SYNTHESIS AND CHARACTERIZATION OF CATIONIC LATEX BEARING GRAFTED CHAINS

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Abstract

Cationic poly(styrene) latices bearing grafted chains were synthesized by surface initiated polymerization of N(2-methoxy-ethyl) acrylamide (MEA) on core latex. The 2-3 \(\mu\)m diameter core particles were produced by successive seeded growth polymerization using a cationic azo-derivative as initiator. The area per charged group was found by conductometric titration to be between 150 and 300 \(\text{\AA}^2\).

The core latex was covered with a shell containing poly(styrene-co-acrolein) by a seeded copolymerization procedure. The surface aldehyde concentration was determined by reaction with hydroxylamine hydrochloride and titration. The surface aldehyde groups were used as starting points for Ce(IV) redox initiated polymerization of MEA. The amount of grafted MEA was estimated indirectly from unreacted monomer concentration. The maximum tether coverage, 9.9 MEA groups/\(\text{\AA}^2\), was obtained for an aldehyde surface concentration of 2.8 groups/\(\text{\AA}^2\). The probable chain conformation is a dense brush with an equilibrium length from 20 to 133 \(\text{\AA}\).

A model latex carrying cleavable chains was synthesized by using a shell containing poly(styrene-co-hydroxy-ethyl acrylate). The procedure for producing the shell and for grafting MEA was the same as for the aldehyde latex. The density of surface hydroxyl groups was assessed by saponification of the ester bonds, followed by conductometric titration of the carboxylic residues. In all experiments competing solution polymerization of MEA occurred due to oligomer leakage from the bead. The grafted chains were characterized by quantitative size exclusion chromatography (SEC) after saponification.
The molecular weight showed an unusual trimodal distribution. The surface density of the long chains was found to be between $4.4 \times 10^{-5}$ and $1.3 \times 10^{-4}$ chains/$\text{Å}^2$. Short and oligomeric chains had a density of about $6 \times 10^{-4}$ chains/$\text{Å}^2$. The probable configuration of each of these species is a mushroom, extending into solution to a maximum of 50 Å.

The beads carrying grafted chains were used as stationary phases for SEC of proteins. Experimental evidence for graft-mediated size exclusion, as predicted by two theoretical models, was provided, demonstrating that SEC can be performed efficiently with non-porous media.
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To Radu, Andrei and Fănel

The path

Lost in thought, hands behind my back,
I walk along the railroad tracks,
The straightest
Of all paths.

Behind me, full-throttle ahead,
There’s a train
That doesn’t know I’m out here.

The train,
My witness is Zeno the Elder,
Will never reach me,
Because I’ll always be a step ahead
Of things that can’t think.

Even if it brutally
Runs me over,
There’ll always be someone
Going along ahead,
Lost in thought,
Hands behind his back.

The way I am now
Before the black monster
That’s closing on me horribly quickly
But will nonetheless never reach me,
Never.

(Marin Sorescu)
CHAPTER 1: Introduction

1.1. THESIS OBJECTIVE

The goal of this work was to provide a synthetic surface that excludes and/or minimizes the adsorption of proteins. The use of this surface as a size exclusion medium for chromatography is demonstrated. The surface is obtained by grafting neutral flexible polymer chains onto a solid non-porous support by initiating polymerization from surface groups. The tethers are characterized with respect to molecular weight, conformation and surface density. The experimental results enhance understanding of the interaction between protein molecules and the grafted layer. This information is important for optimizing new synthetic surfaces to be used as chromatographic supports or as biocompatible materials.

Recent thermodynamic models using simple mean field (Brooks and Müller, 1996) and self consistent field calculations (Steels, 1998; Steels and Haynes, 1998) predict size dependent exclusion of macromolecules by terminally attached polymer chains grafted onto solid supports. This concept applied to size exclusion chromatography runs contrary to the long established belief that exclusion is due to the geometry dependent partition of the analyte between a continuous phase and the porous interior of a gel or cross-linked bead (Porath, 1963; Laurent and Killander, 1964). The porous nature of the chromatographic support has been considered indispensable for obtaining the size dependent exclusion effect in these classical theories. The second objective of this work
was to test the theoretical predictions of the newly developed models and provide experimental evidence for the polymer-brush mediated size-exclusion chromatography.

1.2. PROTEIN ADSORPTION TO SOLID SURFACES

The behavior of protein molecules at the solid/liquid interface is relevant to a variety of natural and synthetic processes. Controlled protein adsorption is a desired event in affinity and ion-exchange chromatographic separations, drug delivery systems, in vivo biosensors and immobilized-enzyme bioreactors. Unwanted protein adsorption occurs during plaque formation on teeth or restorative dental materials and fouling of food processing equipment or contact lenses. The biological response, particularly platelet interactions (platelets are the blood cells responsible for blocking leaks and initiating healing of damage to blood vessels) triggered by implant materials such as vascular grafts, catheters or artificial heart valves is also due to adsorption of plasma proteins on the biomaterial surface (Bamford and Al-Lamee, 1992). As a result, truly biocompatible materials have to be specifically designed to minimize non-specific protein adsorption (Ratner et al., 1993).

The spontaneous adsorption of proteins at the solid/liquid interface is a complex process, intensely investigated and only partially understood (Haynes and Norde, 1994; Norde and Lyklema, 1979; Andrade et al., 1992). Proteins are long chains of amino acids linked by peptide bonds. Their specific linear sequence of amino acids defines the primary structure. The side chains of the various (21) amino acids have different size, shape, charge and hydrogen bonding capacity. Some of them are acidic, while others are basic.
They also have variable degrees of polarity. The hydrophobic side chains prefer to be positioned in the interior, to minimize contact with water, while the charged groups are generally located near the periphery. Hydrogen bond donors and acceptors in the polypeptide main chain are also efficiently matched. These local arrangements make up the secondary structure of the protein molecule (e.g. α-helix). The overall three dimensional configuration of the protein molecule is called the tertiary structure. The process of folding in such an organized arrangement is entropically unfavorable, but this effect is compensated by favorable enthalpic interactions such as hydrophobic dehydration, dispersion forces, hydrogen bond and dipole forces (Haynes and Norde, 1994). The behavior of proteins at interfaces depends on the stability of their structure. Rigid proteins, which retain their spatial arrangement during adsorption, associate with the solid surface due to electrostatic interactions and partial dehydration of the sorbent. “Soft” proteins undergo structural rearrangements upon adsorption, driven mainly by an increase in conformational entropy (Norde, 1992; Fleer and Lyklema, 1988).

1.3. SURFACE MODIFICATION FOR REDUCING PROTEIN ADSORPTION

The nature of the synthetic surface with which the protein solution comes into contact is another important factor in the adsorption process. Properties such as polarity, surface charge density, acid-base character, surface heterogeneity and topography can influence the outcome of the interaction with protein solutions. Adsorption on neutral hydrophilic surfaces is expected to be weak because electrostatic and hydrophobic interactions are minimal (Andrade et al., 1992). Hydrophilic neutral cross-linked gels such as poly(acrylamide), poly(ethylene glycol) (PEG), poly(vinyl alcohol) and poly(N-vinyl
pirrolidone) show lower platelet adhesion than hydrophobic polymers (Kulik and Ikada, 1996), implying lower protein adsorption. Long flexible chains attached to the synthetic surface are also believed to minimize protein adsorption due to excluded volume effects, which tend to compensate the conformational entropy gain that adsorption would provide to the system (Gombotz et al., 1992; Bergström et al., 1992). Covalently bound crosslinked chains are found to be less efficient due to lower entropic stabilization (Bamford and Al-Lamee, 1992).

Poly(ethylene glycol) is the polymer which is used the most by far for modifying synthetic surfaces in order to minimize protein adsorption and platelet adhesion (Llanos and Sefton, 1993a). It can be incorporated as a block (Okkema et al., 1989) or a spacer (Park et al., 1988) or PEG acrylates and methacrylates can be used as macromers (Frere and Gramain, 1992; Maste et al., 1994) or as comonomers (Walton et al., 1997) to provide a protective coating on various synthetic surfaces. Synthetic networks obtained by polymerization of PEG monoacrylates using oligomeric glycerol-lactide triacrylate or trimethylolpropane triacrylate as cross-linkers can be used as scaffolds for tissue engineering or as cell-adhesion resistant surfaces (Keun Han and Hubbell, 1997; Drumheller and Hubbell, 1995). Covalent attachment of PEG with various terminal groups to activated surfaces (Gombotz et al., 1992; Gölander et al., 1992; Van Delden et al., 1996; Llanos and Sefton, 1993b; Bergström et al., 1992; Tseng and Park, 1992) reduced significantly the amount of protein adsorbed. High densities of the grafted chains are not generally achieved, however, probably because in good solvents bound chains progressively exclude mobile chains as the layer density builds up. The effect is stronger
for longer grafted chains. Grafting densities of $7.7 \times 10^{-4}$, $1.25 \times 10^{-3}$ and $1.8 \times 10^{-3}$ chains/Å² were reported for covalent attachment of amino-PEG with molecular weights of 3400, 1000 and 148 g/mole, respectively, to carboxylated polystyrene latices (Van Delden et al., 1996). By plasma deposition of tetraethylene glycol dimethyl ether on various supports, surfaces with a high density of short PEG-like grafted chains were prepared (Lopez et al., 1992). These surfaces had very good short-term fouling resistant properties.

An alternate method for obtaining surfaces with terminally attached chains is initiation of polymerization from a chemical group associated with the support. The reaction adds monomers preferentially to the surface and all the chains grow at the same time as opposed to the above procedures where the preformed polymer is bound to the surface by various chemical reactions. The advantage of this method is that in principle it can provide a more uniformly covered surface, with a higher density of grafted chains. Three procedures for initiation from surface groups are discussed in the literature:

1) Halo-isocyanates can be used to activate a surface containing hydroxyl, amino, carboxyl or amido groups. Metal carbonyls are added to the activated support, followed by thermal or photochemical initiation of a vinyl monomer polymerization (Bamford et al., 1987 and 1992; Bamford and Al-Lamee, 1992). The method has been used for polymerization of acrylic monomers on poly(ether-uretanes), dextran and PEG. PEG acrylates were grafted on PVC surface to improve haemocompatibility.

2) A chloromethylated surface is activated by reaction with tetraoxyethylene ether and potassium. An open crown ether forms on the surface, which can initiate anionic
polymerization of ethylene oxide. PEG chains of up to 3000 daltons were immobilized on poly(styrene) latex by this procedure (Bayer and Rapp, 1992).

3) Surfaces carrying hydroxyl, aldehyde, sulfhydryl or β-diketone groups in conjunction with Ce(IV) can initiate redox polymerization of vinyl monomers. Compared with the above methods, it has the advantage that the reactions can be performed in an aqueous medium. The method was first described by Mino and Kaizerman (1958). It is extensively used for grafting on natural (Vaquez et al., 1992; Wallace and Young, 1966; Iwakura et al., 1965; Athawale and Rathi, 1997; Okieimen et al., 1996 a, b, c and d) and synthetic surfaces (Kubota and Kuwabara, 1997; Panda and Singh, 1996).

Grafting of acrylamide on porous chromatography supports carrying hydroxyl groups on the surface has been used to produce localization of dextran-rich phases for liquid-liquid chromatography of proteins (Müller, 1986 and 1988). By the same procedure, N-(2-methoxy-ethyl acrylamide) was polymerized on hydroxylated chromatography supports to produce tentacle-type size exclusion gels, available commercially from Merck, Darmstadt (Müller, 1994). Acrylamide grafting on negatively charged poly(styrene) latex with aldehyde groups on the surface was also attempted (LeDissez and Brooks, personal communication). It was found that Ce(IV) adsorbed electrostatically to the surface, failing to initiate polymerization. The use of positively charged beads was suggested, in order to overcome this problem.
1.4. CONFORMATION OF THE TERMINALLY-ATTACHED CHAINS ON A SOLID SURFACE

Flexible polymer molecules in solution can adopt various conformations. Their structure can be described statistically, averaging over all possible conformations. In the ideal case where the chain is considered as a sequence of N randomly oriented bonds, each of length l, the random walk model (Kuhn, 1934), further developed by Flory (1953) predicts that the root-mean-square distance of the segments from the center of mass is given by:

\[ \langle R_g^2 \rangle = \frac{1}{6} l^2 N \]  

[1.1]  

where \( R_g \) = radius of gyration of the macromolecule (Å).

This simple equation can be improved to take into account more realistically the restrictions imposed by the fixed bond angles, the chain stiffness due to bulky substituents and the fact that the segments can not overlap (excluded volume effects). The result for real chains is the well-known equation (Flory, 1953; de Gennes, 1979, 1980):

\[ R_g \approx a N^\nu \]  

[1.2]  

where \( a \) = constant which depends on the nature of the polymer and the solvent (Å);  

\( \nu \) = constant which depends on the nature of the polymer/solvent interactions (between 0.5 in a theta solvent and 3/5 in a good solvent).
When one end of the polymer molecule is attached to an interface, distinct changes imposed by this constraint appear. The segment distribution depends on the distance between adjacent chains and on their length. Two limiting cases can be distinguished:

- If the distance between the grafting points is bigger than the radius of gyration of the polymer chains, a “mushroom” conformation is adopted. The height of the grafted layer, on a non-adsorbing surface, does not depend on the surface coverage and the isolated chains keep their unperturbed dimension given by equation [1.2] (de Gennes, 1980).

- If the distance between the grafting points is less than their radius of gyration, chain overlap occurs and the “brush” conformation is adopted. The chains stretch away from the surface as a result of a balance between unfavorable excluded volume interactions and the entropic penalty associated with chain stretching (Fleer et al., 1993; Milner et al., 1988; Rabin and Alexander, 1990). The equilibrium height of the grafted layer is proportional to the length of the chains and to $\sigma^{\frac{1}{3}}$, where $\sigma$ is the surface coverage (chains/unit area). The density profile within the brush as a function of the distance from the surface is still a subject of theoretical and experimental research (Kent et al., 1995; Baranowski and Whitmore, 1995; Uchida and Ikada, 1997).

1.5. SIZE EXCLUSION CHROMATOGRAPHY MODELS

Size exclusion chromatography is usually described as the partition of macromolecules, on the basis of their size, between a stagnant phase trapped in the pores of the support and the mobile phase. The volume of the pore which is accessible to the solute is limited
by its size, so larger macromolecules are expected to penetrate less and to have a shorter retention time than smaller ones (the larger the size, the more excluded the solute is from the matrix) (Porath, 1963; Laurent et al., 1964). However it is recognized that even materials with large pores, in which there is no physical barrier to penetration, will still exclude flexible macromolecules due to steric constraints imposed on the conformation of the solute approaching the pore walls, resulting in loss of conformational entropy of the macromolecule (Giddings et al., 1968). The thermodynamic theories describing SEC focus on calculating the partition coefficient of the solute between the bulk fluid and porous inert networks. The relationship between this parameter and a molecular property of the partitioning species (hydrodynamic radius, Stokes radius or radius of gyration), to make possible a “universal calibration”, is a subject of current investigation (Casassa et al., 1969; Casassa, 1976; Boyd et al., 1995; Busnel et al., 1996; le Maire et al., 1996). In all these models, the matrix is described as a network with rigid pores.

Recently, a “soft-body theory” of SEC (Potschka, 1996) was developed, in which the electrostatic and van-der-Waals interactions between solute and matrix were considered. The retention was defined as the probability of finding a molecule in the pores or adsorbed at the surface of the support, as a result of the energetic fields acting upon it. The existence of such “soft” interactions with the support is proven by the fact that they can be maximized under certain conditions. The “non-ideal” SEC used for certain separations takes advantage of such forces. At low ionic strength of the mobile phase, electrostatic interactions become stronger than the size dependence (Kopaciewicz et al., 1982). At high ionic strength, hydrophobic interactions with the matrix become significant (Herold,
Thus, the “hard-wall” models, which refer only to “ideal” exclusion based on geometric considerations, refer to a special case of SEC.

An alternate approach, which takes into consideration both enthalpic and entropic interactions between the solute and the matrix, is presented by Brooks and Müller (1996). In this model the matrix is treated as a macromolecular solution and the partition of the analyte between this phase and the solvent is determined by the free energy of mixing with the gel polymer. An advantage of this treatment is that pores and pore geometry are not involved (most pore models assume particular geometries in order to simplify the calculations). Rigid walls are not invoked and the contribution of the gel to the mixing entropy is explicitly taken into account in a more realistic manner. Also, the picture provides a natural description for partition of proteins within porous stationary phases which are substituted with hydrophilic polymers in order to minimize non-specific interactions. This model is presented below and its predictions will be compared with experimental data in Chapter 5.

The system under investigation consists of two phases, a mobile phase (denoted with the subscript “m”) and the gel phase (the stationary phase treated locally as a macromolecular solution; denoted with subscript “g”). The partition of the analyte between the two phases, at equilibrium, is required. The mean field theory of Flory and Huggins is used to calculate the Gibb’s free energy of mixing of solvent (component 1) with two polymers (component 2 is the gel and component 3 is the analyte) in a lattice (Flory, 1953):
where $n_i$ = number of molecules of component i on the lattice;

\[ \phi_i = \text{volume fraction of component i;} \]

\[ \phi_i = \frac{n_i P_i}{\sum_{i=1}^{3} n_i P_i} \]  \hspace{1cm} [1.4]

where $P_i$ = ratio of the volume of a molecule of polymer i to the volume of the solvent molecule; $P_1 = 1$

The size of one lattice site is assumed to be equal to the volume occupied by one molecule of solvent; $P_i$ represents therefore the number of lattice sites occupied by a polymer molecule.

$\chi_{ij}$ = Flory interaction parameter, defined by:

\[ \chi_{ij} = \frac{z \Delta \varepsilon_{ij}}{kT} \]  \hspace{1cm} [1.5]

where $z$ = lattice coordination number;

$\varepsilon_{ij}$ = interaction energy of an ij contact;

\[ \Delta \varepsilon_{ij} = \varepsilon_{ij} - (\varepsilon_{ii} + \varepsilon_{jj})/2 \]

$kT =$ Boltzmann’s constant x absolute temperature.
By differentiating the free energy of mixing with respect to \( n_3 \) at constant pressure, temperature and composition, an expression for the chemical potential of the analyte is derived:

\[
\frac{(\mu_3 - \mu_3^0)}{kT} = 1 - \phi_3 + \ln \phi_3 + P_3 \left[ -\phi_1 \phi_2 / P_2 - \chi_{13} (\phi_1 \phi_2 - \phi_1) + \chi_{23} \phi_2 \right]
\]  

[1.6]

where \((\mu_3 - \mu_3^0)\) = difference in chemical potential of component 3 between mixed and standard (pure) state;

The assumptions under which this expression is derived are: (i) the concentration of the analyte is low \((\phi_3 \ll 1)\) and (ii) the Flory parameter for the interaction of the analyte with the solvent is approximately the same as for the interaction of the gel with the solvent, i.e. \(\chi_{13} \approx \chi_{12}\). Expression [1.6] is then written for the mobile phase \((\phi_3^m = 0 )\) as well as for the gel phase. At equilibrium:

\[
\mu_3^m = \mu_3^g
\]  

[1.7]

Solving for the partition coefficient of the solute gives, retaining only first-order terms in concentration:

\[
K = \frac{\phi_3^g}{\phi_3^m} = \exp P_3 \left[ (\phi_1^g - \phi_1^m) (1 - \chi_{13}) + \phi_2^g (1 / P_2 - \chi_{23}) \right]
\]  

[1.8]

The expression can be further simplified by recognizing that, since \(\phi_3 \ll 1\), \(\phi_1^g \equiv 1 - \phi_2^g\) and \(\phi_1^m = 1 - \phi_3^m \equiv 1\), thus:

\[
K = \exp \left[ -P_3 \phi_2^g (1 - 1 / P_2 - \chi_{13} + \chi_{23}) \right]
\]  

[1.9]
Equation [1.9] may be used to predict the influence of various factors on the partition coefficient of the solute. These predictions will be referenced when analyzing the experimental data in Chapter 5.

The main potential disadvantage when applying the mean field approach to aqueous polymer solutions is that the strong hydrogen bonding of the solvent molecules is not taken into account. However, a similar treatment proved remarkably successful in predicting the features of protein partition in aqueous two-phase polymer systems (Brooks et al., 1985; Walter et al., 1991). An analogue of equation [1.9] was derived for the partition coefficient of a third polymer between the two phases formed when two incompatible polymers are mixed in water. The variations of $K$ with protein molecular weight, polymer concentration and polymer molecular weight were successfully predicted and confirmed by the experiments. The dependence of $K$ on molecular weight of the proteins given by [1.9] was tested by Brooks and Müller (1996) using four commercially available SEC supports. The data showed satisfactorily linear plots of $\ln K$ versus $M$, as predicted by the model. It should be noted, however, that the above expression applies only as long as both the analyte and the gel have approximately the same hydrophilicity (i.e. $\chi_3=\chi_{12}$). In this case, the error introduced by neglecting the solvent structuring effects is approximately the same for both components and tends to cancel out.

The interesting feature of this model is that it can explain the size-dependent exclusion effects of porous supports which have immobilized chains attached to their surfaces. It has been shown in the literature that a material with very large pores (geometrically accessible to solutes of virtually any size) derivatized on the surface with linear neutral polymer
chains, has improved performance compared to traditional size-exclusion media (Müller, 1994; Müller, personal communication). In this case, the "gel" phase refers to the grafted layer phase and the model can be used to predict the influence of the size and the density of the grafted chains upon the partition coefficient. It also implies that the size-excluding materials do not necessarily have to contain pores since only a phase possessing a mixing entropy barrier is required to produce size dependent exclusion effects.

A more quantitative picture of the analyte molecule interacting with the grafted layer is presented by Steels, 1998; Steels and Haynes, 1998. In these references the self consistent field (SCF) theory of Scheutjens and Fleer (1979, 1980) is further developed in cylindrical coordinates. The density distribution of the grafted chains within the brush is calculated. The energy of interaction required to move a particle into each layer of the grafted brush is obtained. The concentration of the solute in each layer with respect to the bulk concentration is calculated as the Boltzmann factor:

\[ C_p(z_p) = C_p^{bulk} \exp\left(-A^{int}_{(z_p)} / kT\right) \]  

where \( z_p \) = layer position in the lattice;

\( A^{int} \) = Helmholtz interaction energy, a function of \( z_p \).

The partition coefficient of the solute is calculated as the average concentration of solute within the brush limits (integral from \( z = 0 \) to \( z = h \); where \( h \) = brush height) over the concentration in the bulk phase, \( C_p^{bulk} \). The results show that the interaction energy is net repulsive and increases sharply with the particle size. The numerical model therefore
predicts size-dependent exclusion. The model forecasts also that the value of the partition
coefficient is lower when more dense and/or longer grafted chains are present on the
surface.

1.6. POLY(STYRENE) LATEX SYNTHESIS AND CHARACTERIZATION

Polystyrene beads are commonly used as model colloids, calibration standards, drug
carriers, in immunoassays, or, when crosslinked and synthesized with large pores, as
stationary phases for HPLC. All these applications take advantage of the narrow size
distribution and mechanical and chemical resistance of the polymer. The methods of
preparation include emulsion polymerization with surfactant (van der Hoff, 1958; Erickson
and Seidewand, 1981) or without (Goodwin et al., 1973, 1974 and 1979), successive
seeded emulsion polymerization (Chung-li et al., 1976; Kim et al., 1988), a two-step
swelling method (Ugelstad et al., 1980; Ugelstad, 1982) and dispersion polymerization
(Thomson et al., 1995). The methods involving surfactant offer certain advantages such as
the possibility of obtaining a wide range of sizes (0.2-200 μm) with very uniform size
distribution. Their main disadvantage is the laborious cleaning procedure required after the
synthesis. Surfactant-free synthesis methods, on the other hand, give a more limited range
of size (0.3-5 μm), but produce a relatively clean surface. For sizes up to 1 μm, a one step
synthesis is sufficient. In order to obtain larger particles, a seed polymerization procedure
is usually utilized.

For surfactant-free synthesis, utilized in this thesis, the initiator of choice has to contain
an ionic group in order to stabilize the particles in the absence of surfactant. Potassium
persulfate is by far the most common initiator used for this purpose. However, it carries the disadvantage that it can produce mixed groups on the surface (sulfate, hydroxyl and sometimes carboxyl) via oxidation reactions. An alternate choice is to use an azo initiator which contains an ionic group such as 2, 2’-Azobis (2-amidinopropane) dihydrochloride (ABA.2HCl). This has been used by Goodwin et al. (1979) to produce uniform cationic polystyrene latices with diameters up to 1 μm.

