimate of the surface concentration of aldehyde is obtained from the data in Table 1. The concentration of 2.3×10^{-5} mol/g translates to an area per group of about 60 Å^2, and if a cross-sectional area of 24 Å^2 is taken for the aldehyde group (the value for an alcohol[18]), the implication is that approximately 24/60 = 40% of the surface would be occupied by aldehydes if they were uniformly distributed.

At this level of detail it is probably unwarranted to discuss surface structure near the interface further. It does seem as if all the aldehyde groups in batch 78 were accessible to aqueous reagents, however, and lie within the outermost 30 Å of the dry particle. The fact that the estimation from NMR peak integration for dissolved latex agreed well with the two wet chemical assays implies that all the groups were available to the reagents involved. Although these reactions were both carried out with the latex swollen to some degree with ethanol, the results did not depend on the ethanol concentration, implying that swelling was not a major determinant of the result. On consideration of all the above issues, it seems reasonable to describe the aldehyde functions as surface groups in these preparations.

Acknowledgment. We thank Bruce Gibb, Doina Hritçu, and Sarah Kerrigan for useful discussion. This work was supported by Grant MT 5759 to D.E.B. from the Medical Research Council of Canada and by grants for XPS to K.A.R.M. from the Natural Sciences and Engineering Research Council of Canada.

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MA950403X