The methods of characterization of the latex particles include: conductometric titration (Vanderhoff, 1981; Blaakmeer and Fleer, 1989), colloidal stability determination, sedimentation behavior and suspension viscosity, scanning electron microscopy for particle size measurement (van den Hull and Vanderhoff, 1971) and electrophoretic mobility measurements (Ma et al., 1981).

1.7. THEORY OF SEEDED EMULSION POLYMERIZATION

The purpose of the theories of emulsion polymerization is to predict the number of latex particles which will be formed given certain experimental conditions. The nucleation stage is considered of central importance; its mechanism was in dispute for some time. The classical theory (Smith and Ewart, 1948) states that, in systems containing emulsifier, nucleation takes place only in monomer micelles, which absorb the radicals formed in the aqueous phase. Later, Fitch and Tsai (1971; 1981) introduced the concept of homogeneous nucleation, according to which radicals formed in the water phase continue to grow there until they precipitate due to their length (they become insoluble above a certain “critical” chain length). These precipitates form the primary particles. Their number
will continue to grow until the rate of formation of radicals in the aqueous phase equals
the rate of disappearance of radicals due to their capture into the preformed primary
particles. The emulsifier, if present, leads to increased stability of the primary particles,
preventing them from coagulation.

Hansen et al. (1978, 1979 a, b and c) developed further the model of homogeneous
nucleation in systems with or without added seed. Their concept is called limited
coagulation. When emulsifier is absent, the only stabilizing factor for the particles is their
surface charge, which originates from initiator residues. According to this model, the
primary particles are unstable due to insufficient charge. They therefore coagulate until the
charge density on the aggregate is big enough to maintain suspension stability. The total
number of particles goes through a maximum, after which it continuously decreases until a
constant value is reached. From this point on, the polymerization occurs only in the
preformed particles, leading to a monodisperse product. If a secondary nucleation takes
place, that is if new particles are formed at a later time, the resulting latex will display a
bimodal distribution (two populations: large particles from the primary nucleation and
small particles due to the secondary nucleation). In seeded polymerizations, the key factor
in obtaining monodisperse particles is the choice of experimental conditions to ensure that
all the primary particles coagulate with the seed, without forming a new population of
smaller size.

The above theory can be expressed quantitatively by considering the mechanisms by
which primary particles are produced and consumed during the reaction. The nucleation
rate is calculated as the difference between the rate of primary particle formation and the
rate of coagulation, either with each other or with seed particles:

\[
\frac{dN}{dt} = \frac{dN_1}{dt} - \rho_r - \rho_s
\]  \[1.11\]

where \( \frac{dN}{dt} \) = nucleation rate, s\(^{-1}\)dm\(^{-3}\);

\( \frac{dN_1}{dt} \) = rate of primary particles production, s\(^{-1}\)dm\(^{-3}\);

\( \rho_r \) = rate of coagulation of primary particles with each other, s\(^{-1}\)dm\(^{-3}\);

\( \rho_s \) = rate of coagulation of primary particles with seed particles, s\(^{-1}\)dm\(^{-3}\);

The first term in [1.11] is calculated, considering the mechanisms of radical production
and consumption, as the difference between initiation rate and the radical capture rate of
primary or seed particles:

\[
\frac{dN_1}{dt} = \frac{k_p M}{k_p M + k_c N + k_s N_s}
\]  \[1.12\]

where: \( k_p \) = polymerization propagation constant (\(-190 \text{ at } 50^\circ\text{C}\)), dm\(^3\)mole\(^{-1}\)s\(^{-1}\);

\( M \) = concentration of monomer in water (\(-4 \times 10^{-3}\)), mole dm\(^{-3}\);

\( N \) = number of primary particles, dm\(^3\);

\( N_s \) = number of seed particles, dm\(^3\);

\( k_c \) = rate constant for the capture of oligomers in primary particles, dm\(^3\)s\(^{-1}\);
\[ k_{cs} = \text{rate constant for the capture of oligomers in seed particles, } \text{dm}^3\text{s}^{-1}; \]
\[ \rho_i = \text{initiation rate, } \text{dm}^3\text{s}^{-1}. \]

Equation [1.12] can be further simplified by recognizing that \( k_c N + k_{cs} N \gg k_p M \).

Assuming that the absorption of the radicals into the seed particles is diffusion controlled (as suggested by previous experimental results) and irreversible, equation [1.12] can be written as:

\[ \frac{dN}{dt} = \rho_i \frac{k_p M}{4\pi D_w (N_r r_s + N r)} \quad [1.13] \]

where \( k_{cs} \) has been replaced with \( 4\pi D_w r_s \) and \( k_c \) with \( 4\pi D_w r_1 \). The following new symbols are used:

\[ D_w = \text{diffusion coefficient of the radicals in water, } \text{dm}^2\text{s}^{-1}; \]
\[ r_s = \text{radius of seed particles, } \text{dm}. \]
\[ r = \text{radius of primary particles, } \text{dm}. \]

In equation [1.11], the coagulation rate of the seed particles with each other is given by:

\[ \rho_f = k_f N^2 = \frac{16\pi D_1 r}{ W_{11}} N^2 \quad [1.14] \]

where \( D_1 = \text{diffusion coefficient of primary particles, } \text{dm}^2\text{s}^{-1}; \)
\[ W_{11} = \text{stability ratio for primary - primary particles.} \]

Also, \( \rho_{fs} \) can be expressed in terms of the number of seed and primary particles:
\[ \rho_{is} = k_s N_s = \frac{4\pi D_i r_s}{W_{is}} N_s N \]  

[1.15]

where \( W_{is} \) = stability ratio for primary - seed particles.

By replacing equations [1.13], [1.14] and [1.15] in equation [1.11], the following expression for the nucleation rate is obtained:

\[
\frac{dN}{dt} = \rho_i \frac{k_p M}{4\pi D_w (N_s r_s + N r)} - \frac{4\pi D_i}{W_{is}} N N_s r_s - \frac{16\pi D_i}{W_{is}} N^2 r 
\]  

[1.16]

Equation [1.16] describes the competition between primary and seed particles in the coagulation stage. In order to obtain a monodisperse product, primary particles shouldn't coagulate with themselves up to the point where they would have enough charge to become colloidally stable. Experimental conditions required to achieve this goal are suggested by further simplifications to [1.16]. When the number of seed particles is sufficiently high, they become dominant both in radical capture and coagulation (\( N_s r_s \gg N r \)) and therefore:

\[
\frac{dN}{dt} = \rho_i \frac{k_p M}{4\pi D_w N_s r_s} - \frac{4\pi D_i}{W_{is}} N N_s r_s 
\]  

[1.17]

Equation [1.17] can be integrated numerically if the variation of all parameters with time is known. In order to obtain an analytical expression, a steady-state approximation is made, which implies that \( dN/dt = 0 \) at a certain particle size. Solving for \( N \), an expression for the total number of particles is obtained:
Ignoring the constants, the result shows that the parameters that have to be balanced in order to obtain uniform particles are the initiation rate and the product \( N_s r_s \). This result will be used to analyze the experimental data in Chapter 2.

1.8. LATEX CARRYING ALDEHYDE GROUPS: SYNTHESIS AND CHARACTERIZATION

Latices carrying surface aldehyde groups are currently used in immunoassays, cell labeling, as drug carriers or as supports for affinity chromatography. All these areas of application take advantage of the reactivity of the aldehyde group with amino groups in the proteins (Margel et al., 1982; Slomkowski and Basinka, 1992; Slomkowski et al., 1994; Miksa and Slomkowski, 1995). The synthesis procedures include acrolein or glutaraldehyde polymerization by alkaline, redox initiation or Co irradiation (Margel et al., 1982; Margel, 1984). Surfactant-free emulsion copolymerization of mixtures of acrolein with styrene was also used to produce microspheres with aldehyde groups on the surface. The initiator of choice is potassium persulfate (Yan et al., 1990; Basinska et al., 1993). A semicontinuous method (acrolein was added at a particular time during the seed polymerization) was used by LeDissez et al. (1996) to add an aldehyde shell near the end of the polymerization reaction for a polystyrene latex, using potassium persulfate initiation.
Analysis methods for determination of the aldehyde group surface concentration include: reaction with m-aminophenol followed by fluorescence measurements (Chang et al., 1985), reaction with 1-aminopyrene or dinitrophenyl hydrazine (DNPH) followed by colorimetric analysis (Miksa and Slomkowski, 1995; LeDissez et al., 1996), reaction with hydroxylamine hydrochloride followed by nitrogen content analysis (Margel, 1982, 1984) or conductometric titration of the supernatant (Yan et al., 1990), X-ray photoelectron spectroscopy (semi-quantitative method) (LeDissez et al., 1996; Basinka et al., 1993), proton NMR and tritiated sodium borohydride reduction (LeDissez, 1996).

1.9. EMULSION COPOLYMERIZATION

Emulsion or suspension copolymerization is used for obtaining poly(styrene) latices with improved hydrophylicity and/or with certain surface groups. One example of such synthesis was described in the previous section for obtaining beads with aldehyde groups on the surface. Other monomers which have been included are: acrylamide (Ohtsuka et al., 1981), glycidyl methacrylate (Zurkova et al., 1983; Sumi et al., 1994), butyl methacrylate (Horak et al., 1995; Badran et al., 1997) and hydroxy-ethyl methacrylate (HEMA) (Tuncel et al., 1992).

The comonomer is usually soluble in water (e.g. acrolein, acrylamide, HEMA), while its homopolymer may be water soluble (e.g. poly(HEMA)) or not (e.g. poly(acrolein)). As emulsion copolymerization proceeds in a heterogeneous medium, the partition of the two monomers and their oligomers between the phases plays an important role in the outcome of the reaction. Complete analysis of all the phases in styrene/acrylonitrile
copolymerization (Guillot, 1985), lead to the conclusion that the composition of the monomer mixture in the particles is different from the composition in the emulsion as a whole. The reactivity ratios were found to have approximately the same value as for the bulk copolymerization. In order to control the composition of the product, an automated device was used, in which the composition of the various phases was continually monitored by gas chromatography and the feed adjusted during the synthesis (Guyot et al., 1981).

1.10 THESIS OVERVIEW

In Chapter 2 the synthesis and characterization of the core particles are described. Cationic surfactant-free polystyrene latex with a diameter of 0.8 um is prepared, then successive seeded growth polymerization used to increase its size to 2-3 um.

In Chapter 3 surface modification of the core latex is described. A shell containing poly(styrene-co-acrolein) is first introduced by a surfactant-free seeded copolymerization procedure. The surface aldehyde groups are then used as starting points for Ce(IV) redox polymerization of N(2-methoxy-ethyl-acrylamide) (MEA). The density of the grafted chains is estimated from the surface aldehyde concentration before grafting. The amount of grafted MEA is estimated from monomer conversion.

Chapter 4 records experiments in which the surface of the core latex is modified by introducing cleavable grafted chains. The procedure is the same as in Chapter 3, except for the comonomer used for the shell, which is 2-hydroxy-ethyl acrylate. Characterization of
the cleaved chains is carried out by quantitative size exclusion chromatography. A model for the grafted layer is developed.

In Chapter 5 the use of the grafted beads as size exclusion chromatography media is demonstrated. The results are used to test the predictions of two theoretical models.

Chapter 6 presents the summary of conclusions and ideas for future study.
CHAPTER 2: Synthesis and characterization of surfactant-free cationic poly(styrene) latex

2.1. INTRODUCTION

The objective of the first part of this project was to produce the core particles, with a uniform size of about 2-3 μm and a well characterized surface. The desired final diameter was chosen in a range where the product could be used as stationary phase for size exclusion chromatography.

Seed latex was first synthesized by surfactant-free emulsion polymerization of styrene using 2,2'-Azobis (2-amidinopropane) dihydrochloride (ABA.2HCl) as initiator, according to the method described by Goodwin et al. (1979). The selection of the initiator was determined by the need to obtain positively charged latices bearing only one kind of charged groups on the surface.

A seeded growth polymerization procedure was then used to increase the size of the particles to the required range (2-3 μm) in three steps. The synthesis conditions required for obtaining uniform product with high yield were optimized. Current theories regarding seeded polymerization of styrene in surfactant-free systems (Hansen et al., 1979 b) were used to determine the most important parameters: initiation and coagulation rates. The product was characterized with respect to size distribution and surface charge density.
2.2. EXPERIMENTAL

2.2.1. Materials

Once distilled water used was further purified using a Milli-Q Plus water purification system (Millipore Corp., Bedford, MA).

Styrene was purchased from Aldrich (Milwaukee, WIS), reagent grade material. It was purified by vacuum distillation at 40°C in an atmosphere of argon. Purified styrene was cooled upon collection using a dry ice-methanol bath, then stored under argon in the dark at -70°C. 2,2’-Azobis (2-amidinopropane) dihydrochloride was supplied by Wako Pure Chemical Industries Ltd. (Osaka, Japan) and was used without further purification. The commercial name for the initiator is V-50; its chemical formula is shown in Figure 2.1. Sodium chloride (Fisher Scientific, Fair Lawn, NJ) reagent grade was used without further purification. Standard sodium hydroxide and hydrochloric acid solutions used for titrations and the reagents used to prepare the buffers for electrophoretic mobility measurements were purchased from Fisher (Fair Lawn, NJ).

![Chemical structure of 2,2’-Azobis (2-amidinopropane) dihydrochloride](image)

**Figure 2.1: Cationic azo initiator used for seed production and growth**

2,2’- Azobis (2-amidinopropane) dihydrochloride (ABA.2HCl)
2.2.2. Preparation of seed latices

The method used was as described in the literature (Goodwin et al., 1979). Several recipes were tried; the one described produced the largest beads with the best reproducibility.

The apparatus consisted of a four-necked flask (1 dm³ capacity) equipped with an overhead stirrer, condenser, an additional side-arm funnel and an argon inlet with stopcock (the argon outlet was through the top of condenser). Argon flow was controlled by a needle valve and the stirring rate was monitored with a tachometer. The flask was maintained at a constant temperature by immersion to the neck in a thermostated water bath.

The following quantities of material were used:

0.72 dm³ water

0.872 g NaCl (2.07x10⁻² moles/l)

0.54 g ABA.2HCl (2.76x10⁻³ moles/l)

34.71 g styrene (0.44 moles/l)

Water (0.62 dm³) and the required amount of sodium chloride were placed in the flask, which was then evacuated eight times and flushed with argon under stirring (350rpm). The temperature of the bath was increased to 70°C, under stirring and slow argon flow (1 bubble/s). Styrene was added under argon protection. Five minutes later the initiator dissolved in 0.1 dm³ water, previously degassed and flushed with argon, was released into
the reaction mixture. The reaction was allowed to proceed for 24 hours, at 70°C, under argon flow, at 350 rpm stirring rate. The reaction mixture was then cooled to room temperature and filtered through glass wool to remove large aggregates. The product was dialyzed against distilled water for one week in a 10 l tank with daily changes. The latex was further cleaned by centrifugation and supernatant replacement (two times). The latex suspension was then weighed and solid content was determined by freeze drying. The yield was calculated, relative to the total amount of styrene used.

All the latex suspensions, after cleaning, were stored at 4°C, in polypropylene tubes, until future use.

2.2.3. Seeded polymerizations

The work described in this section was carried out in order to optimize the conditions for obtaining reasonably monodisperse polystyrene beads with a size in the range 1-3 μm using ABA.2HCl as initiator. The published work describing growth of surfactant-free latex utilizes potassium persulfate as initiator (Chung-li et al., 1976, Hansen et al., 1979b, Kim et al., 1988), but general theories have been developed and the most important factors influencing the reaction are known. Using these theories as a starting point, the following parameters in the seed growth steps were varied: temperature, seed content, styrene and initiator concentration, ionic strength, swelling time and reaction time. A typical recipe is described below:

The reaction vessel was the same design as described above, except the capacity was either 0.25 l or 0.5 l. The seed latex was weighed into the flask and the desired
concentration of solids was adjusted using distilled water. Sodium chloride was used in some experiments to achieve the requisite ionic strength. The apparatus was evacuated eight times and flushed with argon under stirring at 350 rpm, then the temperature was increased to the desired value. Styrene was added under argon protection. The seed latex was swollen under the same stirring regime and argon flow (1 bubble/s). Initiator was then added and the reaction continued for the prescribed time. The product was treated exactly as described above. In some reactions, if the product was aggregated, it was sonicated for 30 min before filtering through the glass wool. To prevent further aggregation or degradation, the sonication bath was cooled with ice.

Grown latexes were characterized with respect to distribution of size and solid content. The yield was calculated for the growth reactions. In all experiments except for 10G12 (100g/l), the concentration of styrene relative to total aqueous phase was 80g/l.

Detailed recipes for these steps are given in Table A.1 (Appendix).

2.2.4. Analysis methods

2.2.4.1. Size distribution of the latices

Size distributions were measured on Scanning Electron Microscope (SEM) images of the latex (one drop was dried on carbon plate, then coated with gold by evaporation in vacuum). An image analysis program (Image Tool, University of Texas, Houston) was used to measure at least 100 beads on several pictures taken in different spots on the plate.
2.2.4.2 **Analysis of the surface charge density**

The chemical reaction for seed production and growth is presented in Figure 2.2. The surface functional groups on the polystyrene beads derive from initiator residues only because the method used to synthesize them did not utilize emulsifier and there are no known side reactions that would provide ionogenic groups. They consist of positively charged amidine groups. The suspension stability of the latex results from the presence of these groups on the surface of the microspheres. For this reason surface charge density is an important characteristic of the product.

\[
\begin{align*}
    n \text{H}_2\text{C}=&\text{CH} + \text{ABA.2HCl} & \rightarrow \text{Bead}-\text{CH}=&\text{CH}_2-\text{R}^+ \\
\end{align*}
\]

Styrene \hspace{1cm} Initiator \hspace{1cm} Positively charged poly(styrene) bead

\[R^+ \text{ is the initiator residue:} \]

\[
\begin{array}{c}
\text{CH}_3\text{NH}_2 \\
\text{C} \quad \text{C} \quad \text{C} \\
\text{CH}_3\text{NH}_2
\end{array}
\]

**Figure 2.2 : Seed production and growth**

Emulsion polymerization of styrene using ABA.2HCl as a radical initiator.

Poly(styrene) beads are stabilized by the positive charge on the surface.

A conductometric titration technique was used to determine the concentration of surface functional groups. The method was as described in the literature (Vanderhoff *et al.*, 1970; Blaakmeer and Fleer, 1989), with the modification that the cleaning step, involving ion exchange resins, was omitted, because the beads were surfactant-free. The
samples (already dialyzed as described above) were prepared for titration only by one centrifugation and supernatant replacement. The solid content was determined by freeze drying and weighing. At least 0.5g of solid latex was suspended in 10 ml water, purged with argon for 5 minutes and placed in the conductometric cell, under slow argon flow. A conductivity meter (YSI Scientific Model 35) was used to monitor conductance, while 0.01M titrant (either HCl or NaOH) was added to the sample, using a precision pump (Harvard Apparatus, Infusion/Withdrawal pump, model 9322, South Natick, MA), under vigorous stirring. The constant of the conductivity cell was determined using potassium chloride conductivity standards (Bio-Rad Laboratories, Richmond, CA). At 25°C the value of the constant was found to be 0.9473 cm\(^{-1}\). Titrations were performed at constant flow rate (0.0204 ml/min.) and the elapsed time was monitored. For each sample, the equivalence point was found twice, once from direct titration using HCl and the second time from back titration using NaOH. The result is reported as the average for two samples. The area per charged group was calculated using the following equation:

\[
A = \frac{10^{16} \text{SSA}}{N_A (\text{Eq/g})}
\]  

[2.1]

where \(A\) = area per charged group, Å\(^2\)/charge;

\(\text{Eq/g}\) = surface charge concentration as equivalents per gram latex, g\(^{-1}\);

(amount of hydrochloric acid per gram latex at the end point);

\(\text{SSA}\) = specific surface area of the latex, cm\(^2\)/g;

\(N_A\) = Avogadro’s number, mole\(^{-1}\).
The specific surface area was calculated with the equation:

\[ SSA = 4\pi r^2 \frac{N}{1.05r} \]  \[2.2\]

where \( r \) = mean radius of the beads, from the size distribution histogram, cm;

\( N \) = number of particles per gram solid latex, g\(^{-1}\);

In [2.2] \( N \) has been replaced with \( 3/(4 \pi r^3 \rho) \) where \( \rho = 1.05 \text{ g/cm}^3 \) is the density of bulk polystyrene.

Both solutions used for titration were standardized by potentiometric titration. NaOH was first used to titrate a standard solution of potassium biphtalate (Anachema Science Inc., Montreal, Canada), then HCl was used to titrate the NaOH solution of known concentration.

The surface charge density was calculated with the equation:

\[ \sigma_0 = 1.6 \times 10^{-19} \frac{10^{20}}{A} \]  \[2.3\]

where \( \sigma_0 \) = surface charge density, C/m\(^2\);

A has the same meaning as in [2.1].

In equation [2.3], the elementary charge was used, \( e = 1.6 \times 10^{-19} \text{ C} \).
2.2.4.3 Electrophoretic mobility measurements

The electrophoretic mobilities of latex particles were determined in a Rank Mark I apparatus with a cylindrical chamber at 25°C in 1mM, 10 mM or 100 mM sodium chloride (pH = 7). All measurements were made with 40 volts applied and an electric field strength of approximately 3.6 V/cm. The electrical length and field strength were calculated from the currents measured with KCl solutions in the chamber (Seaman, 1975). The reported results are based on a minimum of two separate mobility determinations, each of which consisted of measuring the velocity of ten particles, alternating the direction of the applied field after each timing. The mobility was calculated from the averaged velocities, the applied voltage and the chamber electrical length, from:

\[
\mu = \frac{v}{l_E / V} \tag{2.4}
\]

where \( \mu \) = electrophoretic mobility, \( m^2/V \) s;
\( v \) = averaged velocity, \( m/s \);
\( l_E \) = the electrical length of the chamber, \( m \);
\( V \) = applied voltage.

The electrophoretic mobility was converted to zeta potential using the Smoluchowsky equation (Russel et al., 1989):

\[
\zeta = \frac{u \eta}{\varepsilon \varepsilon_0} \tag{2.5}
\]

where \( \eta \) = viscosity of the medium = 10^{-3} Ns/m² at 25°C;
\[\varepsilon = \text{dielectric constant of water} = 78.5 \text{ at } 25^\circ\text{C};\]
\( \varepsilon_0 = \text{permittivity constant} = 8.85 \times 10^{-12} \text{ C/(N m}^2). \)

Equation [2.5] is a good approximation when the particle radius is much bigger than the double layer thickness (i.e. \( \kappa a > 300 \), where \( 1/\kappa = \text{double layer thickness, } \AA; a = \text{particle radius, } \AA \)).

The surface charge density behind the electrokinetic shear plane was calculated with the Gouy-Chapman equation valid at “large” potentials (Russel et al., 1989):

\[
\sigma_\zeta = 2(2\varepsilon_0 kT n_b / \pi)^{1/2} \sinh \frac{z e \zeta}{2kT}
\]  \[2.6\]

where the following new symbols were used:

\( \sigma_\zeta = \text{surface charge density, C/m}^2; \)

\( k = \text{Boltzmann’s constant} = 1.38 \times 10^{-23} \text{ J/°K}; \)

\( T = \text{absolute temperature} = 298 \text{°K}; \)

\( z = \text{ion valence} = 1; \)

\( n_b = \text{number of ions of either sign per m}^3 \text{ of bulk solution}; \)

\[
n_b = 1000 c_b N_A
\]  \[2.7\]

where \( c_b = \text{molar concentration of the buffer, mole/l}; \)

\( N_A = \text{Avogadro’s constant} = 6.023 \times 10^{23} \text{ mole}^{-1}. \)
2.3. RESULTS AND DISCUSSION

2.3.1. Seed production

In Table 2.1 the characterization of the seed latices is presented. The yield of the seed production was between 53 and 71%, calculated with respect to the amount of styrene used. The recipe used was that due to Goodwin et al. (1979) describing preparation of cationic latices. This reference also presents an empirical formula which can be used to predict the size of the beads:

\[
\log D = 0.384 \left( \log \frac{[M]^{1.09} [I]}{[Am]^{0.833}} + \frac{2563}{T} \right) - 0.195
\]  

[2.8]

where: 

\( D \) = final diameter of the particles in nm;

\( [M] \) = initial monomer concentration, moles/l;

\( [I] \) = initial ionic strength, moles/l;

\( [Am] \) = initiator concentration, moles/l;

\( T \) = absolute temperature, K.

This formula predicts the diameter of the seed latex to be 0.557\( \mu \)m. The experimental value obtained by Goodwin et al. was 0.671\( \mu \)m for the same experimental conditions. The average value for seed diameter in this work was 0.817 \( \mu \)m ± 25%. Assuming pure components, the only variable parameter in the formula which could give such variations is the total ionic strength. The quality of the distilled water used for synthesis was probably slightly different from one experiment to another. Measurements done on the conductivity
of the water over one week yielded values between 1.03 and 4.25 $\mu\Omega^{-1}\text{cm}^{-1}$. At higher initial ionic strength it is more likely that the size of the beads would be higher. This could also explain the slight variation in the standard deviation of the size. Smaller particles were more uniform, as predicted in the paper for lower ionic strength conditions.

**TABLE 2.1: Characterization of seed latices**

<table>
<thead>
<tr>
<th>Seed #</th>
<th>Mean diameter $\mu$m</th>
<th>Standard deviation</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>0.83±0.5% n=115</td>
<td>0.048</td>
<td>70%</td>
</tr>
<tr>
<td>10</td>
<td>0.74±0.4% n=192</td>
<td>0.038</td>
<td>71.2%</td>
</tr>
<tr>
<td>11</td>
<td>0.73±0.4% n=160</td>
<td>0.038</td>
<td>70%</td>
</tr>
<tr>
<td>12</td>
<td>0.81±0.6% n=96</td>
<td>0.049</td>
<td>53.5%</td>
</tr>
<tr>
<td>13</td>
<td>1.02±0.5% n=115</td>
<td>0.051</td>
<td>62%</td>
</tr>
<tr>
<td>14</td>
<td>0.77±0.6% n=135</td>
<td>0.050</td>
<td>67%</td>
</tr>
</tbody>
</table>

In Figure 2.3, a SEM image and size distribution histogram of batch #14 are presented.

### 2.3.2. Seeded polymerization experiments

#### 2.3.2.1. First stage growth experiments

The results for first stage growth experiments are shown in Table A.2 (Appendix). The table gives the parameters of the synthesis in terms of rate of initiation, number of seed particles and their radius (initial and swollen radius) for all experiments. The following equations were used to calculate the parameters (Hansen et al., 1979b):
Figure 2.3: SEM image and size distribution histogram of batch #14.
\[ \rho_i = 2[I]k_dN_A \]  
\[ N_S = \frac{C_S}{m_S} \]  
\[ m_S = 1.05 \frac{4\pi r_S^3}{3} \]  
\[ r_{calc} = r_S \left( \frac{C_{Sty} + C_S}{C_S} \right)^{1/3} \]

where \( \rho_i \) = rate of initiation, dm\(^3\)s\(^{-1}\)

\( [I] \) = initiator concentration in the reaction mixture, moles dm\(^3\);

\( k_d \) = rate constant for the decomposition of the initiator, sec\(^{-1}\);

\( (8 \times 10^{-6} \text{ at } 50^\circ C, 3.9 \times 10^{-6} \text{ at } 45^\circ C \text{ and } 1.7 \times 10^{-6} \text{ at } 40^\circ C) \)

\( N_A \) = Avogadro's constant, moles\(^{-1}\);

\( N_S \) = number of seed particles per dm\(^3\) of reaction mixture, dm\(^{-3}\);

\( C_S \) = concentration of seed particles in the reaction mixture, g dm\(^{-3}\);

\( m_S \) = seed particle weight, g;

\( r_S \) = seed particle radius (mean value from size distribution histogram), cm;

\( C_{Sty} \) = concentration of styrene in the reaction mixture, g dm\(^{-3}\);

\( r_{calc} \) = predicted radius of the grown particle, cm;
Figure 2.4: SEM image and size distribution histogram of batch #14G1.
\( r_{\text{exp}} \) = radius of the grown particle, cm;

(mean value from size distribution histogram).

A SEM image and size distribution histogram of batch 14G1, obtained in the first growth step of seed 14, are presented in Figure 2.4.

The following parameters were studied and optimized for the first growth step:

1. **Swelling time**

As described in the experimental section, seed latexes were swollen with monomer prior to adding the initiator. Batches in series 9 and 10 had swelling times between 24 and 27 hours. The result of this approach was that some of the seed would separate into another phase, on the walls of the flask and on the stirring paddle. The density of styrene is 0.9 g/cm\(^3\) while bulk polystyrene density is 1.05 g/cm\(^3\). The monomer is infinitely soluble in the polymer. During such long swelling times, the swollen seed particles became lighter than the water and left the homogeneous suspension to form an upper layer in the reaction vessel (creaming). The separated beads had very little chance of contacting the radicals formed later in the aqueous phase, after addition of the initiator. As a result, the overall yield of the growing step was poor and at the end of the reaction, a glue-like material containing styrene was left on the walls of the flask. Experiments in series 11, 12, 13 and 14 were performed without swelling (monomer was added, left under stirring for 5 minutes to equilibrate the temperature, then initiator solution was released into the flask). This technique resulted in much better yields. The results are consistent with the homogeneous nucleation model for seeded polymerization. Primary particles formed in the
aqueous phase have to be in contact with seed particles in order to coagulate, contributing to the overall growth of the seed. It is essential to have a homogeneous suspension at the time this process begins, however.

2. Seed latex and initiator concentration

These parameters are discussed together because, as described in the model, the uniformity of the product is determined by competition between primary and seed particles during the coagulation stage. Rigorous application of the model developed by Hansen et al. (1979 b) would require determination of all the constants involved and a complicated mathematical treatment. In this study a more practical, qualitative approach was adopted. An attempt was made to balance the two most important competing dependences: the initiation rate and coagulation rate. With the optimum combination the initiation rate is slow enough to be compensated for by the coagulation rate between primary and seed particles, as suggested by equation [1.18] in Chapter 1. Qualitatively, a plot with $\rho_i$ (initiation rate) as one coordinate and $N_s r_s$ (number of seed particles multiplied by their radius) as the other exhibits the following features (Figure 2.5):

A. Low seed concentrations and high initiation rate.

In this region coagulation of primary particles with themselves is more frequent then their coagulation with seed particles. The product displays a bimodal distribution. Batches: 9G1, 9G11, 10G11, 11G11 and 11G111 are in this range.

B. High seed concentration and low initiation rate

In this region there are two possibilities:
- when the concentration of initiator is relatively low, many seed particles cream before primary particles can form (too low an initiation rate). They separate into a different phase. The latex obtained is uniform, but the yield is low (most of the styrene is concentrated in the glue-like layer and remains unpolymerized).

![Graph showing the relationship between initiation rate and number of seed particles](image)

**Figure 2.5: Optimization of experimental parameters for the first stage latex growth in terms of initiation rate and \(N_s r_s\) (number of seed particles \(\times\) seed radius)**

- when the concentration of initiator is higher than the creaming limit, but still not high enough to stabilize the beads at the desired size, some beads aggregate and the yield is

\[T = 50^\circ C, \text{ styrene concentration} = 80g/dm^3.\]
low because product is lost. This was the case in the synthesis of batch 11G1, which has the highest value of the parameter $N_{srS}$.

The optimum region obtained for the first step growth at 50°C and 80g/dm$^3$ styrene was in the following range of parameters:

- $\rho_s = 2.12 \times 10^{16}$ to $4.24 \times 10^{16}$ dm$^3$s$^{-1}$
- $N_{srS} = 2.86 \times 10^8$ to $5.04 \times 10^8$ dm$^2$.

3. **Temperature**

Experiments 14G11 and 14G111 were done at 45°C at a relatively low seed concentration (1.5% solids in the reaction mixture). An attempt was made to improve the ratio of styrene to solid particles, to make the first step more efficient (i.e. increase the particle diameter). To compensate for the low seed concentration, the initiation rate was reduced by performing the reaction at a lower temperature (the rate constant for decomposition of initiator is half its value at 50°C). In two experiments, done at two different initiator concentrations, seed particles creamed and no latex was obtained. At this temperature, initiation was too slow compared to the swelling rate of the seed particles. Optimum temperature therefore was taken as 50°C.

4. **Addition of salt**

Experiment 10G11 was done at a low initiator concentration, with sodium chloride ($6.6 \times 10^3$ M) added to increase ionic strength. The seed concentration was also low (high ratio of styrene to seed to promote growth). It was found that the parameter $N_{srS}$ was
outside the optimum range. The result was a latex with a bimodal distribution. The addition of $6.6 \times 10^{-3}$ M salt didn’t improve the coagulation of the primary particles with the seed. The first growing step was done optimally without addition of salt.

2.3.2.2. Second stage growth experiments

Latexes obtained successfully in the first growth step were used as seed in the second step, in order to increase size. The results are shown in Table A.3 (Appendix), where all the parameters have the same significance as above. A SEM image and size distribution histogram of batch 14G12, obtained in the second growth step of seed 14, are presented in Figure 2.6.

The following parameters were studied in the second-stage growing experiments:

1. Swelling time

Batches in series 9 and 10 were swollen for 15 hours prior to adding initiator. Some latex was obtained, with bimodal distribution. The yield was negative for all of them, that is the mass of product was less than the seed added. The explanation, as in the first step, is the creaming of seed before initiation. The seed being bigger than that used in the first step, it has larger surface and swollen volume, so the effect appears faster and phase separation is even more evident. The seed left in homogeneous suspension is insufficient to ensure uniform product. The styrene left forms a secondary population. The second step was performed optimally without swelling.
Figure 2.6: SEM image and size distribution histogram of batch #14G12.
2. **Seed latex and initiator concentration**

In the second growth step, the seed was larger than in the first step. This meant that, at the same solid concentration by weight, the number of seed particles was lower (in the range one half to one fourth compared to the first step experiments). Increasing the number of seed particles to get the same number per unit volume as before, would have made aggregation events more probable and also would have resulted in less efficient growth (at the same monomer concentration, the ratio between the weight of styrene and seed determines the predicted increase in size). When designing the experiments in this stage, conditions were sought which would ensure uniform product at the lowest possible seed concentration. One solution envisioned was to decrease the initiation rate (by decreasing the initiator concentration), at seed concentration just slightly higher than utilized previously. This was the case in series 11, 12 and 13. Some of the products aggregated during synthesis, others later upon storage or cleaning by centrifugation (batches 12G122, 13G12 and 13G122). Some others remained stable during cleaning procedures and were used in the following growth step. For these, the yield was generally low (from negative to 37%) because much of the product was lost by aggregation. There was only one exception, batch 12G12, which had a higher yield (65%), but required sonicating after synthesis and after each centrifugation. The behavior of the latexes obtained with low initiator concentration led to the conclusion that they lacked sufficient surface charge to be stabilized in suspension at their final size. The only source of charge on the surface being the initiator, it followed that its amount had to be increased in the recipe. Batches 14G12 and 14G122 had higher initiator concentration (twice and four
times, respectively); this produced better yield (66% and 72%) and increased bead stability. The results obtained in various parameters ranges are summarized in Figure 2.7.

Optimal conditions to perform this step occurred in the following region of the plot:

- $\rho_i = 1 \times 10^{16}$ to $4.3 \times 10^{16} \text{ dm}^{-3} \text{s}^{-1}$.
- $N_{SRS} = 1.8 \times 10^{13}$ to $4.3 \times 10^{13} \text{ dm}^{-2}$.

![Figure 2.7: Optimization of experimental parameters for the second stage latex growth in terms of initiation rate and $N_{SRS}$](image)

$T = 50^\circ \text{C}$, styrene concentration = 80g/dm$^3$.

The fact that the reaction can be performed successfully at lower number of seed particles per unit volume can be explained by considering the model developed by Hansen et al.
Larger particles have more surface per particle available for coagulation with water soluble oligomers, so fewer primary particles will be formed.

3. Addition of salt

Experiments in series 9 and 10 and also 11G12 were performed with addition of sodium chloride ($2.5 \times 10^{-3}$ to $2.4 \times 10^{-2}$ M) to increase ionic strength. All of them were unsuccessful due to stabilization of a secondary population of beads. Batches 11G12 and 11G122 were done using the same recipe, except the first one had salt added, while the second did not. In both cases, the resulting latex had insufficient surface charge density. The result was at higher ionic strength (batch 11G12), the product aggregated.

The second step growth was done optimally without addition of salt.

2.3.2.3. Third stage growth experiments

Latexes obtained in the second step had final diameter between 1.6 and 1.8 μm. To reach the desired size (between 2 and 3 μm), they were grown in a third stage. The results are presented in Table A.4 (Appendix). A SEM image and size distribution histogram of batch 14G1233 obtained in the third stage growth of seed 14 are shown in Figure 2.8. The following parameters were studied for the third growth stage:

1. Seed latex and initiator concentration

In Figure 2.9, the parameters for this step are plotted. All experiments were done in the same range of the parameter $N_r r_s$, using a low initiation rate (batches in series 11, 12 and 13) or a high initiation rate (batches in series 14). The low initiator concentration region
Figure 2.8: SEM image and size distribution histogram of batch #14G1233
was generally characterized by instability of the product due to low surface charge. The outcome ranged from no product (batch 13G123; all creamed) or product which aggregated later during cleaning procedures (batch 12G123) to stable latex with low yield (batch 11G123; yield 13.5%) or with good yield (batch 12G123; yield 57%), but with a strong tendency towards aggregation. The difference in the behavior of the product could originate from two features of the synthesis:

Figure 2.9: Optimization of experimental parameters for the third stage latex growth in terms of initiation rate and $N_{srS}$

$$T = 50^\circ C; \text{ styrene concentration} = 80g/dm^3.$$
- different latex batches initially had different surface characteristics because they were synthesized in different ways (during growth each step adds charge on the preexisting surface);

- this region in the parameter field lies on the edge of stability.

The high initiator concentration region gave consistent and reproducible results with respect to high yield and stability of the product. It was also characterized by a slightly broader size distribution of the resulting latex (batches 14G1233 and 14G1223).

2. Temperature

One trial was done at 40°C (batch 14G123). No product was obtained because of creaming. The optimum temperature again was found to be 50 °C.

2.3.2.4. Surface charge density of the core latices

Successful latexes obtained in the third growth stage were characterized by conductometric titration to determine the concentration of initiator residues on the surface, as described in the experimental section. The chemical reactions describing various stages of the titration are shown in Figure 2.10.

The shape of the conductometric curve is determined by the nature of the functional groups to be titrated and by the reagent used. Amidine residues on the surface of the latex are weakly basic (pKb ≥ 3.3). Before the titration starts, a low concentration of OH groups is associated with the latex due to the dissociation equilibrium in Figure 2.10a. As the titration proceeds, hydrochloric acid added reacts with the hydroxyl groups to give
water and Cl\(^-\) ions (Figure 2.10b), while the equilibrium is pushed to the right. As hydroxyl groups are consumed, more are produced through dissociation. During this stage of the titration, the conductivity remains constant because the mobility of OH\(^-\) ions which disappear is approximately the same as the mobility of the Cl\(^-\) ions added with the titrant.

\[
\begin{align*}
\text{a)} & \quad -\text{C}-\text{C}_2^+\text{NH}^- + \text{Cl}^- + \text{HOH} \rightleftharpoons K_b \quad -\text{C}-\text{C}_2^+\text{N} \text{H}_3^- + \text{Cl}^- + \text{OH}^- \\
\text{Dissociation equilibrium of amidine residues}
\end{align*}
\]

\[
\begin{align*}
\text{b)} & \quad \text{H}^+ + \text{Cl}^- + \text{OH}^- \rightarrow \text{H}_2\text{O} + \text{Cl}^- \\
\text{Direct titration of basic groups}
\end{align*}
\]

\[
\begin{align*}
\text{c)} & \quad \text{H}^+ + \text{Cl}^- + \text{OH}^- + \text{Na}^+ \rightarrow \text{H}_2\text{O} + \text{Na}^+ + \text{Cl}^- \\
\text{Back titration of excess HCl}
\end{align*}
\]

**Figure 2.10: Stages of the conductometric titration of amidine groups**

a) ions in solution before titration; b) ions in solution during the direct titration with HCl, before equivalence point; c) ions in solution during the back titration with NaOH, before equivalence point.

When all functional groups have been used, further addition of hydrochloric acid results in increased conductivity because free H\(^+\) ions have much higher mobility (ascending part of the titration curve). After completing the direct titration, the excess of hydrochloric acid is
Figure 2.11: Conductometric titration of functional surface groups

Titration of 0.776g beads (batch #14G1233): a) direct titration using HCl; b) back titration using NaOH, after 0.204 ml HCl have been added.
back titrated with sodium hydroxide. The conductivity of the solution decreases upon reacting acid with base (Figure 2.10c), until all excess acid is consumed. At this point, further addition of sodium hydroxide increases the conductivity of the solution (free electrolyte added). Using this technique, the equivalence point can be determined twice, once from direct titration and the second time (more precisely, because of the V-shape of the curve) from the back titration. Examples of the titration curves are given in Figure 2.11. The end-point is read at the intersection of the two linear portions of each graph (indicated by the arrow in the figure).

The characterization of the beads is given in Table 2.2. They are consistent with the behavior of the latex during cleaning. Batches in series 14 were more stable in suspension and they are seen to have a higher charge density. The results also support the supposition made earlier, when optimizing the conditions for seeded polymerizations, that a correlation exists between the latex stability and the amount of initiator used in the synthesis. Batches in series 14 were obtained at higher initiator concentration than latex 12G1233.

**TABLE 2.2: Surface properties of latices obtained after the third growth stage**

<table>
<thead>
<tr>
<th>Batch #</th>
<th>r</th>
<th>SSA/g cm²/g</th>
<th>Eq/g moles/g</th>
<th>Area/group Å²/group</th>
</tr>
</thead>
<tbody>
<tr>
<td>12G1233</td>
<td>1.15x10⁻⁴±1.2% n=152</td>
<td>2.48x10⁴</td>
<td>1.3x10⁻⁶</td>
<td>313.6</td>
</tr>
<tr>
<td>14G1233</td>
<td>1.35x10⁻⁴±0.8% n=191</td>
<td>2.12x10⁴</td>
<td>2.3x10⁻⁶</td>
<td>153.9</td>
</tr>
<tr>
<td>14G1223</td>
<td>1.38x10⁻⁴±0.8% n=194</td>
<td>2.07x10⁴</td>
<td>1.9x10⁻⁶</td>
<td>181.9</td>
</tr>
</tbody>
</table>
In Table 2.3 data from electrophoretic mobility measurements are presented. All investigated latexes were positively charged. The results show that the mobility and the zeta potential measured for each latex decreased with increasing the ionic strength of the buffer. The result is in agreement with previously published studies (Ottewill and Shaw, 1972). The maximum detected under some conditions in the zeta potential versus salt concentration curve in the referenced studies (Tuin et al., 1996; Ottewill and Shaw, 1972; Hidalgo Alvarez et al., 1986) was not encountered in the range of the experimental parameters investigated here.

In the last two columns of Table 2.3 the surface charge densities calculated from electrophoretic measurements ($\sigma_\zeta$) are compared with surface charge densities calculated from conductometric titrations ($\sigma_0$). For all latexes, $\sigma_\zeta$ is smaller than $\sigma_0$ by a factor varying between 5 and 20. This discrepancy is also reported in previous studies on both positively and negatively charged poly(styrene) beads (Hidalgo Alvarez et al., 1986; Tuin et al., 1996). The difference ($\sigma_0 - \sigma_\zeta$) decreases with increasing ionic strength. The effect is explained in the literature by the presence of a “hairy” layer of chains bearing a terminal charge on each, so the surface charge occupies a finite thickness. Within this thickness some counterions exist, reducing the effective net charge contributing to the mobility. At higher ionic strength the charges will tend to collapse, increasing the apparent charge density as seen by electrophoresis. It has been reported that the polymeric hairs can
be removed by heating the latex above the glass transition temperature (Seebergh and Berg, 1992).

**TABLE 2.3: Electrokinetic properties of latices obtained after the third growth stage**

<table>
<thead>
<tr>
<th>Batch #</th>
<th>[NaCl] mM/l</th>
<th>Mobility x10^8 m^2/volt-sec</th>
<th>Zeta potential volt</th>
<th>σ_ζ C/m^2</th>
<th>σ_0 C/m^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>12G1233</td>
<td>1</td>
<td>8.27±0.18</td>
<td>0.119</td>
<td>0.010</td>
<td>0.051</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5.08±0.16</td>
<td>0.073</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.66±0.11</td>
<td>0.038</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>14G1233</td>
<td>1</td>
<td>7.77±0.21</td>
<td>0.112</td>
<td>0.009</td>
<td>0.104</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6.11±0.12</td>
<td>0.088</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.95±0.16</td>
<td>0.042</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>14G1223</td>
<td>1</td>
<td>8.35±0.13</td>
<td>0.120</td>
<td>0.011</td>
<td>0.088</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5.21±0.15</td>
<td>0.075</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.98±0.16</td>
<td>0.043</td>
<td>0.020</td>
<td></td>
</tr>
</tbody>
</table>

1^) : measured in sodium chloride of the specified concentration, at pH=7.
2^) : calculated from electrophoretic mobility, using equation [2.6].
3^) : calculated from conductometric titration, using equation [2.3].

**2.4. CONCLUSIONS**

An emulsifier-free procedure for obtaining cationic polystyrene latices with size in the range 2-3 μm was developed. An azo derivative was used to initiate radical polymerization...
of styrene. The synthesis was done in four steps. Seed latex with a diameter of approximately 0.8 \( \mu \text{m} \) was first obtained according to a method described in the literature. The size of the initial latex was then increased in three seeded growth polymerization steps. It was found that, in order to obtain an uniform product with a high yield, the initiation rate had to balance the coagulation rate of primary particles with seed particles. The optimum range of the experimental conditions required to achieve this goal was determined for each step. The size of the final product was 2.3 to 2.8 \( \mu \text{m} \). The latex was characterized by SEM, conductometric titration and electrophoretic mobility measurements. The titration curves confirm that the latex contains only one kind of charged groups on the surface, i.e. weakly basic amidine groups. The area per charged group was found to be in the range 150 to 300 Å²/group.
CHAPTER 3: Synthesis and analysis of core/shell latex carrying Ce(IV)/aldehyde initiated terminally-attached chains

3.1. INTRODUCTION

The objective of this part of the project was to introduce non-ionic flexible polymer chains on the surface of the core latex. Ideally, these chains were to be anchored to the bead by covalent bonds at as high a density as possible. The monomer for the grafts synthesis was chosen to be N(2-methoxy-ethyl-acrylamide) (MEA).

The cationic polystyrene latices synthesized as described in Chapter 2 were first covered with a shell containing poly(styrene-co-acrolein) by a seed copolymerization procedure using ABA.2HCl as initiator. The aldehyde content of the shell was analyzed by proton NMR and an assay utilizing hydroxylamine, followed by conductometric titration. In the second step, grafted chains anchored to the surface were produced by polymerization of MEA in the presence of Ce(IV) as a redox initiator. Analysis of the resulting surface properties provided a simple model for the grafted layer.

3.2. EXPERIMENTAL

3.2.1. Materials

Surfactant-free cationic poly(styrene) latex used as starting material was synthesized as described in Chapter 2. Reagents and purification procedures were as described in Chapter 2 with following additions:
Acrolein was distilled at 56°C in an argon atmosphere in order to remove the inhibitor. It
was cooled upon collection using a dry ice-methanol bath. The purified acrolein was
stored at -70°C under argon, in the dark up to one month until use.

Cerium (IV) ammonium nitrate ((NH₄)₂Ce(NO₃)₆) reagent grade (98.5% purity),
hydroxylamine hydrochloride (99% purity) and ethylenediaminetetraacetic acid trisodium
salt hydrate (EDTA) were purchased from Aldrich (Milwaukee, WIS). The N-methoxy
ethyl acrylamide (MEA) was kindly supplied by Merck (Darmstadt) and was used without
further purification. The concentrated acetic and nitric acid solutions were purchased from
Fisher (Fair Lawn, NJ). Sodium sulfite reagent grade was purchased from Fisher (Fair
Lawn, NJ).

3.2.2. Acrolein/styrene copolymer shell preparation

A seeded growth copolymerization procedure was used to produce the shell on the
latex, according to the reaction shown in Figure 3.1. The apparatus was as described in
Chapter 2. Surfactant-free poly(styrene) latex was used as seed. A mixture of styrene and
acrolein was used to produce a shell around the poly(styrene) core. Detailed recipes are
given in Table A.5 (Appendix). All the concentrations are reported with respect to the
total volume of the reaction mixture.

Typically seed latex was charged in the flask, which was then evacuated and flushed
with argon eight times. The temperature was raised to 50°C, under gentle argon flow (one
bubble/s) and stirring at 350 rpm. Styrene was added and the seed allowed to swell for 15
min, then acrolein dissolved in 10 ml water was introduced, followed after 5 min by
initiator solution in 10 ml water. The reaction was continued at 50°C, under argon flow
and with stirring at 350 rpm, for 6 h. The reaction mixture was then cooled down to room temperature and filtered through glass wool to remove large aggregates. The product was cleaned by dialysis as described in Chapter 2. Further cleaning was done by centrifugation and supernatant replacement (three times). The latex suspension was then weighed and the solid content determined by freeze drying. All latex suspensions, after cleaning, were stored at 4°C in polypropylene tubes until future use.

\[
\text{Positively charged poly(styrene) bead} \quad \text{Acrolein} \quad \text{Styrene}
\]

\[
\text{Positively charged bead with poly(styrene-co-acrolein) shell}
\]

\[R^+\] is the initiator residue:

\[
\begin{align*}
&\text{CH}_3 \quad \text{NH}_2 \\
&\text{C} \quad \text{C}^+ \\
&\text{CH}_3 \quad \text{NH}_2
\end{align*}
\]

**Figure 3.1: Seeded copolymerization of styrene with acrolein**
3.2.3. Ce(IV) initiated MEA polymerization

The chemical reaction for this stage of the synthesis is shown in Figure 3.2. The apparatus consisted of a three-necked cylindrical flask (50 ml) equipped with magnetic stirrer, argon inlet and outlet with stopcocks, and rubber stopper. Detailed recipes are given in Table A.6 (Appendix); all concentrations were calculated with respect to the total volume of the reaction mixture.

Typically a latex suspension and MEA monomer were introduced to the reactor, which was then degassed and flushed with argon twice. The appropriate amount of cerium (IV) ammonium nitrate dissolved in 10 mM nitric acid solution (the volume was adjusted to give a final concentration of 1.82 mM nitric acid in the reaction mixture) was added through the rubber stopper from a syringe, previously filled with argon. The contents of the flask were mixed by hand, then stirred for one hour at 40°C. The reaction was continued for a given time at room temperature, under stirring and gentle argon flow. At the end of the reaction, a sample of the latex suspension was removed for monomer content analysis. The rest of the product was suspended in 250 ml water and cleaned either by filtration (Millipore membrane, 1.25μm) or by centrifugation. It was washed with 25 ml 0.1 M sodium sulfite solution in 0.1 M acetic acid (freshly prepared) and then with 50 ml 0.03 M EDTA and rewashed with water. The latex was resuspended in 25 ml water and stored in polypropylene tubes at 4°C.
Figure 3.2: Cerium (IV) initiated polymerization of N(2 methoxy-ethyl) acrylamide originating from aldehyde groups on the surface of the latex

3.2.4. Analysis methods

3.2.4.1. Aldehyde group content of the copolymer shell

3.2.4.1.1. Nuclear Magnetic Resonance

For the latexes with high aldehyde surface concentrations, proton NMR spectroscopy was used as described in the literature (LeDissez et al., 1996). The samples for NMR were prepared as described below:
A latex suspension containing approximately 30 mg solids was freeze dried in a glass vial for 24 hours, then the solid residue dissolved in 1 ml deuterated tetrahydrofuran (THF\textsubscript{d8}, atom 99% D, Aldrich, Milwaukee, WIS). Traces of water were removed from the solution by keeping it in contact with molecular sieves overnight. The sample was then transferred into an NMR tube, previously flushed with argon. A 400 Mhz Bruker spectrometer was used to record the spectrum.

An example of a proton NMR spectrum is shown in Figure 3.3. Two regions were used for calculations: the aromatic region around 7 ppm and the aldehyde region around 9 ppm. The first one represents the signal from the five protons in the aromatic ring of styrene and the second is the proton in the aldehyde function. The following equation was used to calculate the molar ratio between aromatic and aldehyde residues:

\[ R = \frac{\text{Ar}}{5 \text{Ald}} \]  \[3.1\]

where \( R \) = ratio between aromatic and aldehyde protons, moles styrene residues per mole aldehyde group;

\( \text{Ar} \) = integral of the aromatic peaks around 7 ppm;

\( \text{Ald} \) = integral of the aldehyde peak around 9 ppm.

The aldehyde concentration was calculated with the formula:

\[ \{-\text{CH} = \text{O}\} = \frac{1}{104 R} \]  \[3.2\]

where \(-\text{CH}=\text{O}\) = aldehyde concentration in the copolymer shell, mole/g latex.
Figure 3.3: Proton NMR spectrum of latex containing a poly(styrene-co-acrolein) shell

The molecular weight of styrene (104 g/mole) was used in [3.2] to convert the number of moles of latex to grams. The expected aldehyde content being small compared to the amount of polymer in the bead, the molecular weight of the whole bead is taken to be that due to styrene only.
3.2.4.1.2. Conductometric titration

Conductometric titration was used, as described in the literature (Yan et al., 1990), to determine the aldehyde content in the copolymer shell. This involves reacting the aldehyde groups with hydroxylamine hydrochloride according to the reaction shown in Figure 3.4.

\[ \text{H}_2\text{N-OH.HCl} + \text{R-CH=O} \rightarrow \text{R-CH=N-OH} + \text{HCl} + \text{H}_2\text{O} \]

Figure 3.4: Reaction of hydroxylamine hydrochloride with aldehyde groups in the copolymer shell

The hydrochloric acid produced is then titrated conductometrically with sodium hydroxide.

The experimental procedure is described below:

A 0.1M solution of hydroxylamine hydrochloride was prepared in a volumetric flask (M.W. = 69.5; 0.695g/100mL). A sample of latex suspension of known solid content was weighed in a glass vial (approximately 2 g suspension containing about 0.5 g solid particles), then an exact volume of hydroxylamine hydrochloride solution was added (0.1 to 0.3 ml). The mixture was left to react overnight, while tumbling in a rotating rack at room temperature. The suspension was then filtered through a membrane filter (0.22 μm pore size). The beads were washed two times on the filter with 2 ml aliquots of distilled water, which were added to the initial supernatant. The combined solution was diluted to 10 ml, purged with argon for 5 min, than transferred into the conductometric cell. The
titration proceeded under inert atmosphere (argon), using the experimental setup
described in Chapter 2. The titrant was 0.1 M sodium hydroxide solution, previously
standardized by potentiometric titration using potassium biphtalate. During the titration,
the time and the conductance were monitored at a constant flow rate (0.0102 ml/min). The
equivalence point was read at the first inflection point of the conductance versus titrant
volume plot. Aldehyde content of the sample was calculated with the formula:

\[
\{-\text{CH} = \text{O}\} = \frac{V_{\text{NaOH}} C_{\text{NaOH}}}{w} \quad [3.3]
\]

where \{-\text{CH}=\text{O}\} = \text{aldehyde concentration in the copolymer shell, mole/g latex;}

\[V_{\text{NaOH}} = \text{volume of titrant at equivalence point, ml;}
\]

\[C_{\text{NaOH}} = \text{concentration of titrant, mole/l;}
\]

\[w = \text{amount of solid latex in the sample.}
\]

Blank titrations were performed in the same way on the underivatized beads. The value for
the blank (typically equivalent to 10% of the aldehyde functions) was subtracted from the
value for the sample. Each titration was repeated twice; the result is reported as the
average. Equation [2.1] (Chapter 2) was used to calculate the area per aldehyde group
(where in this case, Eq/g is the aldehyde concentration \{-\text{CH}=\text{O}\} calculated as described
above). The size of the beads was assumed the same as for the seed latex because the
amount of polymer in the shell was small compared to the size of the bead. Applying
equation [2.7] to calculate the predicted radius using the amount of monomers and seed in
each copolymerization experiment, an increase in size between 5 and 10% is obtained for batches A2 to A10.

The latices covered with the copolymer shell were not analyzed to determine the surface concentration of the initiator groups because the amount of latex available was insufficient. It was assumed that only a relatively small number of groups was added during this step.

### 3.2.4.2. Analysis of MEA consumption during grafting

The amount of unreacted monomer was determined by HPLC analysis (performed on a Merck-Hitachi, Inert Version system, E. Merck, Darmstadt; Monitor L4200, Merck-Hitachi) of the supernatant separated from the reaction mixture by centrifugation and filtration. A 50x10 mm Superformance column (E. Merck, Darmstadt) packed with Merck Licrospher 60 RP-Select B beads (Merck, Darmstadt) was used for this analysis (3 ml/min; mobile phases: A was 0.1% TFA (trifluoroacetic acid, 99.9% purity, Fisher, Fair Lawn NJ) in water and B was 50% vol. acetonitrile in A; monitor at 240 nm; 20 μl sample). The sample was run on the column using a 50 min gradient program (100%A for 5 min; gradient B from 0 to 100% for 35 min.; 100%B for 5 min.; 100%A for 10 min.). Calibration runs were performed using known amounts of monomer dissolved in solvent A. The conversion was calculated with respect to the initial concentration.

### 3.2.4.3. Electrophoretic mobility measurements

The experimental procedure was the same as described in Chapter 2. The results were interpreted qualitatively only.
3.3. RESULTS AND DISCUSSION

3.3.1. Copolymer shell preparation and characterization

3.3.1.1. Shell characterization

In Table 3.1, data from copolymer shell analysis are presented. Two methods were used to determine the aldehyde group concentrations: proton NMR and hydroxylamine assay, followed by the conductometric titration of the supernatant. There is a difference of about one order of magnitude between the two sets of results. This discrepancy may be explained by two features of the methods:

1) NMR was done on a solution of polymer and therefore would be expected to detect all the aldehyde groups, while the conductometric titration detected only the groups on the intact latex accessible from bulk solution. Batches with high aldehyde content probably had more than one monolayer of copolymer incorporated, therefore not all the groups were accessible for the reaction with hydroxylamine. A crude estimate of the cross-sectional area for an aldehyde group (by assuming it equal to that of an alcohol) is 24 Å² (Davies and Rideal, 1961). Considering the specific surface area of the beads, an approximate concentration for a complete monolayer is 1.5x10⁻⁵ mole aldehyde per gram latex. All the values obtained by NMR analysis are higher than this limit, which suggests that buried groups were present possibly undetectable by the hydroxylamine assay. The area per aldehyde group shown in the last column of the table was calculated using the results of the conductometric titration. In the case of batches A2 and A3, it is clear that the equivalent of a full monolayer was present on the surface (the area is in the range of 20 Å²). For all the other batches the coverage is less, which makes it improbable that groups
are buried inside the bead (acrolein groups tend to stay on the surface of the latex due the presence of hydrogen bonds with water molecules). NMR analysis probably overestimates the results when the concentration is below $10^{-4}$ mole/g.

2) Low aldehyde concentrations are probably beyond the accuracy of the NMR method. The aldehyde peak located around 9 ppm on the NMR spectra (Figure 3.3) is broad, most likely due to the fact that the aldehyde group is located in various environments in a statistical copolymer. The integral is therefore approximate. The lowest limit of detection was $5.2 \times 10^{-5}$ moles/g ($1.6 \times 10^{-6}$ mole aldehyde per milliliter of polymer solution in THF) as calculated from the NMR spectrum. LeDissez et al. (1996) compared the NMR method with DNPH assay and found also that some low concentrations were not detectable by NMR. The lowest concentration they reported by NMR was $2 \times 10^{-5}$ mole/g. However, due to the different synthetic method used, which favored homopolymer formation (producing a single environment for the aldehyde residue), the aldehyde peak this concentration refers to was sharper, which explains the better sensitivity in their analysis.

The purpose of this step in the synthesis was to introduce a useful concentration of aldehyde groups on the surface of the latex in order to provide efficient initiation of grafting in the second step. Ideally these groups should be uniformly distributed on the surface and entirely accessible from the water phase, which meant that coverage of less than a monolayer was needed. NMR analysis proved not accurate enough for concentrations in this range.
Table 3.1: Characterization of the aldehyde/styrene copolymer shell

<table>
<thead>
<tr>
<th>Batch #</th>
<th>{-CH=O} NMR moles/g latex</th>
<th>{-CH=O} titration moles/g latex</th>
<th>Area per aldehyde group $\AA^2$/group</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>creamed</td>
</tr>
<tr>
<td>A2</td>
<td>$2.7 \times 10^{-4}$</td>
<td>$1.8 \times 10^{-5}$ (1.9$ \times 10^{-5}$)</td>
<td>22.3</td>
<td>bimodal</td>
</tr>
<tr>
<td>A3</td>
<td>$2.9 \times 10^{-4}$</td>
<td>$1.4 \times 10^{-5}$ (1.5$ \times 10^{-5}$)</td>
<td>28.7</td>
<td>some aggregates</td>
</tr>
<tr>
<td>A4</td>
<td>No peak</td>
<td>NA</td>
<td>NA</td>
<td>aggregated</td>
</tr>
<tr>
<td>A5</td>
<td>No peak</td>
<td>NA</td>
<td>NA</td>
<td>aggregated</td>
</tr>
<tr>
<td>A6</td>
<td>$5.3 \times 10^{-5}$</td>
<td>$4.1 \times 10^{-6}$ (6.4$ \times 10^{-6}$)</td>
<td>86.6</td>
<td>stable</td>
</tr>
<tr>
<td>A7</td>
<td>No peak</td>
<td>0</td>
<td>0</td>
<td>stable</td>
</tr>
<tr>
<td>A8</td>
<td>$8.5 \times 10^{-5}$</td>
<td>$1.0 \times 10^{-5}$ (1.2$ \times 10^{-5}$)</td>
<td>35.1</td>
<td>stable</td>
</tr>
<tr>
<td>A9</td>
<td>NA</td>
<td>$4.1 \times 10^{-6}$ (6.4$ \times 10^{-6}$)</td>
<td>86.6</td>
<td>stable</td>
</tr>
<tr>
<td>A10</td>
<td>NA</td>
<td>$7.1 \times 10^{-6}$ (9.0$ \times 10^{-6}$)</td>
<td>48.4</td>
<td>stable</td>
</tr>
</tbody>
</table>

1): The first number is the result of the conductometric titration of the supernatant after the reaction with hydroxylamine hydrochloride. The number in the bracket includes the basic initiator residue concentration determined by conductometric titration of the corresponding parent latex (Chapter 2, Table 2.2).

2): The area per group corresponding to the value for aldehyde concentration determined by reaction with hydroxylamine hydrochloride and titration (first number in the previous column).

In Figure 3.5 an example of a conductometric titration curve is shown. In the first stage sodium hydroxide reacts with free hydrochloric acid and the conductivity of the solution decreases as a result of replacing mobile hydrogen ions with sodium ions. After all the free hydrochloric acid has been reacted (first inflection on the curve), the conductivity remains constant in the second stage because hydroxyl ions which come with the titrant now replace chloride ions associated with the hydroxylamine molecules. In the third stage, the
conductivity increases when the sodium hydroxide added remains free in solution. From the second inflection on the curve, the concentration of hydroxylamine hydrochloride can be read, providing evidence that all the concentrations were correctly estimated.

![Titration curve for aldehyde groups analysis](image)

**Figure 3.5: Titration curve for aldehyde groups analysis**
Latex A10 (0.561 g), reacted with 1.2x10^5 moles hydroxylamine hydrochloride

The uncertainty of the method can be assessed by considering the biggest error, which is the reading of the equivalence volume from the curve. The minimum volume which can be read precisely is about 5x10^{-3} ml. Considering the concentration of the titrant (0.1 M) and the average weight of the sample (0.5 g), the uncertainty in the analysis is estimated to be 1x10^{-6} aldehyde groups per gram solid latex. This value represents 5 to 25% of the measured concentration, depending on its value. Two measurements done on the same
sample didn’t actually differ by more than 5 to 10%. A better precision could theoretically be achieved by using a more diluted titrant or by adding it at a lower flow rate. Practically, however, a more diluted titrant means smaller variation in conductivity, which flattens the curve. Also, the flow rate chosen for the analysis is the lowest achievable with the pump we used. Using more solid latex for each analysis was another option, but the amount available was limited. Comparison with the data in the literature (Yan et al., 1990) shows that the aldehyde concentrations for which the method was used in this reference were in a much higher range. The study describes analysis of smaller beads (0.3 μm) for which the aldehyde concentration was about 1 to 6×10^4 mole/g. Smaller beads have the advantage of a larger specific surface area, yielding a higher surface concentration and aldehyde content in mole per gram solid particles.

The absolute value of the aldehyde concentration determined by the hydroxylamine assay presents one more uncertainty, however. The method described in the literature (Yan et al., 1990) was used on beads prepared by initiation with potassium persulfate. These particles carry acid groups on the surface, due to the presence of initiator residues. In the present study, the core latex was prepared using a cationic initiator which leaves basic residues on the surface. As described in Chapter 2, these groups were analyzed by titration with hydrochloric acid. It is possible, therefore, that some of the hydrochloric acid produced upon reacting the latex with hydroxylamine hydrochloride was consumed by the basic initiator residues. An alternate approach in order to avoid this interference would be to analyze the beads for nitrogen content after producing the oxime (Margel, 1984; Margel et al., 1982). The fact that the initiator used for copolymerization in our study
contains nitrogen makes this approach difficult, however. An estimate of the amount of hydrochloric acid consumed by the initiator residues is given by the result of the conductometric titration for determination of the charge density on the core latex (Chapter 2). Assuming that the initiator groups added by copolymerization do not significantly add to those already present on the surface of the core latex (supported by the electrophoretic mobility measurements to be discussed), it is possible to estimate the aldehyde content by adding the amount given by titration of basic groups on the core particle to the amount determined by the hydroxylamine assay. These results are reported in the bracket in the third column of Table 3.1.

The concentrations estimated by this method are 5 to 30% higher than the results of the hydroxylamine method alone. However, the calculation involves the assumption that all the initiator residues are accessible to hydrochloric acid from bulk solution. Due to the presence of the aldehyde groups on the surface, some of the initiator residues might in fact be sterically inaccessible, depending on the copolymer sequence and conformation adopted on the surface, which is unknown. Due to these uncertainties, the first result was adopted and used in further calculations.

In conclusion, the hydroxylamine assay proved more precise than NMR for determining the surface aldehyde concentrations. At a coverage of less than a monolayer on the surface of beads with a diameter of 2.3 to 2.7 μm, the method has an average uncertainty of 1 to 2x10⁻⁶ mole per gram solid latex.
In Table 3.2 the results of the electrophoretic measurements performed on latex in various stages of synthesis are shown. All core latexes were positively charged. Batch #14G1233 had the highest mobility, which is in agreement with the highest surface charge.

Table 3.2: Electrophoretic mobility of latex in various synthesis stages

<table>
<thead>
<tr>
<th>Synthesis stage</th>
<th>Mobility x10^8 m^2/volt-sec</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Seed latex: #12G1233</strong></td>
<td></td>
</tr>
<tr>
<td>#14G1233</td>
<td>+5.08±0.16</td>
</tr>
<tr>
<td>#14G1223</td>
<td>+6.11±0.12</td>
</tr>
<tr>
<td><strong>Latex covered with copolymer shell: #A2</strong></td>
<td></td>
</tr>
<tr>
<td>#A3</td>
<td>+1.68±0.14</td>
</tr>
<tr>
<td>#A8</td>
<td>+4.34±0.22</td>
</tr>
<tr>
<td># A10</td>
<td>+5.20±0.12</td>
</tr>
<tr>
<td><strong>Grafted latex : #A2GR</strong></td>
<td></td>
</tr>
<tr>
<td>#A3GR</td>
<td>+0.38±0.07</td>
</tr>
<tr>
<td>#A8GR</td>
<td>-0.17±0.03</td>
</tr>
<tr>
<td>#A10GR</td>
<td>+0.39±0.13</td>
</tr>
<tr>
<td>#A10GR1</td>
<td>0</td>
</tr>
</tbody>
</table>

1) : measured in 10mM sodium chloride, pH=7.

density measured by conductometric titration (Table 2.2). After adding the copolymer shell, the mobility decreased for all batches, except for A10. This result is likely due to masking of the surface charge or enhanced electroosmotic drag due to hydrophilic surface groups (Janzen et al., 1996). This effect was more pronounced for batches with higher aldehyde content in the shell (A2 and A3). Batch A10 had the same mobility before and after adding the shell, but it also had the lowest aldehyde concentration. The fact that none
of the batches showed higher mobility after the copolymerization step supports the assumption made earlier that the amount of initiator added in this step is lower than the amount already present on the core latex.

3.3.1.2. Parameters influencing the copolymerization

The results of the copolymer shell synthesis can be explained in terms of the difference in solubility between the two monomers and their copolymerization reactivity ratios (Guillot, 1985; Guyot et al., 1981). Acrolein is water soluble, while styrene is not. The reactivity ratios for the styrene/acrolein copolymerization are: \( R_{\text{sty}} = 0.25; R_{\text{acro}} = 0.25 \) (Young, 1975). This means that the rate constant for the reaction of a styrene terminated radical with an acrolein molecule (when an alternating sequence results) is four times higher than the rate constant of the reaction in which the same radical captures a styrene molecule (when a homopolymer sequence results). The same statement is valid for an acrolein terminated radical. If the copolymerization took place in a good solvent for both monomers, a 1:1 molar mixture would normally yield an azeotropic system, in which the composition of the copolymer was the same as in the feed and most copolymer sequences were alternating. In seeded emulsion copolymerization however, where three different phases are present (seed particles, insoluble monomer droplets and the aqueous phase), composition at the main polymerization locus can be quite different from the overall bulk composition.

As described in Chapter 2, surfactant-free seeded polymerization starts in solution because the initiator is water-soluble. At the beginning of the reaction, partition of
monomers in the emulsion is probably the following: most of the styrene is swollen in the seed particles, some is dissolved in water ($\sim 4 \times 10^3$ mole dm$^{-3}$) and, depending on the amount, some might exist as droplets in emulsion; the acrolein is completely dissolved in water. The first radicals would therefore be expected to be acrolein radicals because this is the monomer which has the highest concentration in the aqueous phase. They will continue to grow in solution, occasionally incorporating some styrene units until reaching the critical length at which they become insoluble. The insoluble oligomers can either coagulate with each other to form primary particles or can be incorporated in the seed, where addition of styrene partitioned into the bead or acrolein from the aqueous phase will take place. The partition of these oligomers between the aqueous phase and the seed ultimately depends on their compatibility with the hydrophobic polystyrene in the core beads. The higher their styrene content, the better is the chance of incorporating them in the seed. The same statement is true for the primary particles. Given the very low water solubility of styrene, it is expected that very few oligomers or primary particles will contain enough styrene to make them partition preferentially in the bead. This means that only a small fraction of the total initiator added is present in the shell, which is supported by the electrophoresis results. It is also in agreement with the fact that batches A6 and A9, which were done using the same seed and the same acrolein to seed ratio but at two different initiator concentrations (the amount used in A9 is one tenth of that in A6) yielded latex with the same aldehyde content. Therefore the initiator concentration did not influence the outcome.
The low yield of incorporation of acrolein in the copolymer shell (between 1 and 10% of the acrolein added was found in the shell) can also be explained by the low probability of producing oligomers and primary particles with high affinity for the seed. Most of the acrolein probably remains in solution, either as monomer molecules, or, more likely, as partially soluble oligomers. This is supported by the observation that the supernatant was cloudy at the end of the copolymerization experiments and by the fact that in the synthesis of batch A2 a second population of small beads was obtained.

The acrolein to seed ratio was varied between $2 \times 10^{-4}$ and $1 \times 10^{-2}$ moles acrolein per gram latex in experiments A1 to A5, all of which used the same seed and the same initiator concentration. Styrene was added to give 1 to 1 or 0.4 to 0.6 molar ratio in the copolymer. The influence of the acrolein to seed ratio was found to be as follows:

- At a high ratio of acrolein to seed (batch A1), no product was obtained (seed latex creamed). This is probably due to the acrolein content of the initial oligomers being much higher. Seed swelling and phase separation was faster than the formation of insoluble oligomers compatible with the seed. The swollen latex lost contact with the aqueous phase in which the radicals were formed.

- At a low ratio of acrolein to seed (batch A4 and A5), the product was aggregated probably because the seed increased in size, but did not have a sufficient density of stabilizing groups on the surface (either initiator or aldehyde oligomers). In all the other experiments, the presence of higher aldehyde concentration on the surface stabilized the beads by a steric mechanism (Vandehoff, 1981) in spite of the fact that
very few initiator residues were added. The observed stabilizing effect of the acrolein shell is probably due to some fraction of the copolymer chains containing homooligomeric acrolein sequences which extend away from the surface in loops or tails (Fleer et al., 1993) due to their hydrophilic character. This hypothesis is supported both by the observed aggregation effect at low acrolein concentration and by the electrophoresis results. The mobility of the beads was lower at higher aldehyde content.

The optimum range for the acrolein to seed ratio was found to be $1 \times 10^{-3}$ to $2 \times 10^{-3}$ moles acrolein per gram latex. It was kept in this range for experiments A6 to A10.

The character of the surface on which the copolymerization took place was the most important factor determining the outcome of these experiments. Three seed latexes were used, which, according to the results presented in Table 2.2, had different charge densities. Batch 12G1233 (seed used in experiments A1 to A5) had approximately half the charge density of the seeds in series 14 (used in experiments A6 to A10). The results show that lower charge density on the seed favored higher acrolein incorporation, other conditions being equal. Primary copolymer particles were probably much bigger than those formed in seeded experiments when styrene only was used due to higher solubility of the copolymer compared to polystyrene. Both polystyrene and polyacrolein homopolymers are insoluble in water, but their copolymer most likely has better solubility due to the following reasons:

- Insolubility of polyacrolein in water is probably due to the fact that adjacent aldehyde groups forming tetrahydropyran structures (Schultz, 1964). Introducing styrene units on the chain lowers the number of neighboring aldehyde groups.
• The hydrophobic character of polystyrene is diminished upon introducing acrolein units due to formation of hydrogen bonds at the aldehyde sites.

Heterocoagulation of the primary and seed particles will be controlled by electrostatic repulsion. Seeds with lower charge density therefore will have diminished repulsion and incorporation would be expected to be higher, as observed. Also, because aldehyde groups are hydrophilic, they will tend to stay on the surface of the bead as much as stereochemistry allows (Basinska et al., 1993). Initiator residues concentrate on the surface of the bead as well. Hence, the seed with lower initiator surface concentration has somewhat more surface area available to accommodate aldehyde groups, although this effect will be small.

3.3.2. Ce(IV) initiated MEA polymerization

The reaction is a redox initiated polymerization which proceeds at the vinyl bond of the MEA molecule. A proposed mechanism for the case in which the reducing agent is an aldehyde is given in Figure 3.6. In the initiation step, the rate determining process is the disproportionation of the Ce(IV) complex with the aldehyde (B in Figure 3.6). The reducing agent (i.e. aldehyde group) forms a radical in this initial step. The more stable this radical is, the slower will be the initiation. The exact location of the initiating radical is not discussed in the literature. Although the aldehyde template has been used in conjunction with Ce(IV) for the initiation of acrylonitrile solution polymerization (Ahmed et al., 1978), the structure of the complex was not investigated in the reference. In other studies on initiation using acetanilide (Dong et al., 1994) or β-diketone (Zhao and Qiu,
1996) as the reducing group, it has been shown that the radical is formed in the position adjacent to an electron withdrawing group (e.g. hydroxyl, ketone or carboxyl). In the case of using the aldehyde as the reducing group, the hydrogen in the α-position is more easily extracted than the one in the β-position. The hydrated form of the aldehyde probably forms the complex with Ce(IV). The proposed configuration of the complex is shown in the Appendix (Figure A.1). If the grafting point is located on the carbonyl group, a ketone results at the starting extremity of the poly(MEA) chain, as indicated in Figure 3.2. This statement is supported by the results of the hydroxylamine assay done on the grafted beads to be discussed.

The termination reaction by Ce(IV) is prevalent at high ceric ion concentrations, relative to termination in which two polymeric chains are involved (recombination or disproportionation) (Mino and Kaizerman, 1958). Chains with a terminal double bonds result by this mechanism, as shown in Figure 3.2.
1. INITIATION

\[ \text{Ce}^{4+} + R_1\text{CH}-R_2 \xrightarrow{K} \text{B} \xrightarrow{k_d} \text{Ce}^{3+} + H^+ + R_1\text{CH} = R_2 \]

\[ R_1\text{H} - R_2 + M \rightarrow M^* \]

2. PROPAGATION

\[ M^* + M \xrightarrow{k_p} \text{GROWING POLYMER} \]

3. TERMINATION

\[ M^* + \text{Ce}^{4+} \xrightarrow{k_{11}} \text{Ce}^{3+} + H^+ + \text{DEAD POLYMER} \]

\[ M^* + M^* \xrightarrow{k_{12}} \text{DEAD POLYMER} \]

B is the complex between Ce\(^{4+}\) and the aldehyde group.

Figure 3.6: Mechanism of redox initiated polymerization using Ce(IV)/aldehyde system

In Table 3.3 the results of the grafting reaction are shown. The monomer conversion varied from 0 to 99%. In experiment A9GR the concentrations of monomer, aldehyde and Ce(IV) were lower than in all the other experiments. No grafting occurred under these conditions.
experimental conditions. Batches A10GR1 and A10GR3 were synthesized under the same conditions except for the monomer concentration, which was higher for A10GR3. Evidently higher monomer concentration increased the amount of product by mass action and enhanced the yield of the grafting reaction. This is in agreement with previous studies of Ce(IV) initiated grafting reaction on starch (Athawale and Rathi, 1997) and poly(vinyl alcohol) (Panda and Singh, 1996).

The most important factor in determining the outcome of the grafting reaction seems to be the surface aldehyde concentration of the beads. In Figure 3.7, a plot of the amount of grafted MEA versus initial surface aldehyde concentration is presented. The results show that there is an optimum surface concentration of aldehyde groups at which a maximum grafting efficiency is achieved. This observation may be explained as follows:

As the grafted chains start to grow from the surface, those which are close together on the surface probably terminate with each other by the bimolecular reaction illustrated in Figure 3.6. If a larger distance between the aldehyde groups occurs, the probability of escaping early bimolecular termination is higher and grafting efficiency is enhanced. On the other hand, too low surface concentration of the starting groups leads to low yields due to mass action. The optimum surface concentration was found to be 0.03 groups/Å² (batch A8GR).
### Table 3.3: Characterization of the grafted beads

<table>
<thead>
<tr>
<th>Batch #</th>
<th>MEA conversion</th>
<th>Polymerized MEA</th>
<th>Tether coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>mole/g latex</td>
<td>NMEA groups/Å²</td>
</tr>
<tr>
<td>A2GR</td>
<td>44±5</td>
<td>1.40x10⁻³</td>
<td>3.41</td>
</tr>
<tr>
<td>A3GR</td>
<td>55±5</td>
<td>1.96x10⁻³</td>
<td>4.78</td>
</tr>
<tr>
<td>A8GR</td>
<td>99±5</td>
<td>3.53x10⁻³</td>
<td>9.93</td>
</tr>
<tr>
<td>A9GR</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A10GR1</td>
<td>12±5</td>
<td>0.43x10⁻³</td>
<td>1.26</td>
</tr>
<tr>
<td>A10GR2</td>
<td>31±5</td>
<td>0.49x10⁻³</td>
<td>1.44</td>
</tr>
<tr>
<td>A10GR3</td>
<td>28±5</td>
<td>2.00x10⁻³</td>
<td>5.88</td>
</tr>
</tbody>
</table>

1): determined from HPLC of the supernatant at the end of the grafting reaction;

2): calculated under the assumption that all polymerized MEA was distributed on the beads, using data from the second column and the amount of seed in each synthesis;

3): calculated with the formula:

\[ N_{MEA} = \frac{T \cdot N_A}{SSA}, \text{ MEA groups/Å}^2. \]

where: \( T \) = polymerized MEA amount, moles/g latex;

\( SSA \) = surface area available on the latex (using the radius of the seed latex), Å²/g latex;

\( N_A \) = Avogadro's number, mole⁻¹.

The electrostatic repulsion between the tetravalent cerium ion and the positively charged beads is another factor which influenced the outcome of the grafting reaction.

Batches A8 and A10 had higher surface charge density than A2 and A3, as indicated both by the conductometric titration of the “parent” latex and by the electrophoretic mobility measurements. The stronger electrostatic repulsion probably has lead to a lower surface
concentration of Ce(IV), resulting in a lower initiation rate. In turn, this effect decreased the probability of mutual termination of the chains (fewer chains started at one time).

![Graph](image)

**Figure 3.7:** Grafted MEA amount (tether coverage) *versus* the aldehyde group concentration in the copolymer shell

A disadvantage of the method used for grafting is that the number of grafted chains couldn’t be assessed. Hydroxylamine reaction followed by conductometric titration to determine the number of unreacted aldehyde groups after grafting yielded results approximately equal (±30%) to the aldehyde concentrations before grafting (1.2x10⁻⁵ moles/g for A2GR and 1.4x10⁻⁵ moles/g for A8GR; the result is the average of two trials). The result is probably due to the presence of ketone groups at the anchoring point of the grafted chains, as shown in Figure 3.2. These functional groups react with hydroxylamine in the same way as does aldehyde (Morrison and Boyd, 1975). The results support the
hypothesis that the grafting point is located on the \(\alpha\)-carbon of the aldehyde template, as stated earlier. The observed difference between the carbonyl concentration before and after grafting can be due to the different environment of the analyzed group. On the surface bearing tethered chains, the carbonyl group is likely to be shielded and less accessible than on the bare surface. Under these conditions, a lower concentration would be found on the grafted beads, as observed for batch A2GR. Because the grafting reaction proceeds in acidic medium, protons adsorbed on the bead can interfere with the titration performed after grafting and hydroxylamine treatment. This was observed in the analysis of batch A8GR, where the first trial yielded \(1.8 \times 10^{-5}\) moles/g and after extensive washing only \(9.5 \times 10^{-6}\) moles/g were found (very close to the result reported for the ungrafted beads, \(1 \times 10^{-5}\) moles/g).

The efficiency of grafting was estimated by using the tether coverage \(N_{\text{MEA}}\) (groups/Å\(^2\)) calculated from monomer conversion and the specific surface area of the beads. These results are reported in the last column of Table 3.3. No direct estimate of the grafted amount was made and it was assumed that all the polymerized MEA was associated with the beads. Other methods which could be considered include nitrogen analysis and X-ray photoelectron spectroscopy. Due to the fact that the grafted polymer represents, by weight, a very small fraction of the bead, nitrogen analysis is not sensitive enough for the determination of MEA associated with the surface. In addition, it requires a relatively high amount of sample which can not be recovered. On the other hand, XPS provides only semiquantitative information regarding the elemental composition of the surface and the
molecular environment of each atomic species (Ratner et al., 1992). The method is also very sensitive to surface contamination while handling the sample (Ratner, 1990).

3.3.3. Model of the surface

An approximate model of the grafted surface was derived from the data by assuming that all the aldehyde groups present on the surface of the beads initiated chains and that all the polymerized MEA was located on the latex (i.e., that no solution polymerization occurred). The average degree of polymerization of the terminally-attached chains was calculated from the ratio of polymerized MEA amount (mole/g latex) to the surface aldehyde concentration before grafting (mole/g latex). The results are shown in the second column of Table 3.4. The estimates for the length of the grafted chains varied between 60 and 350 monomer units. The distance between the grafted chains was estimated from the area per aldehyde group. The radius of gyration of the tethers was calculated assuming linear chains in near theta solvents, although this approximation doesn’t take into consideration the attachment of the chains to the surface. For all batches the value of the radius of gyration is higher than the average chain separation (third and fourth column in Table 3.4), which means that the grafted chains probably overlap and a brush configuration was adopted (Fleer et al., 1993). The estimated equilibrium height of the brush is shown in the fifth column of Table 3.4. According to the model, the beads are covered with a dense grafted layer which extends into solution 20 to 133 Å from the surface. These estimates are consistent with the elecrophoretic mobility measurements.
done on the grafted beads (Table 3.2), which show that the terminally-attached chains shielded the positive charge initially present on the beads.

Table 3.4: Modeled parameters of the grafted surface

<table>
<thead>
<tr>
<th>Batch #</th>
<th>DP of grafted chains</th>
<th>Chain separation 2) Å</th>
<th>Radius of gyration 3) Å</th>
<th>Brush height 4) Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2GR</td>
<td>76</td>
<td>4.7</td>
<td>25.8</td>
<td>33.4</td>
</tr>
<tr>
<td>A3GR</td>
<td>137</td>
<td>5.3</td>
<td>34.7</td>
<td>55.7</td>
</tr>
<tr>
<td>A8GR</td>
<td>350</td>
<td>5.9</td>
<td>55.4</td>
<td>132.6</td>
</tr>
<tr>
<td>A10GR1</td>
<td>60</td>
<td>6.9</td>
<td>23.0</td>
<td>20.4</td>
</tr>
<tr>
<td>A10GR2</td>
<td>69</td>
<td>6.9</td>
<td>24.6</td>
<td>23.5</td>
</tr>
<tr>
<td>A10GR3</td>
<td>283</td>
<td>6.9</td>
<td>49.9</td>
<td>97.4</td>
</tr>
</tbody>
</table>

1): Calculated with the formula: DP = T/{-CH=O}

where DP = average degree of polymerization of the grafted chains;

T = polymerized MEA amount determined by HPLC analysis, mole/g latex;

{-CH=O} = aldehyde concentration in the copolymer shell determined by conductometric titration, mole/g latex;

2): Calculated with the formula: D = A^{1/2}, Å

where D = chain separation on the surface of the beads, Å;

A = area per aldehyde group, Å² (Table 3.1).

3): Estimated from (Fleer et al., 1993) \( R_g = aMn^{1/2} \) for linear polymers in near theta solvents, where:

Mn = average molecular weight of the chains, g/mole;

Mn = 129DP; a = 261x10⁻³, Å (PMMA).

4): Estimated brush height is (Fleer et al., 1993):

\( h = (DP-1) (\sigma I^2)^{1/3} \), Å
where \( \sigma = A^{-1} \) = density of the grafted chains, \( \text{Å}^{-2} \);
\[ l = 1.4 \] = length of repeating unit in the polymer chain, \( \text{Å} \).

3.4. CONCLUSIONS

A two step method for chemical modification of the surface of cationic poly(styrene) beads was developed. In the first step, the beads were covered with a shell containing poly(styrene-co-acrolein) by a seed polymerization procedure. The aldehyde content of the copolymer shell was estimated by proton NMR and hydroxylamine reaction, followed by conductometric titration of the supernatant. It was found that the NMR analysis is not accurate enough for the range of concentrations encountered. Conductometric titration, on the other hand, could underestimate the result by 5 to 30% due to interference with basic initiator residues present on the latex. The surface aldehyde concentrations were found to be between \( 1.15 \times 10^{-2} \) and \( 4.48 \times 10^{-2} \) groups/\( \text{Å}^2 \) (\( 4 \times 10^{-6} \) to \( 2 \times 10^{-5} \) mole/g latex).

The most important determinant of the outcome of the copolymerization was found to be the character of the surface of the seed. Batches with lower charge density incorporated a higher amount of acrolein. In the second step the aldehyde groups located on the surface of the latex were used as starting points for Ce(IV) initiated polymerization of MEA chains. The amount of grafted polymer was determined indirectly, by measuring the concentration of MEA left unreacted in the supernatant. The tether coverage \( N_{\text{MEA}} \) was calculated as MEA groups polymerized per unit area of latex. Values of coverage between 0 and 9.9 groups/\( \text{Å}^2 \) were obtained. It was not possible to determine the length of the grafted chains by direct measurement. The most important parameter which influenced the
grafting reaction was found to be the surface concentration of the aldehyde groups. The maximum grafting efficiency was obtained for a concentration of 0.028 aldehyde groups/Å². An approximate model for the grafted surface was developed by assuming that all the surface aldehyde groups initiated chains and that all the polymerized MEA was located on the beads. The probable conformation of the chains is a dense brush with the height varying between 20 and 133 Å.
CHAPTER 4: Synthesis and analysis of core/shell latex carrying cleavable grafted chains

4.1 INTRODUCTION

While in the work described to date latices carrying grafted chains were successfully made, little characterization of the tethered later was possible. The objective of the work described in this chapter was to synthesize a model latex carrying cleavable terminally-attached chains. The advantage of such an approach is that the grafted chains in principle can be cleaved and separated from the solid support. The characterization of the chains then can provide information useful both for optimizing the grafting conditions and for understanding the properties of the surface. Such detailed information was not available when using the aldehyde support for grafting.

Surface modification of the core latex was carried out in two steps, as described in Chapter 3. The comonomer used for the shell was 2-hydroxy-ethyl acrylate (HEA). The monomer was chosen because it meets the conditions required for the study: it is water soluble, it provides hydroxyl groups on the surface of the latex which should be reactive with Ce(IV) to initiate MEA polymerization and it contains an ester bond which can be cleaved after grafting to allow separation of the terminally-attached chains from the support. The reactions were carried out successfully and the cleaved chains were characterized by size exclusion chromatography. Both the surface density and the molecular weight distribution of the grafted layer were determined. A model of the surface layer was developed.
4.2. EXPERIMENTAL

4.2.1. Materials

Surfactant-free cationic poly(styrene) latex used as starting material for this study was synthesized as described in Chapter 2.

The source and the purification procedure for all the reagents, except for HEA were described in Chapters 2 and 3. 2-hydroxy-ethyl acrylate (HEA) reagent grade was purchased from (Aldrich Milwaukee, WIS). The first batch of HEA (used for synthesis of latex H1, see Discussion) was purified by simple vacuum distillation at 90°C in an argon atmosphere. The second batch of monomer was purified by vacuum fractionation using a 10 cm Vigreux column. The main fraction was collected at 77°C (vapor temperature). The monomer was cooled upon collection during distillation using a dry ice-methanol bath. The purified HEA was stored protected from light at -70°C under argon until future use.

4.2.2. HEA/Syrene copolymer shell preparation

A seed copolymerization procedure was used to produce the shell on the latex, according to the reaction shown in Figure 4.1. The synthesis procedure was the same as the one used for acrolein/styrene copolymerization, which is described in the experimental section of Chapter 3. The only difference was the reaction time, which in the case of HEA/styrene copolymerization was 5 h. Detailed recipes are given in the Appendix, Table A.7. All the concentrations are reported with respect to the total volume of the reaction mixture.
Positively charged poly(styrene) bead + 2 hydroxy-ethyl acrylate + Styrene

Positively charged bead with poly(styrene-co-hydroxy ethyl acrylate) shell

\( R^0 \) is initiator residue:

\[ \text{CH}_3 \text{NH}_2^+ \]

\[ \text{CH}_3 \]

Figure 4.1: Seed copolymerization of styrene with HEA

4.2.3. Ce(IV) initiated MEA polymerization

The chemical reaction for this stage of the synthesis is shown in Figure 4.2. The same apparatus and synthesis procedure as the one described for grafting on aldehyde latex was used. The recipes are given in Table A.8 (Appendix).
4.2.4. Analysis methods

4.2.4.1. Acrylic acid content in distilled HEA

One of the impurities present in commercial 2-hydroxy-ethyl acrylate monomer is acrylic acid. Because it was found (see Discussion of batch #H1) that inadequate purification of the monomer can alter the properties of the product, the acrylic acid content of the purified monomer was assessed by a titration procedure. The method was as follows: 0.1 to 0.2 g purified HEA was weighed and diluted to 10 ml with water. The

Figure 4.2: Cerium (IV) initiated polymerization of N(2-methoxy-ethyl) acrylamide activated by hydroxyl groups on the surface of the latex
solution was purged with argon for 5 min, then placed in the stirred conductometric cell. The titration proceeded under inert atmosphere (argon), using the experimental setup described in Chapter 2. The titrant was 0.037 M sodium hydroxide solution, previously standardized by potentiometric titration using potassium biphtalate. During the titration, the time and conductance were monitored at constant flow rate (0.0204 ml/min). The equivalence point was read on the conductance versus titrant volume plot. Acid content of the sample was calculated with a formula similar to \[3.3\], with \{-CH=O\} replaced by \{COOH\} and \(w\) = amount of monomer (g).

4.2.4.2. Analysis of monomer (HEA or MEA) consumption during reaction

The amount of unreacted monomer was determined by HPLC analysis of the supernatant separated from the reaction mixture by centrifugation and filtration, as described in the experimental section of Chapter 3.

4.2.4.3. Hydroxyl group content of the copolymer shell

The technique involved saponification of the ester bonds in HEA residues, followed by conversion of the sodium carboxylate groups left on the latex to carboxylic acid upon acidification. The chemical reactions are shown in Figure 4.3. Conductometric titration was then used to assess the concentration of the acid groups.

A series of experiments was done on latex #H1 in order to determine the minimum concentration of sodium hydroxide required for complete cleavage of the ester bonds; Table A.9 (Appendix) contains the experimental conditions used. Batches #H2 and #H3 were cleaved under exposure to 0.67M sodium hydroxide. The treatment of the latex after
saponification was the same in the analysis of all three batches. The detailed procedure is as follows:

\[
\text{Step 1: Saponification of the ester groups on the surface of the latex}
\]

\[
\text{Step 2: Conversion of the carboxylate groups into carboxylic acid}
\]

**Figure 4.3: Analysis of hydroxyl groups in the copolymer shell**

A 2M sodium hydroxide solution was prepared. A sample of latex suspension of known solid content was weighed in a glass vial (approximately 2 g suspension containing about 0.2 g solid particles), then 1 ml of 2.0M sodium hydroxide solution added. The mixture was left to react overnight while tumbling in a rotating rack at room temperature. The latex was then washed 4 to 6 times in the centrifuge with distilled water until the pH of the supernatant was neutral. The pellet was then resuspended in 2 ml 0.1 M hydrochloric acid and washed again 2 to 4 times with water to neutral pH. After the final wash, the volume was adjusted to 10 ml with water. The suspension was purged with argon for 5 min and transferred to the conductometric cell. The titration and data
calculation proceeded as described in section 4.2.4.1. (where in this case, \( w \) is the amount of solid latex in the sample).

The beads covered with the copolymer shell were not analyzed for the basic initiator group concentration because the amount of latex was insufficient. It was assumed that only a relatively small number of initiator groups were added during the copolymerization step, compared to the amount already present on the core latex.

4.2.4.4. Cleavage of grafted chains

The conditions for cleavage of the grafted chains from the latices were the same as described in section 4.2.4.3 and shown in Figure 4.4. The reaction was performed on approximately 10 g of latex suspension of known solid content (about 1 g solids). An exact amount of 2.0M sodium hydroxide solution was added to give 0.67 M final concentration in the mixture. Completion of the cleavage reaction was determined by titration of the beads and comparing the result with that obtained for the ungrafted beads; 36 hours were required. Otherwise the latex was treated exactly as described in the previous section. The supernatant and the first wash were mixed, neutralized with hydrochloric acid and concentrated by evaporation (40°C, Rotovap). The concentrated solution containing the released grafted chains was then weighed and analyzed by size exclusion chromatography.
Figure 4.4: Chemistry of cleavage of grafted chains by saponification

4.2.4.5. Size exclusion chromatography of the grafted chains

A 300x10 mm column packed with BIO-SEC EMD tentacle type gel (Merck, Darmstadt) was used to analyze the grafted polymers. Aliquots of 200 µl of the concentrated solutions obtained by cleaving the grafts from latex were run on the column in 0.2 M sodium chloride at 0.5 ml/min, monitoring at 220 nm. The characteristics of the
column were: void volume \( (V_0) \) 7.18 ml (determined with DNA) and pore volume \( V_p=13.05 \text{ ml} (V_p = V_{NaNO_3} - V_0) \), where \( V_{NaNO_3} \) was the volume at which 0.02M NaNO₃ eluted. In order to convert the partition coefficients of the grafted polymer to molecular weights, a set of commercial poly(acrylamide) standards (Polysciences, Inc.) was used. The calibration equation obtained as described above, was:

\[
\log (M_n) = 5.072 + 2.246K_D \quad [4.1]
\]

where \( M_n = \) number average molecular weight;

\[ K_D = \text{partition coefficient calculated from the elution volume, } V_e, \text{ of the sample}, \]

\[ K_D = \frac{(V_e - V_0)}{V_p}. \]

Equation [4.1] contains the correction factor of 129/71 to account for the ratio between the molecular weight of MEA and acrylamide. It can be used to obtain a good approximation of the number average molecular weights of the grafts. It assumes however, that the methoxy-ethyl side chains do not change the mean end-to-end distance of the poly(MEA) molecules in the column compared to poly(acrylamide). For freely coiling solutes of similar architecture, this assumption is reasonable (Casassa, 1976).

To produce a quantitative estimate of the terminally-attached chain concentration a calibration of the optical density of the polymer was performed as follows:

The grafted polymer solution resulting from cleavage of latex H3GR4, which was made in a large batch for this purpose, was purified to remove low molecular weight components (salt and monomer) using a 600x26 mm Sephadex G25 (Pharmacia, Uppsala, Sweden) size exclusion gel column run at 3 ml/min in water, monitored at 220 nm, 1 ml sample. The first fraction, which eluted around 12 min, contained high molecular weight
components (this was checked by running the eluate, after concentration by evaporation, on the BIO-SEC column and comparing the profile with that run before purification). The eluates from three runs on the Sephadex column were combined, concentrated approximately five fold by evaporation, and a portion weighed (1.9578g) and freeze-dried to determine the solid content (4.8x10^{-3}g), which corresponded to 2.45x10^{-3}g/ml in the pooled eluate. A 200 μl aliquot of this solution containing 4.9x10^{-4}g polymer was injected twice in the BIO-SEC column. At a flow rate of 0.5 ml/min, monitored at 220 nm, an average area of 29.7x10^{6}μV s (28.29 and 31.10) was obtained in the polymer region (6.06x10^{10}μV s/g polymer).

The effect of potential terminal double bonds, formed by cerium termination or by disproportionation (Odian, 1991), on the optical density at 220 nm was checked by investigating a high molecular weight poly(MEA) sample synthesized by hydrogen peroxide initiation. A solution of this polymer was prepared (containing 1.47x10^{-3}g/ml). A 200 μl aliquot of this solution, when injected in the BIO-SEC column, gave an average area of 14.66x10^{6}μV s (14.12 and 15.20 in two trials), that is 4.99x10^{10}μV s/g polymer, at a flow rate of 0.5 ml/min. Because of the high molecular weight of the sample (its elution volume was 7.23 ml, almost equal to the void volume of the column), the effect of any terminal double bond chromophore on the optical density of the solution would be minimal in this measurement. Compared to the calibration done using the polymer cleaved from batch H3GR4, the area of the polymer peak per gram was 20% lower. It is concluded that the method of calibration described above can be used to estimate the amount of polymer cleaved from the beads to within an uncertainty of at most 20%.
The areas of the polymer peaks were converted to absolute mass of grafted material with the formula:

\[ GW = \frac{(PAxS/6.0610^{10})}{(0.2w)} \]  

where \( GW \) = mass of grafted material, g grafted polymer/ g latex

\( PA \) = total area of the polymer peaks, \( \mu \text{V s} \);

\( S \) = mass of grafted polymer solution obtained after cleavage and evaporation, g;

\( w \) = the amount of solid latex exposed to cleavage, g.

It was assumed that the density of the grafted polymer solution was 1.0 g/cm\(^3\).

4.2.4.6. Electrophoretic mobility measurements

The experimental procedure was the same as described in Chapter 2. The mobility results were used qualitatively only.

4.3. RESULTS AND DISCUSSION

4.3.1. Copolymer shell preparation and characterization

The goal of the first step in the latex surface modification was to introduce a useful concentration of hydroxyl groups - ideally uniformly distributed and solidly anchored to the bead - in order to provide efficient initiation of grafting in the second step. This had to be done without reducing the charge and stability of the beads. To be easily analyzed before and after grafting, the HEA residues in the shell had to be entirely accessible from the water phase, which meant that a very small amount was needed, ideally covering less than a monolayer on the surface of the latex.
Conductometric titration is considered (Vanderhoff et al., 1970) to be a suitable method for analyzing latices containing carboxylic groups on the surface. In Figure 4.5, a titration curve of saponified core/shell latex is shown. To relate the result of the titration quantitatively to the HEA content of the copolymer shell, the cleavage conditions had to be optimized in order to ensure that all the ester bonds had been hydrolyzed. Batch #H1 was used for this purpose, in the set of experiments performed under the conditions in Table A.9 (Appendix). In Figure 4.6, the carboxylic acid content of the cleaved latex is plotted as a function of the concentration of the sodium hydroxide used for saponification. The number of cleaved groups increased proportionally with the base concentration until saturation occurred, that is all groups were cleaved. The last point on the graph would be expected to be higher, but the time for saponification was longer than for all the other points, which might have caused secondary reactions to occur. We also encountered an experimental difficulty in the titration of the sample because the latex aggregated upon the addition of the base and remained that way during the titration. Hence the number of carboxylic groups could have been somewhat underestimated.

The intercept of the straight line defined at low concentrations in Figure 4.6 is $3 \times 10^{-5}$ mole/g latex, which means that the beads contained carboxylic groups or other weak acid groups before cleavage. This result is consistent with the electrophoretic mobilities, shown in Table 4.1. The bare latex (seed #11) was positively charged, it became negatively charged upon shell preparation (H1) and it had a higher negative charge density (higher negative mobility) after cleavage due to the new COO$^{-1}$ groups created by saponification.
Figure 4.5: Titration curve of cleaved latex
Latex H2 cleaved (0.200 g)

Figure 4.6: Carboxylic acid content versus concentration of NaOH used for saponification (optimization of the cleavage conditions; latex H1)
The net negative surface charge observed on latex H1 after addition of the copolymer shell, in spite of the cationic initiator used with the already positively charged beads, was due to an acrylic acid impurity present in the HEA monomer. The analysis sheet accompanying the monomer indicated that it contained 1% w/w acrylic acid. Titration of the monomer after purification by simple distillation showed the presence of $1.69 \times 10^{-4}$ moles acid/g monomer, or 1.2% by weight. Assuming that all of it was distributed on the surface of the beads, their carboxylic acid content would have been approximately $3.9 \times 10^{-5}$ mole/g, which is close to the value of the intercept. These results demonstrate that simple distillation of monomer was not sufficient to remove the acrylic acid impurity from the HEA.

Table 4.1: Electrophoretic mobility of latex in various synthesis stages

<table>
<thead>
<tr>
<th>Synthesis stage</th>
<th>Mobility $\times 10^8$ m$^2$/volt-sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed latex: #11</td>
<td>+3.82±0.29</td>
</tr>
<tr>
<td></td>
<td>+4.52±0.12</td>
</tr>
<tr>
<td></td>
<td>+5.21±0.15</td>
</tr>
<tr>
<td>#14G1233</td>
<td></td>
</tr>
<tr>
<td>#14G1223</td>
<td></td>
</tr>
<tr>
<td>Latex covered with copolymer shell: #H1</td>
<td>-1.19±0.09</td>
</tr>
<tr>
<td>#H2</td>
<td>+0.55±0.07</td>
</tr>
<tr>
<td>#H3</td>
<td>+0.49±0.07</td>
</tr>
<tr>
<td>Cleaved latex: #H1CL</td>
<td>-2.87±0.10</td>
</tr>
<tr>
<td>#H2CL</td>
<td>-3.83±0.17</td>
</tr>
<tr>
<td>#H3CL</td>
<td>-4.29±0.12</td>
</tr>
</tbody>
</table>

1): measured in 10mM sodium chloride, pH=7.
A fractionation procedure therefore was used for a second batch of monomer. Conductometric titration of the product showed that no weak acid was present. This monomer was used to prepare batches H2 and H3. As shown in Table 4.1, these beads had positive surface charges following addition of the shell. The lower mobility of the bead covered with copolymer shell, compared to the corresponding seed, is likely due to masking of the surface charge or enhanced electroosmotic drag due to hydrophilic surface groups (Janzen et al., 1996).

In Table 4.2, data from HPLC analysis of the supernatant and from the conductometric titrations are presented. A mass balance of the consumed HEA, comparing data in the third column with the actual amount found on the beads by titration (fourth column) shows that only approximately 3% of the reacted monomer was contained in the shell on the latex.

This finding is consistent with the theory of seed polymerization (Hansen et al., 1979b) in surfactant-free systems. Free radical polymerization starts in solution and the oligomers grow until they reach the critical length at which they are not soluble in water. At this point they either coagulate with each other to form new nuclei or they coagulate with the seed, which is swollen with monomer, and continue polymerization in or at the surface of the bead. When the monomer is not very soluble in water (e.g. styrene), the critical length is very small (3-4 monomer units). On the other hand, if the monomer and its polymer are soluble in water (e.g. HEA), polymerization in solution independent of the seed will take place. When copolymerization of a mixture of two monomers is attempted, both their
Solubility and the reactivity ratios governing the copolymerization have to be considered to predict their behavior (Guillot, 1985). We are not aware of any reports describing the copolymerization of styrene with 2-hydroxyethyl acrylate. However, reactivity ratios for styrene/4-hydroxybutyl acrylate have been reported (Young, 1975) and we used them to predict the behavior of our system: $R_{\text{sty}} = 0.72$; $R_{\text{HEA}} = 0.73$. This means that the rate constant for the reaction of a styrene terminated radical with a HEA molecule (when an alternating sequence results) is only slightly higher than the rate constant of the reaction in which the same radical captures a styrene molecule (when a homopolymer sequence results). The same statement is valid for a HEA terminated radical.

Table 4.2: Characterization of the HEA/styrene copolymer shell

<table>
<thead>
<tr>
<th>Batch #</th>
<th>HEA conversion % (HPLC)</th>
<th>Reacted HEA 1) mole/g seed</th>
<th>OH content (conductometric titration) mole/g latex</th>
<th>Area per OH group Å²/group</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>NA</td>
<td>NA</td>
<td>1.1x10⁻⁴ (8x10⁻⁵)</td>
<td>11.6</td>
</tr>
<tr>
<td>H2</td>
<td>38±5</td>
<td>7.6x10⁻⁴</td>
<td>2.74x10⁻³</td>
<td>12.9</td>
</tr>
<tr>
<td>H3</td>
<td>38±5</td>
<td>5.7x10⁻⁴</td>
<td>1.69x10⁻³</td>
<td>20.1</td>
</tr>
</tbody>
</table>

1): calculated under the assumption that all reacted HEA is located on the beads (using data from second column and the amount of seed used).  
2): the number in the bracket is calculated by substracting the COOH content due to acrylic acid in the monomer (intercept in Figure 4.6).

The probability of finding significant concentrations of oligomeric radicals with homopolymeric sequences of either of the two monomers will be moderately high. Those containing more styrene units will become insoluble faster and will coagulate with the seed, while the more hydrophilic oligomers will tend to grow in solution. Alternating
monomer strings will also be reasonably water soluble, so only those oligomers with significant regions of homooligostyrene would be expected to incorporate into the seed surface, where addition of styrene partitioned into the bead or the HEA from the aqueous phase will take place. Because of the high water solubility of HEA, insoluble oligomers will be relatively rare, qualitatively explaining the low HEA incorporation observed. As the surface concentration of hydroxyl groups required for subsequent grafting was small, the amount incorporated was considered to be sufficient and no attempt to optimize the conditions further was made.

Other factors which influenced this step were the specific area of the seed (more groups were incorporated per gram latex when the seed was smaller, due to the higher surface area available) and the concentration of HEA monomer (lower HEA concentration produced lower incorporation).

4.3.2. Ce(IV) initiated MEA polymerization

4.3.2.1. Kinetics of grafting reaction

The reaction is a redox initiated polymerization which proceeds at the vinyl bond of the MEA molecule, as described in Chapter 3. A general mechanism for the case in which the reducing agent is a primary alcohol is given in Figure 4.7 (Mino and Kaizerman, 1959a). The configuration of the complex between the alcohol group and Ce(IV) (B in Figure 4.7) is shown in the Appendix (Figure A.1). If all the alcohol radicals produced are consumed in the initiation of polymer chains and mutual termination is neglected, the rate of initiation (R_i), propagation (R_p) and termination (R_t) are described by the following equations (Oedian, 1991):
1. INITIATION

\[
\text{Ce}^{4+} + \text{R-CH}_2\text{OH} \xrightarrow{K} \text{B} \xrightarrow{k_4} \text{Ce}^{3+} + \text{R-CH-OH} + \text{H}^+
\]

\[
\text{R-CH-OH} + \text{H}_2\text{C=CH} \rightarrow \text{R-CH-CH}_2\text{CH}\cdot
\]

2. PROPAGATION

\[
\text{R-CH-CH}_2\text{-CH}\cdot + \text{H}_2\text{C=CH} \xrightarrow{k_p} \text{R-CH-CH}_2\text{-CH-CH}_2\text{-CH}\cdot
\]

3. TERMINATION

3.a. Monomolecular termination

\[
\text{R-CH-CH}_2\text{-CH}\cdot + \text{Ce}^{4+} \xrightarrow{k_{11}} \text{Ce}^{3+} + \text{H}^+ + \text{R-CH-CH=CH}
\]

3.b. Bimolecular termination

\[
2 \text{R-CH-CH}_2\text{-CH}\cdot \xrightarrow{k_{12}} \text{R-CH-CH}_2\text{-CH-CH-CH}_2\text{-CH-R}
\]

\[
2 \text{R-CH-CH}_2\text{-CH}\cdot \xrightarrow{k_{13}} \text{R-CH-CH}_2\text{-CH}_2 + \text{R-CH-CH=CH}
\]

Figure 4.7: Mechanism of redox initiated polymerization using Ce(IV)/alcohol system
\[ R_i = K \, k_d \, [\text{Ce}^{4+}] \, [\text{alcohol}] \]  \hspace{0.5cm} [4.3]

\[ R_p = k_p \, [M] \, [M^*] \]  \hspace{0.5cm} [4.4]

\[ R_t = k_{tt} \, [\text{Ce}^{4+}] \, [M^*] \]  \hspace{0.5cm} [4.5]

where M is the monomer, \( k_d \), \( k_p \) and \( k_{tt} \) are the rate constants for disproportionation of the Ce(IV) complex, polymerization and monomolecular termination, respectively, and K is the equilibrium constant for the Ce(IV)/alcohol complex formation. Making the steady state approximation \( (R_i = R_t) \), the polymerization rate is given by:

\[ R_p = (K \, k_p \, k_d / k_t) \, [M] \, [\text{alcohol}] \]  \hspace{0.5cm} [4.6]

In Figure 4.8, data illustrating the conversion of MEA versus reaction time are plotted. Monomer conversion was determined by HPLC analysis of the supernatant during the grafting reaction (experiment H3GR1).

![Graph](image)

**Figure 4.8: MEA conversion during grafting (H3GR1 experiment)**
The linearity is consistent with data in the literature (Misra and Bhattacharya, 1980). The conversion obviously would plateau at longer times but the experiment was stopped before monomer exhaustion, due to the high viscosity of the reaction mixture. The high viscosity probably contributed to scattering of the data points because difficulties with stirring allowed local variations in concentration. From the first two concentrations, the initial rate of polymerization was calculated to be $8.9 \times 10^{-4}$ mole l$^{-1}$ min$^{-1} \pm 10\%$. This value is one order of magnitude less than found in the above mentioned reference, for polymerization of acrylamide using Ce(IV)/L-cysteine initiation. The difference may be due to the relatively limited mobility of the hydroxyl groups anchored to the latex.

### 4.3.2.2. Efficiency of the grafting reaction

In Table 4.3, data on monomer conversion following completion of grafting are compared with the mass of grafted chains cleaved from the beads. Data referring to batches H2GR1 and H2GR2 are less certain ($\pm 30\%$) because only the approximate volume of the grafted polymer solution was known (for all other batches, the grafted polymer solution was weighed). The last column shows the percentage of the total monomer polymerized associated with the grafted chains. The mass balance calculations indicate that two kinds of polymer resulted: polymer in solution and grafted polymer on the beads. The percentage of MEA found covalently attached to the surface was only 0.3 to 2.1\% of the total amount of polymerized MEA. This is consistent with the observation that a high viscosity supernatant resulted during grafting reactions H3GR1 and H3GR2, implying the presence of high molecular weight polymer in the liquid phase. Three reactions could lead to this event:
The presence of Ce(IV) ions could have initiated bulk polymerization of MEA monomer, independent of the presence of the hydroxyl groups on the latex. If this was the case, the supernatant should have been viscous in all the experiments, not in only some of them. Also, while homopolymerization in the presence of Ce(IV) without a reducing agent has been reported for acrylonitrile, acrylamide is not known to react this way (Wallace and Young, 1966). Since MEA is an acrylamide derivative, this reaction is unlikely. Finally, in the experiments described in Chapter 3, highly viscous supernatants were never observed.

Very reactive 1,2-glycol units physically adsorbed on the surface of the latex could have acted as activators for solution polymerization. As the glycol unit splits upon oxidation with Ce(IV), forming a free radical and an aldehyde (Iwakura et al., 1965; Mino and Kaizerman, 1959b) two initiating centers result. The ethylene glycol would have to have been present as an impurity in the HEA monomer used to produce the shell or it could have been produced by hydrolysis of HEA residues during grafting (by cleavage of the ester bond). As the monomer used for shell copolymerization was purified by fractionation, it is very unlikely that traces of ethylene glycol would still be present (the boiling point of ethylene glycol at 18 mmHg is 100°C; the main fraction during HEA purification came over at 77°C, at approximately 18 mmHg). Also, the copolymer shell preparation was done at neutral pH and MEA grafting at pH 2.7. Splitting the ester bonds under either condition is very improbable since it was found that highly basic conditions and long reaction times were necessary to hydrolyze the ester bonds in HEA residues.
- Chains with an appropriate content of hydroxyl groups desorbed from the beads during grafting could have promoted solution polymerization. The existence of such chains on the surface of the latex is consistent with the values of the copolymerization constants for styrene/HEA. Statistically, it is reasonable to expect that some adsorbed copolymers will contain relatively hydrophobic regions anchoring more hydrophilic tails. The conformation of such species adsorbed on a solid surface is known (Fleer et al., 1993). The styrene-rich part will form an anchor layer, while the HEA-rich parts, being in a good solvent, will extend into solution as free dangling loops or tails. As the grafting of MEA proceeds, these HEA-rich regions have a higher probability of being the first ones to react, as the hydroxyl groups which initiate polymerization will be more mobile in this region. The newly formed poly(MEA) grafted regions will increase the size of the hydrophilic part of the chains, ultimately leading to their desorption in some cases. In solution, they can continue to react and form higher molecular weight species.

This last possibility is the most likely to have occurred and caused the poor grafting efficiency. Considering the synthesis conditions for all the grafting experiments, batches H3GR1 and H3GR2 were prepared using comparatively small Ce(IV) concentrations (3x10^{-3} to 4.6x10^{-3} mole/l), so the termination rate would be relatively low. This explains the high molecular weight of the polymer obtained in solution, resulting in high supernatant viscosities. In all the other preparations, higher Ce(IV) concentrations likely caused lower molecular weight products to be formed in solution.
Table 4.3: Mass balance of MEA during the grafting experiments

<table>
<thead>
<tr>
<th>Batch #</th>
<th>MEA conversion</th>
<th>Poly(MEA) / g latex</th>
<th>Grafted chains 2) g/g latex</th>
<th>% reacted MEA in grafted chains 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% (HPLC)</td>
<td></td>
<td>Polymer</td>
<td>Short chains</td>
</tr>
<tr>
<td>H2GR1</td>
<td>20±5</td>
<td>0.068</td>
<td>~12.3x10^-4</td>
<td>~2.3x10^-4</td>
</tr>
<tr>
<td>H2GR2</td>
<td>43.5±5</td>
<td>0.159</td>
<td>~8.1x10^-4</td>
<td>~1.0x10^-4</td>
</tr>
<tr>
<td>H3GR1</td>
<td>72±5</td>
<td>0.562</td>
<td>17.8x10^-4</td>
<td>1.7x10^-4</td>
</tr>
<tr>
<td>H3GR2</td>
<td>50±5</td>
<td>0.153</td>
<td>2.6x10^-4</td>
<td>1.5x10^-4</td>
</tr>
<tr>
<td>H3GR3</td>
<td>29±5</td>
<td>0.133</td>
<td>8.3x10^-4</td>
<td>3.7x10^-4</td>
</tr>
<tr>
<td>H3GR5</td>
<td>31±5</td>
<td>0.285</td>
<td>9.8x10^-4</td>
<td>3.4x10^-4</td>
</tr>
</tbody>
</table>

1): Calculated under the assumption that all polymerized MEA was distributed on the beads (using MEA conversion data and the amount of seed in each recipe);
2): Calculated with formula [4.2], using the area of each peak on the chromatogram of grafted polymer solution;
3): Percentage of polymerized MEA associated with the grafted chains.

An alternate explanation for poor grafting efficiency has recently been suggested by Okieimen et al. (1996a, 1996b and 1996c), who also found high levels of homopolymer formation during grafting of various acrylates on modified cellulose or starch. They explain the result by assuming that most of the grafts are the result of interaction of homopolymer radicals with radicals on the support. In this model it is crucial, if a high frequency of grafting is to be achieved, that these sites are still reactive by the time the radicals formed in solution reach them. In the referenced work, addition of low molecular weight reducing agents such as isopropanol or thiourea increased the efficiency of grafting by promoting fast homopolymerization. Also, incremental addition of the monomer and the initiator enhanced the yield of the grafting reaction by producing active sites on the
support later in the process. Further experiments are needed in order to decide if the described mechanism is relevant to grafting on latex, however.

It is concluded that bulk MEA polymerization forming soluble polymers occurred in all the experiments to a much greater extent than the actual grafting reaction on the beads. Hence no straightforward assessment of the reaction conditions necessary to optimize MEA conversion can be made from the present results.

4.3.2.3. Characterization of the terminally-attached chains

In Table 4.4, a characterization of the grafted chains is given. The results combine the data obtained by size exclusion chromatography of the cleaved polymer with the absolute amount calculated from the area of the peaks to give an estimate of the area per molecule and the average chain separation between terminally-attached chains on the bead. All the calculations assume uniform distribution of the grafted chains on the latex surface. In the last column, the approximate radius of gyration of the polymer graft is calculated (assuming that the radius of gyration of the graft is the same as for the macromolecule in solution). As all the values calculated for the radius of gyration are smaller than the values of the chain separation, we conclude that the grafted chains take on a mushroom configuration (Fleer et al., 1993).

In all the grafting experiments, two categories of terminally attached chains were obtained: polymeric grafted chains (with DP between 18 and 360) and short grafted chains (with DP between 7 and 10). A possible explanation for this unusual distribution of molecular weights is that the mechanism of polymerization is strongly influenced by the local concentration of hydroxyl groups on the surface. As these groups start initiating,
Table 4.4: Characterization of the chains grafted on the beads

<table>
<thead>
<tr>
<th>Batch #</th>
<th>Grafted chains (^1) mole/g</th>
<th>Size distribution (^1) of grafted polymer mole %</th>
<th>Chain separation (^2) A</th>
<th>Radius of gyration (^3) A</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2GR1</td>
<td>~6.3 x 10^-9</td>
<td>~1</td>
<td>~236</td>
<td>45.9</td>
</tr>
<tr>
<td></td>
<td>~3.7 x 10^-7</td>
<td>~57.2</td>
<td>~31</td>
<td>14.5</td>
</tr>
<tr>
<td></td>
<td>~2.7 x 10^-7</td>
<td>~42.8</td>
<td>~36</td>
<td>7</td>
</tr>
<tr>
<td>H2GR2</td>
<td>~2.4 x 10^-9</td>
<td>~0.6</td>
<td>~383</td>
<td>47.9</td>
</tr>
<tr>
<td></td>
<td>~3.1 x 10^-7</td>
<td>~73.7</td>
<td>~34</td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td>~1.08 x 10^-7</td>
<td>~25.7</td>
<td>~57</td>
<td>7</td>
</tr>
<tr>
<td>H3GR1</td>
<td>4.3 x 10^-8</td>
<td>11.9</td>
<td>89</td>
<td>46.6</td>
</tr>
<tr>
<td></td>
<td>1.5 x 10^-7</td>
<td>41.3</td>
<td>48</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>1.7 x 10^-7</td>
<td>46.8</td>
<td>45</td>
<td>8</td>
</tr>
<tr>
<td>H3GR2</td>
<td>1.4 x 10^-7</td>
<td>48.3</td>
<td>49</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>1.5 x 10^-7</td>
<td>51.7</td>
<td>47</td>
<td>8</td>
</tr>
<tr>
<td>H3GR3</td>
<td>1.5 x 10^-8</td>
<td>3.7</td>
<td>150</td>
<td>50.3</td>
</tr>
<tr>
<td></td>
<td>1.1 x 10^-7</td>
<td>27.2</td>
<td>56</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>2.8 x 10^-7</td>
<td>69.1</td>
<td>34</td>
<td>9</td>
</tr>
<tr>
<td>H3GR5</td>
<td>1.6 x 10^-8</td>
<td>3.4</td>
<td>146</td>
<td>47.0</td>
</tr>
<tr>
<td></td>
<td>1.6 x 10^-7</td>
<td>33.6</td>
<td>46</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>3.0 x 10^-7</td>
<td>63.0</td>
<td>34</td>
<td>9</td>
</tr>
</tbody>
</table>

\(^1\): Determined by SEC analysis of the cleaved tentacles.
\(^2\): Calculated with the formula: \(D = \left[ \frac{SSA}{(G N_A)} \right]^{1/2}, \text{Å} \)

where:

- \(SSA = \text{surface area per gram solid beads, } \text{Å}^2\)
- \(G = \text{amount of grafted chains cleaved from } 1\ \text{gram latex, mole/g latex}\)
- \(N_A = \text{Avogadro's number, mole}^1\)
- \(G = \text{GW}/M_n, \text{where:}\)
  - \(\text{GW} = \text{mass of grafted polymer per gram latex, calculated with formula [4.2];}\)
  - \(M_n = \text{number average molecular weight of grafted chains, calculated with formula [4.1] using the elution volume of each fraction;}\)

\(^3\): Estimated, according to reference (Fleer et al., 1993), \(R_g = a M_n^{1/2}, \text{linear polymers in near theta solvent, where:}\)

- \(a: 261 \times 10^{-4}, \text{nm (PMMA).}\)

those which are sufficiently close to each other can mutually terminate by combination or disproportionation and account for the short graft population. Groups which are further apart or are separated by terminated oligomers initiate chains which continue to grow. At
some point, some of the very long chains will restrict monomer diffusion towards shorter growing chains located under them, i.e., within a radius of gyration of the longer grafted chains. This could result in the two populations of chains observed. The fact that the grafted polymer cleaved from the beads containing the highest surface concentration of hydroxyl groups in the shell (batch H2) contained mostly short chains (99 mole % on H2GR1 and 99.4 mole % on H2GR2), independent of the Ce(IV) concentration, is consistent with the above mechanism.

The results show that higher Ce(IV) concentrations improved the grafting efficiency over the concentration range utilized. The overall coverage with grafted chains was higher on batch H2GR1 compared to H2GR2, which was synthesized at lower Ce(IV) concentration. The same result was obtained for batch H3GR3 when compared with batch H3GR2 (grafted under the same conditions, except for lower Ce(IV) concentration for batch H3GR2). Given the high valence of the cerium ions and the positive charge of the surface, electrostatic repulsion plays a significant role in the initiation step. By varying Ce(IV) concentration, important changes in the ionic strength of the supernatant resulted. The electrostatic repulsion will be weaker at high ionic strength, due to lower surface potential values (Israelachvili, 1991). For this reason, surface initiation is probably more frequent at high Ce(IV) concentrations. The observed influence of the parameter is also consistent with the mechanism of initiation and with results reported for grafting on cellulose (Okieimen et al., 1996c). According to these studies, the grafting efficiency would level off or decrease at higher Ce(IV) concentrations, due to prevalence of
termination over the propagation reaction. This range wasn’t investigated in the present study.

Batches H3GR1 and H3GR2 were synthesized under the same conditions, the only difference being a higher monomer concentration for H3GR1. The oligomeric graft content was approximately the same. The coverage with polymeric grafted chains was higher at higher monomer concentration, however. A similar result was obtained comparing batches H3GR3 and H3GR5, the second one being synthesized using higher monomer concentration. The effect was the same as observed for the grafting on aldehyde latex in Chapter 3.

In conclusion, the composition of the terminally-attached chains was affected by the copolymer shell composition, and the Ce(IV) and monomer concentration. More investigation is required in order to understand the grafting conditions sufficiently to tailor the grafted chains concentration and molecular weight distribution.

4.2.3.4. Comparison between grafting reaction on aldehyde and on hydroxyl beads

A quantitative comparison between the two surfaces with respect to the efficiency of grafting can not be made because the data for the aldehyde latex are incomplete (the amount of grafted material was measured indirectly). However, the differences can be estimated by considering the mechanism of copolymer shell preparation and Ce(IV) initiated grafting. By comparing the reactivity ratios for the pair acrolein/styrene (0.25/0.25) with the one for HEA/styrene pair (0.72/0.73), the fact that a higher content of alternating sequences is more probable in the acrolein/styrene copolymerization is evident. It is therefore reasonable to expect that the aldehyde groups would be better
anchored to the bead and more uniformly distributed on the surface than the hydroxyl groups. The fact that poly(HEA) is soluble in water, while poly(acrolein) is not, provides further support to the interpretation that desorption of copolymer chains during grafting will occur to a lesser extent in the case of aldehyde latex. In conclusion, even if solution polymerization of MEA occurred during grafting on the beads carrying the acrolein/styrene copolymer shell, it was less significant than during grafting on the beads covered with HEA/styrene.

The probable configuration of the complex formed between the cerium ion and the reducing group located on the surface is shown in the Appendix, Figure A.1. In the case of the hydroxylated latex, the grafting point is located further away from the backbone of the shell than in the case of the aldehyde latex. The complex with the aldehyde is bulkier due to the hydrated structure of the complex. The location and the structure of the complex has three consequences:

- The cerium ion has to penetrate deeper into the electric double layer surrounding the aldehyde bead in order to initiate polymerization. Due to electrostatic repulsion between the Ce$^{4+}$ ion and the positively charged latex, fewer sites are likely to be initiated.

- The bulky configuration of the complex makes initiation on neighboring sites less probable in the case of the aldehyde latex compared to the hydroxylated one.

- Once the polymerization is started, the grafts originating from the hydroxyl group have a higher probability of mutual termination in the early stages. Their contact is
facilitated by the fact that they are closer to each other and are located at the ends of chains which are more mobile.

For these reasons, the density of the long grafted chains is more likely higher when aldehyde latex is used.

### Table 4.5: Characterization of the surface of the grafted beads

<table>
<thead>
<tr>
<th>Batch #</th>
<th>Bead area $\text{Å}^2$</th>
<th>$a_0$, $\text{Å}^2$</th>
<th>$a_1$, $\text{Å}^2$</th>
<th>$a_2$, $\text{Å}^2$</th>
<th>$a_{\text{OH}}$, $\text{Å}^2$</th>
<th>Mushroom coverage %</th>
<th>Hydrophil. coverage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2GR1</td>
<td>2.3x10⁹</td>
<td>2.7x10⁸</td>
<td>15.7x10⁸</td>
<td>2.7x10⁸</td>
<td>3.4x10⁹</td>
<td>93.2</td>
<td>244.7</td>
</tr>
<tr>
<td>H2GR2</td>
<td>2.3x10⁹</td>
<td>1.1x10⁸</td>
<td>9.8x10⁸</td>
<td>1.1x10⁸</td>
<td>3.5x10⁹</td>
<td>53.5</td>
<td>207.3</td>
</tr>
<tr>
<td>H3GR1</td>
<td>2.4x10⁹</td>
<td>2.4x10⁸</td>
<td>6.2x10⁸</td>
<td>21x10⁸</td>
<td>2.3x10⁹</td>
<td>122.1</td>
<td>218.7</td>
</tr>
<tr>
<td>H3GR2</td>
<td>2.4x10⁹</td>
<td>2.2x10⁸</td>
<td>3.9x10⁸</td>
<td>0</td>
<td>2.4x10⁹</td>
<td>25.0</td>
<td>122.5</td>
</tr>
<tr>
<td>H3GR3</td>
<td>2.4x10⁹</td>
<td>5.2x10⁸</td>
<td>4.1x10⁸</td>
<td>8.5x10⁸</td>
<td>2.4x10⁹</td>
<td>73.4</td>
<td>170.9</td>
</tr>
<tr>
<td>H3GR5</td>
<td>2.4x10⁹</td>
<td>5.4x10⁸</td>
<td>6.6x10⁸</td>
<td>7.9x10⁸</td>
<td>2.3x10⁹</td>
<td>82.3</td>
<td>179.0</td>
</tr>
</tbody>
</table>

1): $a_x$ is the area covered by a specific group on one bead (the subscript “x” is assigned as: 0 for chains with DP<10, 1 for short grafted chains, 2 for long grafted chains and OH for unreacted hydroxyl groups);

Area covered by one grafted chain was modeled as a circle of radius $R_g$.

2): Area covered by one unreacted hydroxyl group was considered (Davies and Rideal, 1961) $20\text{Å}^2$.

3): Mushroom coverage is the percentage of the bead area covered by tethers ($a_0$, $a_1$ and $a_2$);

4): Hydrophilic coverage is the percentage of the bead area covered by all groups on the surface (grafted chains and unreacted hydroxyl groups); see discussion in section 4.3.2.5.

### 4.3.2.5. Model of the surface

In Table 4.5, a model for the surface of the grafted beads is presented. The area covered by grafted chains was modeled as a circle of radius $R_g$, as it is done for an
unconstrained mushroom (Fleer et al., 1993). The goal was to calculate the percentage of the area of the bead that was covered.

Two levels of coverage are considered in the table: hydrophilic coverage, which is calculated as the sum of the areas per species for all groups (including unreacted HEA) and mushroom coverage, which is the percentage of the area covered by oligomeric or polymeric grafted chains only. The unreacted hydroxyl groups were included in the calculation because they are hydrophilic, and, according to the electrophoretic measurements done on the latex covered with the shell, they contribute to shielding the positive charge on the surface of the seed.

The results show that, for all the batches, the hydrophobic poly(styrene) surface of the bead was completely covered on average with hydrophilic groups (all values in the last column of Table 4.5 are higher than 100%). This statement is consistent with the electrophoretic mobility of the grafted beads, which was zero for all batches. That is, the effect of the positive charge initially present on the seed was completely shielded. The fact that most of the numbers are much higher than 100% is likely due either to small groups overlapping in the shadow of long chains or to a poor approximation of the area occupied by various species.

The mushroom coverage was between 25 and 120%. Given the size distribution of the grafted chains and the model for the distribution outlined above, we the surface can be visualized as a "lawn" of hydroxyl groups with interspersed "shrubs" (oligomers) and "trees" (polymers) extending above it about 50Å away from the surface (approximately...
equal to $R_g$ for the largest grafted chains). The role these species play when the surface is placed in contact with protein solutions is discussed in Chapter 5.

4.4. CONCLUSIONS

A two step method for introducing cleavable grafted chains on the surface of cationic polystyrene beads was developed. In the first step, the beads were covered with a shell containing poly(styrene-co-hydroxy-ethyl acrylate) by a seed polymerization procedure. The density of the hydroxyl groups introduced on the surface was assessed by saponification of the ester bonds under optimal conditions, followed by conductometric titration of the resulting carboxylic residues left on the latex. It was found that an acrylic acid impurity in HEA monomer can change the charge of the beads during the synthesis of the shell. Careful purification of the monomer removed the impurity efficiently. The amount of HEA associated with the beads was found to be between $1.7 \times 10^{-5}$ and $8 \times 10^{-5}$ mole/g latex (surface density $1.5 \times 10^{-2}$ to $8.6 \times 10^{-2}$ groups/$\AA^2$), representing approximately 3% of the consumed HEA. In the second step, cleavable grafted chains were introduced on the surface by Ce(IV) initiated polymerization of MEA activated by hydroxyl groups. MEA consumption kinetics were monitored by HPLC of supernatant. The results agree with previous studies. The factors which influence the grafting reaction were studied: density of starting groups, Ce(IV) and MEA concentration. The last two factors, when increased within the studied range, improved the efficiency of grafting. The first factor influenced the distribution of the grafted chains (a higher density of hydroxyl groups on the surface produced a larger population of short chains). In all the grafting experiments,
competing solution polymerization of MEA occurred, due to oligomer leakage from the beads.

Quantitative size exclusion chromatography was used to characterize the cleaved chains. The distribution of the grafts showed an unusual trimodal distribution on most of the batches, probably due to the proximity of the starting points on the surface of the beads. Long grafted chains with a degree of polymerization of approximately 250 units and a surface density between $4.4 \times 10^{-5}$ and $1.3 \times 10^{-4}$ chains/Å$^2$ were found on the surface. Populations of short chains (DP approximately 20) and oligomers (DP about 8) were also found, at densities of about $6 \times 10^4$ chains/Å$^2$. The probable conformation of each of these species is a mushroom, but the height to which they extend into solution is different, depending on their size. The maximum height was estimated to be 50 Å from the bead surface. The degree of coverage of the surface was calculated at two levels: the hydrophilic coverage (i.e. by all hydrophilic groups, including unreacted hydroxyl groups) was more than 100% for all the batches, which is consistent with the electrophoretic measurements; the mushroom coverage (i.e. coverage with grafted chains only) varied between 25 and 122%.
CHAPTER 5: Interaction of grafted latex with protein solutions

5.1. INTRODUCTION
The grafted beads prepared and characterized as described previously were exposed to protein solutions. The first objective was to verify that the tethered layer reduces non-specific protein adsorption on the surface, with the eventual goal of improved biocompatibility. Secondly, the well characterized model surface was used to test the predictions of the Flory-Huggins mean field theory calculations as applied to size-exclusion chromatography by Brooks and Müller, 1996. Thirdly, the experimental data were compared qualitatively with the results predicted by self-consistent field (SCF) calculations for the interaction of terminally-attached polymer chains with proteins, as presented by Steels, 1998 and Steels and Haynes, 1998. Both models were briefly described in section 1.5 (Chapter 1).

The tethered beads were used as stationary phases for SEC of standard proteins. The parameters which influence the value of the partition coefficient were studied: the molecular weight of the proteins, the properties of the grafted layer and the ionic strength of the mobile phase.

5.2. MATERIALS AND METHODS
The proteins used in the study were (MW, isoelectric pH): insulin (6500, 5.3), myoglobin (17200, 7.0), trypsin inhibitor (21500, 4.5), carbonic anhydrase (30000, 5.9), β-lactoglobulin (36000, 5.2), ovalbumin (43000, 4.6), bovine serum albumin (68000, 4.9), human serum albumin (66000, 4.9), keyhole lympid hemocyanin (KLH) (3x10^6) and
Lambda DNA (32×10^6). DNA was from Promega Corp. (Madison, WI); all the other proteins were from Sigma (St. Louis, MO). Sodium phosphate (NaH₂PO₄) and sodium chloride used to prepare the mobile phase were reagent grade, purchased from Aldrich (Milwaukee, WIS). The source and the purification methods for all the other materials was described in the previous chapters.

The beads used as stationary phases in this study were synthesized by Ce(IV) initiated grafting of MEA on core-shell polystyrene latex bearing aldehyde groups (batches A2GR, A8GR, A10GR1 and A10GR3) or hydroxyl groups (batches H2GR1, H2GR2, H3GR2, H3GR3 and H3GR5) described in Chapter 3 and 4. A latex without a grafted layer was also used, as a blank. For this purpose, latex A8 (core-shell beads bearing aldehyde groups, precursor of latex A8GR) was reacted with hydroxylamine hydrochloride in order to replace the aldehyde groups, potentially reactive towards amino residues in the protein molecules (Slomkowski et al., 1992), with oxime groups, according to the reaction described in Chapter 3. The procedure was the following: an exact volume (0.6 ml) of 0.1M hydroxylamine hydrochloride solution was added to a concentrated suspension of latex A8 (23.3% solid content), previously weighed in a polypropylene tube (4 g). The mixture was left to react overnight, while tumbling in a rotating rack at room temperature. The product was then cleaned by centrifugation and supernatant replacement (three times; completion checked by neutrality of the supernatant).

HPLC analysis of protein solutions was performed on a Merck-Hitachi (inert version) system (E. Merck, Darmstadt), equipped with a Merck-Hitachi L4200 detector. The columns were prepared as described below:
Typically a concentrated suspension of latex (approximately 20% solids in distilled water) was packed under pressure in a steel HPLC column (Upchurch Scientific, Oak Harbor, WA), size 75 x 4.6 mm. The column was equilibrated with the mobile phase at room temperature, at a flow rate of 0.1 ml/min (unless otherwise stated). Most of the measurements were done using a relatively high ionic strength buffer (10 mM sodium phosphate, 300 mM sodium chloride, pH=7.2). In order to test the effect of the ionic strength of the mobile phase on the partition coefficients of proteins, some measurements were done using a low ionic strength buffer (2.5 mM sodium phosphate, 2.5 mM sodium chloride, pH=7.2). The pH of the buffer was adjusted using a 1 M sodium hydroxide solution.

Protein solutions in the mobile phase were prepared just prior to use at a concentration of 1 mg/ml in most cases, except myoglobin (0.5 mg/ml) and DNA (15.6x 10^{-3} mg/ml). DNA concentration was chosen in the range in which the elution time didn’t depend on concentration. Aliquots of 20 μl of each protein solution were run on every column at a flow rate of 0.1 ml/min (0.03 ml/min for H3GR2 column), monitoring at 260 nm for DNA and at 280 nm for all others.

The following characteristics of the columns were determined as described in the literature (Hagel, 1989):

- void volume, \( V_0 \), equal to the elution volume of a nonretained peak; a high molecular weight protein, KLH, or DNA was used for this purpose (elution volume was measured for both materials and the lowest value was reported as the void volume of the column);
- total liquid volume, \( V_t \), equal to the elution volume of a low molecular weight substance (sodium nitrate, 1.5 mg/ml, 20 \( \mu \)l injected, monitored at 280 nm) which can totally penetrate the column;

- number of theoretical plates per unit length, calculated from:

\[
N = \left( \frac{V_t}{h_r} \right)^2 \times 5.54 \times \frac{1}{(7.5 \times 10^{-2})}, \text{ m}^{-1} \quad [5.1]
\]

where \( h_r \) = half width of the NaNO\(_3\) peak, ml.

- permeability, calculated with the formula:

\[
P = \frac{V_t - V_0}{V_0} \quad [5.2]
\]

The partition coefficient, \( K_D \), for each protein was calculated from:

\[
K_D = \frac{(V_e - V_0)}{(V_t - V_0)} \quad [5.3]
\]

where \( V_e \) is the elution volume of the protein solution.

Equation [5.1] gives a good approximation for the number of theoretical plates when the shape of the peaks is approximately Gaussian. Most of the experimental chromatograms met this condition.

5.3. RESULTS AND DISCUSSION

5.3.1. Theoretical background

The result of the mean field theory calculations (Brooks and Müller, 1996) expressed by equation [1.7] was further developed to specifically show the effect of the density and the length of the grafted chains upon the partition coefficient of the solute. By recognizing
that the volume fraction of the grafted polymer, \( \phi_2^e \), as defined in equation [1.4] is the ratio of the number of sites on the lattice occupied by component 2 over the total number of sites, it follows that:

\[
\phi_2^e = \frac{n_2 P_2 v_1}{V}
\]  

[5.4]

where \( V = \) total volume of the lattice, \( \AA^3 \);
\( v_1 = \) volume of the solvent molecule, \( \AA^3 \).

In equation [5.4], the volume of the lattice can be replaced by the product of area (A) and the height (h) of the grafted layer, \( V = Ah \), thus:

\[
\phi_2^e = \frac{\sigma P_2 v_1}{h}
\]  

[5.5]

where \( \sigma = n_2 / A = \) density of the grafted chains (chains/unit area), \( \AA^{-2} \);
\( h = \) height of the grafted layer, \( \AA \).

By replacing the expression for \( \phi_2^e \) given by equation [5.5], equation [1.7] becomes:

\[
K = \exp \left[ -P_3 \frac{\sigma P_2 v_1}{h} \left( 1 - 1 / P_2 - \chi_{13} + \chi_{23} \right) \right]
\]  

[5.6]

Equation [5.6] can be used to predict the qualities of the surface for an optimum exclusion effect. The equation has to be analyzed separately for two different regimes of surface coverage, each with a specific configuration of the chains. When the distance \( d \) between the grafting points (\( d \approx \sigma^{-1/2} \)) is longer than the radius of gyration of the grafted chains, in the absence of any favorable energetic interaction between chain segments and
the surface, a random coil configuration is adopted, commonly called a “mushroom”.

When the grafted chains overlap due to a distance d shorter than their radius of gyration, they adopt a stretched configuration, extended away from the surface, called “brush” (de Gennes, 1979). The height of the grafted layer depends therefore upon the regime of coverage. In the mushroom regime the extension of the grafts into solution is approximately \(2R_g\) (where \(R_g\) = radius of gyration of the terminally attached chains) (Fleer et al., 1993). The height of the tethered layer, at equilibrium, is:

\[
h_m = 2R_g = 2a \text{DP}^{1/2} = 2a \text{P}_2^{1/2} \left( \frac{v_1}{v_2} \right)^{1/2} \tag{5.7}
\]

where \(\text{DP}\) = average degree of polymerization of the grafted chains;

\(a = \text{constant depending on the nature of the grafted polymer and the solvent, } \AA;\)

\(v_2 = \text{volume of one grafted monomer, } \AA^3.\)

In the brush regime, the equilibrium height of the grafted layer can be written as (Fleer et al., 1993):

\[
h_b \equiv \text{DP} \left( \sigma l^2 \right)^{1/3} = \text{P}_2 \left( \frac{v_1}{v_2} \right) \left( \sigma l^2 \right)^{1/3} \tag{5.8}
\]

where \(l = \text{length of one unit in the grafted polymer chain, } \AA.\)

By replacing the height given by equation [5.7] in the expression for the partition coefficient, [5.6], an equation applicable in the mushroom regime may be obtained:

\[
K = \exp \left[ -\frac{P_3}{3} \sigma P_2^{1/2} \frac{v_1^{1/2} v_2^{1/2}}{2a} \left( 1 - 1/P_2 - \chi_{13} + \chi_{23} \right) \right] \tag{5.9 a}
\]
Equation [5.9 a] shows that, in the mushroom regime, the partition coefficient of the solute decreases, i.e., exclusion increases, when more dense and/or longer chains are grafted on the surface. As the dependence on $\sigma$ is stronger, it implies that increasing the density is a more efficient way to achieve better excluding surfaces than introducing longer chains.

Similarly, by replacing the height given by equation [5.8] in the expression for the partition coefficient, [5.6], an equation applicable in the brush regime may be obtained:

$$K = \exp\left[-\frac{P_3 \sigma^{2/3} \sum_{i=1}^{N_{MEA}} (1 - \frac{1}{P_2 - \chi_{13} + \chi_{23}}) \right]$$  \[5.9 b\]

Equation [5.9 b] shows that in the brush regime, the partition coefficient of the solute decreases when more dense and/or longer chains are introduced on the surface. The dependence is weaker than in the mushroom regime.

Equation [5.6] will be referred to when analyzing data for beads produced by grafting on hydroxylated latex, for which both the density and molecular weight of the tethers are known. For the batches prepared by grafting on aldehyde groups, the experimentally determined parameter is the tether coverage $N_{MEA} (\text{Å}^2)$, calculated as the number of MEA groups grafted per unit area of the beads. The density of the grafted chains is related to the tether coverage by the formula:

$$\sigma = \frac{N_{MEA}}{DP}$$  \[5.10\]

where $DP =$ average degree of polymerization of the tethers.
Also, $P_2$ can be replaced with:

$$P_2 = DP^\frac{V_2}{V_1}$$  \[5.11\]

where $V_2 = $ volume of one grafted polymer segment, Å$^3$.

By substituting both $\sigma$ and $P_2$ in equation [5.6], the dependence of the partition coefficient in terms of the tether coverage can be written as:

$$K = \exp\left[ -P_3 \frac{N_{MEA} V_2}{h} \left( 1 - \frac{1}{P_2} - \chi_{13} + \chi_{23} \right) \right]$$  \[5.12\]

5.3.2. Latex prepared by grafting on aldehyde groups

The properties of the beads and the characteristics of the columns are presented in Table 5.1. The results show that the beads with the highest tether coverage (A8GR) gave the column with the highest number of theoretical plates and the highest permeability. In terms of traditional pore models, the permeability (porosity) is interpreted as the ratio of the pore volume to the void volume; the higher this ratio, the more selective the column (Goodwing et al., 1990). This interpretation of the separation mechanism has led to the synthesis of supports with as high a pore volume as possible. Mechanical stability imposes a limit to this approach however, and introduction of crosslinking chemistry to rigidify the gels has led to adsorption problems. The present approach shows that, by tailoring the grafted surface, the selectivity of the column can be varied without affecting the mechanical strength of the support.
Table 5.1: Characterization of the beads and parameters of columns in the aldehyde series

<table>
<thead>
<tr>
<th>Column</th>
<th>Particle diameter µm</th>
<th>Polymerized MEA 1) mole/g latex</th>
<th>Tether coverage 2) N_{MEA} (Å$^2$)</th>
<th>Length of grafted chains 3) DP</th>
<th>N m$^{-1}$</th>
<th>Permeability</th>
<th>Pressure bar</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2GR</td>
<td>2.30±1%</td>
<td>1.40x10$^{-3}$</td>
<td>3.41</td>
<td>76</td>
<td>10306</td>
<td>0.16</td>
<td>15</td>
</tr>
<tr>
<td>A8GR</td>
<td>2.66±0.8%</td>
<td>3.53x10$^{-3}$</td>
<td>9.93</td>
<td>350</td>
<td>14200</td>
<td>0.35</td>
<td>20</td>
</tr>
<tr>
<td>A10GR1</td>
<td>2.76±0.8%</td>
<td>0.43x10$^{-3}$</td>
<td>1.26</td>
<td>60</td>
<td>6187</td>
<td>0.31</td>
<td>8</td>
</tr>
<tr>
<td>A10GR3</td>
<td>2.76±0.8%</td>
<td>2.00x10$^{-3}$</td>
<td>5.88</td>
<td>283</td>
<td>10040</td>
<td>0.27</td>
<td>18</td>
</tr>
</tbody>
</table>

1) : calculated under the assumption that all polymerized MEA was distributed on the beads, using monomer conversion determined by HPLC of the supernatant and the amount of seed in each grafting experiment;

2) : calculated with the formula:

$N_{MEA} = \frac{T \cdot N_A}{SSA}$, MEA groups/Å$^2$.

where $T = \text{polymerized MEA amount, moles/g latex;}$

$SSA = \text{surface area available on the latex, Å}^2/g \text{ latex;}$

$N_A = \text{Avogadro’s number, mole}^{-1};$

3) : assuming that all the aldehyde groups initially present on the latex started grafted chains.
There is a limitation in using grafted beads, however, as shown in Table 5.1, where the back pressures are recorded. Column A8GR had the highest back pressure among packings with approximately the same diameter (A8GR, A10GR1 and A10GR3). The explanation of this effect is likely that longer grafted chains create a stronger viscous drag around the beads when mobile phase flows through the bed, requiring a higher pressure in order to achieve the same nominal flow velocity in the column. This explanation is consistent with the fact that the blank column, packed with ungrafted beads, had a back pressure of only 11 bars, compared to 20 bars for the column packed with grafted beads of the same size running at the same flow rate. This effect could create problems when packing bigger columns for preparative applications. Using bigger beads will decrease the back pressure but at the cost of decreasing surface area of the packing, which in turn will lower the capacity. Hence, the challenge with the grafted beads will be to optimize the properties of the grafted layer to obtain the maximum efficiency with the minimum back pressure.

In Figure 5.1, the elution volume as a function of analyte molecular weight for column A8GR and its ungrafted blank are presented. The beads bearing grafted chains produce molecular weight-dependent variation of the elution volumes, while the ungrafted beads display almost constant elution volume, independent of the size of solute. This result can be explained qualitatively by analyzing equation [1.9]. The terms containing \( \chi \) parameters describe the contribution of the enthalpic interactions between the analyte and the other two components in the system (grafted polymer and solvent), while the other terms
describe the contribution of the entropic interactions. If no grafted chains are present, no exclusion effect should occur, as observed experimentally. On the other hand, upon the interaction with a grafted surface, the system loses entropy because fewer conformations are available both for the analyte and the immobilized chains. Interaction with the surface is therefore unfavorable from this point of view. The enthalpic terms usually favor adsorption, either by electrostatic attraction between the analyte and the surface, by weak hydrophilic interactions or by hydrophobic interactions. If the effect is strong enough, values of K>1 can be obtained. In the case of the A8 blank column, the beads are positively charged but covered with a wettable layer. No chemical interactions between the stationary phase and the proteins were evident, but weak electrostatic attraction was probably present for myoglobin (at pH=7.2, myoglobin has a small negative charge, as the isoelectric point for myoglobin is 7.0), for which the elution volume was larger than for the other solutes. In the case of the A8GR column, the loss in entropy upon mixing with the grafted layer phase more than compensated for any gain in enthalpy, resulting in a protein excluding effect. This effect was stronger for larger molecules (elution volumes decreased as the molecular weight of the analyte increased), as predicted by [1.7]. A separation based on size occurred, as less column volume was available to large molecules than to small ones.
Figure 5.1: Elution volume as a function of analyte molecular weight for columns A8 blank, packed with beads without poly(MEA) graft (open squares) and A8GR, packed with beads carrying terminally attached chains (filled squares). Solutes loaded include: sodium nitrate, myoglobin, HSA and KLH.

In Figure 5.2, the variation of the partition coefficients of two proteins (myoglobin and human serum albumin) as a function of the tether coverage of the support is shown. The partition coefficient of the higher molecular weight albumin is lower than the one of myoglobin on all four columns, as the model predicts (in equation [1.9], increasing $P_3$ results in smaller values for $\ln K$). The partition coefficients decrease with increasing tether coverage for both proteins. This is consistent with the observation that, in equation [5.12], at constant $P_3$, $\ln K$ decreases as the parameter $N_{MEA}$ increases. The difference ($\ln K_{myo} - \ln K_{HSA}$) in absolute value increased with increasing the tether coverage, that is the
molecular weight selectivity of the columns was higher when more polymer was present in the grafted layer. In equation [5.12], the slope of $\ln K$ with respect to $P_3$ increases as

\[ \text{InK} = \alpha + \beta P_3 \]

Figure 5.2: Variation of the partition coefficient of myoglobin (open squares) and HSA (filled circles) \textit{versus} grafted poly(MEA) coverage for columns A2GR, A8GR, A10GR1 and A10GR3.

$N_{\text{MEA}}$ is increased or $h$ is decreased. The results suggest therefore that the increase in molecular weight selectivity was due to more dense grafted chains on the surface and/or to shorter chains. The parameter calculated to characterize the tether coverage does not give separate information about the two effects.

In Table 5.2 the estimated parameters of the model in terms of density of grafted chains, height of the layer and volume fraction of the tethered polymer are presented. Values of $\phi_2^8$ higher than 1 were obtained for all the batches. The likely explanation of this
result is that the tether coverage determined from monomer conversion overestimated the actual amount of polymer present on the beads. It is likely that solution polymerization of MEA occurred on the aldehyde beads too (see discussion of the grafting efficiency in Chapter 4). A quantitative estimation of the degree to which this event took place was not possible. Further simulation of the chromatographic data for the aldehyde beads was not pursued, therefore.

Table 5.2: Modeled parameters of the grafted layer for beads in the aldehyde series

<table>
<thead>
<tr>
<th>Batch #</th>
<th>$\sigma$ 1)</th>
<th>$P_2$ 2)</th>
<th>$h$ 3)</th>
<th>$\phi_2$ 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2GR</td>
<td>0.045</td>
<td>485.2</td>
<td>33.4</td>
<td>19.3</td>
</tr>
<tr>
<td>A8GR</td>
<td>0.028</td>
<td>2234.6</td>
<td>132.6</td>
<td>14.1</td>
</tr>
<tr>
<td>A10GR1</td>
<td>0.021</td>
<td>383.1</td>
<td>20.4</td>
<td>11.6</td>
</tr>
<tr>
<td>A10GR3</td>
<td>0.021</td>
<td>1806.8</td>
<td>97.4</td>
<td>11.6</td>
</tr>
</tbody>
</table>

1) : Calculated with equation [5.10], using the tether coverage and the DP of grafted chains shown in Table 5.1.
2) : Calculated with equation [5.11], where $v_1 = 29.9 \, \text{Å}^3$ and $v_2 = 190.9 \, \text{Å}^3$. The volumes were calculated using the formula:

$$v = \frac{10^{24} \, \text{MW}}{(\rho \, N_A)}$$

where $\text{MW} = \text{molecular weight, g/mole (MW}_1 = 18; \text{MW}_2 = 129)$;

$$\rho = \text{density, g/cm}^3 \, (\rho_1 = 1; \rho_2 = 1.122; \rho_2 \, \text{assumed equal with the density of acrylamide [Polymer Handbook]})$$;

$$N_A = \text{Avogadro's number, mole}^{-1}.$$

3) : Calculated for brush configuration of the grafted layer, equation [5.8];

$$l = 1.4 \, \text{Å} \, (\text{length of C-C simple bond}).$$

4) : Calculated with equation [5.5].
In Figure 5.3, the variation of the partition coefficient obtained on column A10GR1 and A10GR3 versus molecular weight of the proteins is plotted. On both columns, the plot is approximately linear, as predicted in equation [1.9]. The parameters of the lines are:

- A10GR1: slope = -4.9x10^{-6}, intercept = -0.059, R = -0.811; \( P_N (|R| \geq 0.811) = 10\% \);

- A10GR3: slope = -1.1x10^{-5}, intercept = -0.584, R = -0.927; \( P_N (|R| \geq 0.927) = 3.7\% \).

where \( P_N \) = the percentage probability that \( N \) measurements of two uncorrelated variables give a correlation coefficient higher than the experimental value, \( R \) (Taylor, 1982);

\( N = 5 \) (number of experimental points).

Figure 5.3: Partition coefficients as a function of protein molecular weight for columns A10GR1 (filled squares) and A10GR3 (open circles).

Proteins include: insulin, myoglobin, trypsin inhibitor, ovalbumin, BSA and HSA.
The results show therefore a significant ($P_N \leq 5\%$) linear correlation for column A10GR3.

The tether coverage was higher for A10GR3 beads compared to A10GR1 implying longer and/or more dense grafted chains. The grafting conditions were the same, except for monomer concentration, which was higher for A10GR3. Given the influence of this factor in the synthesis of the hydroxyl beads (see discussion in Chapter 4), it seems more likely that the density of the long chains was higher in A10GR3 synthesis. As predicted by equation [1.9], an increase in $\phi_{g}$ or in $P_2$ results in a stronger exclusion effect, causing lower values of partition coefficients for all the proteins.

In conclusion, the experimental data for the beads obtained by grafting on aldehyde functions demonstrate qualitatively that the mean field theory of polymer solutions as applied to size-exclusion chromatography (Brooks and Müller, 1996) describes correctly the trends observed when proteins interact with surfaces bearing grafted chains.

The effect of the ionic strength of the mobile phase was studied by running a series of proteins (insulin, myoglobin, trypsin inhibitor and ovalbumin) in high and low ionic strength on column A3GR3. Since most of the proteins chromatographed bore a net negative charge at the experimental pH ($pH > pI$; $pI = $ isoelectric point of protein) and since the latex has a net positive surface charge from its cationic initiator, any significant exposure of the surface would be expected to result in protein adsorption. This effect is generally stronger at low ionic strength due to stronger electrostatic attraction (Fleer et al., 1993; Andrade et al., 1992). The elution volumes at low ionic strength did not differ by more than 10% from those at high ionic strength. Also, no peak broadening was
observed. This implies that the grafted chains effectively shielded the surface charge. The blank column was not run at low ionic strength.

5.3.3. Latex prepared by grafting on hydroxyl groups

The properties of the beads and the characteristics of the columns are presented in Table 5.3. The grafting reaction was less efficient when using the hydroxylated beads, due to greater extent of solution polymerization of MEA (see discussion of grafting efficiency, Chapter 4). As a result, the tether coverage, $N_{MEA}$ (Å$^2$), was two orders of magnitude less on these beads. It should be noted however, that in the case of the aldehyde batches, no direct assessment of the grafted polymer present on the beads was done, therefore the comparison is only qualitative. It is possible, for example, that the grafted amount determined by analysis of the supernatant was overestimated and that some MEA polymerized in solution, too. On the other hand, beads in the aldehyde series showed much better chromatography performance (results for the hydroxylated beads follow). Also, none of the supernatants following the aldehyde grafting was viscous, which was not the case for hydroxylated latex. Thus, it is concluded that the graft coverage was higher and more efficient in protein exclusion in the aldehyde series.

The data in Table 5.3 show that the back pressure on column H3GR2 was very high so that the flow rate had to be reduced to 0.03 ml/min (compared to 0.1 ml/min for all the other columns). Batch H3GR2 had the lowest tether coverage among the hydroxylated beads and analysis of the cleaved tethers proved that it had only short ones. This is inconsistent with the previously observed effect, that the longer the grafted chains, the
Table 5.3: Characterization of the beads and parameters of columns in hydroxyl series

<table>
<thead>
<tr>
<th>Column</th>
<th>Particle diameter μm</th>
<th>Polymerized MEA (^1) mole/g latex</th>
<th>Tether coverage (^2) (N_{\text{MEA}} (\text{Å}^2))</th>
<th>Length of grafted chains (^3) DP</th>
<th>N (\text{m}^1)</th>
<th>Permeability</th>
<th>Pressure bar</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2GR1</td>
<td>2.66±0.8%</td>
<td>~8.33x10^{-5}</td>
<td>~0.034</td>
<td>18.7</td>
<td>12948</td>
<td>0.21</td>
<td>6</td>
</tr>
<tr>
<td>H2GR2</td>
<td>2.66±0.8%</td>
<td>~5.42x10^{-5}</td>
<td>~0.019</td>
<td>16.6</td>
<td>17747</td>
<td>0.29</td>
<td>10</td>
</tr>
<tr>
<td>H3GR2</td>
<td>2.76±0.8%</td>
<td>3.74x10^{-3}</td>
<td>0.009</td>
<td>10.9</td>
<td>3266</td>
<td>0.60</td>
<td>51 (^4)</td>
</tr>
<tr>
<td>H3GR3</td>
<td>2.76±0.8%</td>
<td>5.22x10^{-3}</td>
<td>0.027</td>
<td>22.7</td>
<td>2627</td>
<td>0.22</td>
<td>10</td>
</tr>
<tr>
<td>H3GR5</td>
<td>2.76±0.8%</td>
<td>6.14x10^{-3}</td>
<td>0.029</td>
<td>21.3</td>
<td>17067</td>
<td>0.42</td>
<td>19</td>
</tr>
</tbody>
</table>

\(^1\) determined by analysis of tentacles by quantitative SEC;
\(^2\) calculated with the formula:
\[(\text{MEA}) = G \cdot N_A / \text{SSA}, \text{MEA groups/Å}^2.\]
where \(G\) = polymerized MEA amount associated with the latex, moles/g latex;
\(\text{SSA} = \text{surface area available on the latex, Å}^2/\text{g latex};\)
\(N_A = \text{Avogadro's number, mole}^{-1};\)
\(^3\) calculated as an average, using size distribution of grafted chains.
\(^4\) measured at a flow rate of 0.03 ml/min.
higher the back pressure. A higher tendency towards aggregation due to less steric stabilization is likely to be the cause of this effect. An alternate explanation can be found in the grafting conditions for this latex. A very small Ce(IV) concentration was used, which means that the termination rate was low. As discussed earlier, under these conditions high molecular weight polymer was formed in solution, resulting in high supernatant viscosities. It is possible therefore that some long chains adsorbed on different beads (the high solid content required to achieve a useful concentration of the reacting groups favored this event, too), creating bridges between particles (Vanderhoff, 1981 b), which would account for the higher hydrodynamic resistance. This explanation is supported by the fact that batch H3GR1 (not shown in Table 5.3 for reasons explained below), synthesized at the same Ce(IV) concentration but higher MEA amount, couldn’t be packed in a column because the back pressure exceeded the maximum allowed pressure for our HPLC unit of 100 bars before the column was completely packed. According to the analysis of the cleaved tethers, batch H3GR1, unlike batch H3GR2, carried a population of long grafted chains. The hydrodynamic resistance of these long chains, combined with the above mentioned bridging effect, caused the excessive back pressure. In conclusion, to obtain grafted beads useful as chromatography packings, the synthesis conditions should be chosen in a range where bridging between particles is avoided. The low number of theoretical plates on this column is consistent with the low tether coverage observed experimentally, while the slightly higher permeability value is not. The effect contradicts the results for all the other columns and could not be explained presently.
As shown in the previous chapter, the grafted chains on hydroxylated beads showed an unusual trimodal distribution. The chain length given in Table 5.3 is an average degree of polymerization, considering the mole fraction of each population of grafted chains. All model calculations which will consider this average number assume therefore uniform distribution for all three populations of chains on the surface of all the beads. This assumption seems reasonable for all batches of hydroxylated latex, except H2GR1, which showed the lowest back pressure and permeability in spite of the fact that it had the highest tether coverage. The statistical data from the linear regression of the protein molecular weight dependence of the partition coefficients shown in Table 5.4 indicate a very low regression coefficient for this column. These findings can be explained by poor quality of the grafted surface, perhaps characterized by variable tether concentration from one bead to another. The result is probably due to an experimental error at the beginning of the grafting reaction, i.e. omitting the mixing step after the monomer was added to the latex (see Chapter 4 for the description of the grafting procedure). This could cause two populations of grafted beads to be obtained, each with a different degree of tether coverage. The beads carrying fewer chains on the surface have lower hydrodynamic resistance and the overall back pressure of the column decreases. The amount of latex was insufficient for repeating this experiment.

The intercepts of the linear regressions of partition coefficients shown in Table 5.4 have values between -0.081 and +0.16, which is approximately one order of magnitude less than that obtained for column A10GR3. This finding supports the earlier statement that the coverage was more efficient on the aldehyde beads. The positive value of the
intercept on column H3GR3 is due to adsorption of insulin. The slope of the linear regression on this column is equal to the one on column A10GR3, but only because the first point (insulin) was so high. All the other columns had slopes of approximately half those for column A10GR3. The molecular weight selectivity was therefore lower due to poorer coverage. The slope, the absolute value of the intercept and the correlation coefficient had the highest value for column H3GR5, which had the highest tether coverage among the hydroxylated batches with presumed uniform distribution of the grafted chains. This finding is in agreement with the prediction of the model. The results in the last column of Table 5.4 show that $P_N$ was higher than 5% in all cases, that is the linear correlation is statistically insignificant. This finding is contrary to the prediction of the model. It is noteworthy to mention that in deriving equation [1.8], only first-order terms in concentration were retained. The predictions of the mean field model could probably be improved if higher-order terms are introduced. It would be interesting to see if a numerical model based on such a derivation would predict the experimentally observed curvature on the plot $\ln K$ versus analyte molecular weight.

In Table 5.5 the estimated parameters of the model in terms of density of grafted chains, height of the layer and volume fraction of the tethered polymer are presented. The experimental partition coefficients of insulin, myoglobin and BSA are plotted versus calculated $\phi_z$ in Figure 5.4. The separation was size-dependent on all the supports, except batch H2GR1, for which an inversion between insulin and myoglobin occurred, probably due to non uniform tether coverage and adsorption of the larger protein.
Table 5.4: Statistical data from linear regression of the partition coefficient (LnK) versus protein molecular weight dependence for the columns in hydroxyl series

<table>
<thead>
<tr>
<th>Column</th>
<th>Slope $\times 10^6$</th>
<th>Intercept</th>
<th>R</th>
<th>Probability 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(regression coefficient)</td>
<td>$P_N (R \geq R_0)$</td>
</tr>
<tr>
<td>H2GR1</td>
<td>-3.7</td>
<td>-0.081</td>
<td>0.505</td>
<td>39%</td>
</tr>
<tr>
<td>H2GR2</td>
<td>-4.8</td>
<td>-0.072</td>
<td>0.777</td>
<td>19%</td>
</tr>
<tr>
<td>H3GR2</td>
<td>-4.5</td>
<td>-0.037</td>
<td>0.517</td>
<td>39%</td>
</tr>
<tr>
<td>H3GR3</td>
<td>-1.1</td>
<td>0.16</td>
<td>0.636</td>
<td>28%</td>
</tr>
<tr>
<td>H3GR5</td>
<td>-5.1</td>
<td>-0.073</td>
<td>0.785</td>
<td>19%</td>
</tr>
</tbody>
</table>

1) Percentage probability that N measurements ($N=5$) of two uncorrelated variables give a correlation coefficient with $R \geq R_0$ (Taylor, 1982; p.249).

The decrease in the value of the partition coefficient as $\phi_2^8$ increased, as predicted by equation [1.7], was a general trend for all three proteins on the supports thought to have uniform distribution of grafted chains. The trend is stronger for insulin, probably because low molecular weight solutes can penetrate deeper into the grafted layer, although this effect is not represented in the model. As a result, the grafted non-porous beads are expected to give the best selectivity in the low molecular weight range. The variation with the amount of grafted polymer was not as prominent as in the case of the aldehyde series (Fig. 5.2) probably because the beads in the hydroxylated series didn’t differ as much from each other.
With the data in Table 5.5, predicted values of the partition coefficients were calculated using equation [5.6], where the difference $\chi_{13} - \chi_{23}$ was assumed to be zero. The value of the term $(1 - 1/P_2)$ was approximately 0.99 in all the cases. This means that the dominant term is the volume fraction of the grafted polymer, $\phi_2^g$, which can be increased by introducing more dense grafted chains and/or longer ones in the mushroom regime. In order to calculate the value of $P_3$, values of protein densities (Mahler & Cordez, 1971) were used in the following equation:

$$P_3 = \frac{MW}{\nu_1 N_A \rho}$$  \hspace{1cm} [5.13]

where $MW = \text{molecular weight of protein, g/mole;}$

$\rho = \text{density of protein, g/cm}^3$;

$\nu_1 = 29.9 = \text{volume of water molecule, Å}^3$;

$N_A = \text{Avogadro’s number.}$

The calculated $P_3$ values ranged from 264 for insulin to 2773 for BSA. When plugged into equation [5.6], they gave much lower values for InK than the experimental ones (three orders of magnitude less). The discrepancy is due to the fact that in the Flory-Huggins approach the solute is considered a freely jointed polymer. This assumption doesn’t hold for proteins with secondary and tertiary structure. The number of possible configurations which a macromolecule can sample on the lattice is expected to be much lower for a protein than for a completely flexible polymer. As a result, the loss in entropy when the protein enters the brush was overestimated in the model.
Table 5.5: Modeled parameters of the grafted layer for beads in hydroxyl series

<table>
<thead>
<tr>
<th>Batch #</th>
<th>$\sigma$ 1)</th>
<th>$P_2$ 2)</th>
<th>$h$ 3)</th>
<th>$\phi_2$ 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2GR1</td>
<td>1.82x10^{-3}</td>
<td>119.5</td>
<td>25.64</td>
<td>0.25</td>
</tr>
<tr>
<td>H2GR2</td>
<td>1.14x10^{-3}</td>
<td>106.1</td>
<td>24.16</td>
<td>0.15</td>
</tr>
<tr>
<td>H3GR2</td>
<td>0.83x10^{-3}</td>
<td>69.6</td>
<td>19.57</td>
<td>0.09</td>
</tr>
<tr>
<td>H3GR3</td>
<td>1.19x10^{-3}</td>
<td>145.2</td>
<td>28.26</td>
<td>0.18</td>
</tr>
<tr>
<td>H3GR5</td>
<td>1.40x10^{-3}</td>
<td>135.8</td>
<td>27.34</td>
<td>0.21</td>
</tr>
</tbody>
</table>

1) : Calculated with equation [5.10], using the tether coverage and the DP of grafted chains shown in Table 5.3.
2) : Calculated with equation [5.11], where $v_1 = 29.9 \text{ Å}^3$ and $v_2 = 190.9 \text{ Å}^3$. The volumes were calculated using the formula:
$v = \left(10^{24} \text{ MW}\right) / (\rho N_A)$
where $\text{MW}$ = molecular weight, g/mole ($\text{MW}_1 = 18$; $\text{MW}_2 = 129$);
$\rho$ = density, g/cm$^3$ ($\rho_1 = 1$; $\rho_2 = 1.122$; $\rho_2$ assumed equal with the density of acrylamide [Polymer Handbook]);
$N_A$ = Avogadro's number, mole$^{-1}$.
3) : Calculated for mushroom configuration of the grafted layer, equation [5.7], where $R_g$ was estimated according to (Fleer et al., 1993) (linear polymers in near theta solvents):
$R_g \approx a \left(\text{MW}\right)^{1/2}$
$a \approx 261x10^{-3}, \text{ Å (PMMA)}$.
4) : Calculated with equation [5.5].

A correction parameter was introduced in order to account for the rigid structure of the protein. The experimental value of the partition coefficient for myoglobin, measured on column H3GR3 was used to calculate $P_3$ with equation [5.6], where all the other variables had the values shown in Table 5.5. The ratio between $P_3$ calculated with equation [5.6] and $P_3$ calculated with equation [5.13] yielded the correction parameter of 1.8x10^{-3}, which was then used to adjust the value of $P_3$ for all the other proteins.
Figure 5.4 Variation of the partition coefficient of insulin (filled squares), myoglobin (open circles) and BSA (filled triangles) versus volume fraction of grafted poly(MEA).

Myoglobin was chosen for this calculation because it is the only protein which is approximately neutral at the experimental pH and therefore has the least electrostatic interaction with the beads. The calculated values of the partition coefficients for all the proteins when chromatographed on columns H3GR3 and H3GR5 were then plotted in Figure 5.5., along with the experimental data. The results show that the mean field model gives good agreement with the experiment in the low molecular weight range (up to 30,000 Da). Above this limit, the experimental slope is lower than the one given by the linear regression.
Figure 5.5: Measured and calculated partition coefficients as a function of protein molecular weight for columns H3GR3 and H3GR5.

Proteins include: insulin, myoglobin, trypsin inhibitor, carbonic anhydrase, β lactoglobulin, ovalbumin and BSA. Calculations are based on mean field theory of polymer solutions, with parameters given in Table 5.5. The difference between the Flory interaction parameters for the solute-solvent (1,3) and solute-grafted chains (2,3) interaction was assumed zero: $\chi_{13} - \chi_{23} \approx 0$.

A better agreement with the experimental trend is obtained by using the self-consistent field approach outlined by Steels, 1998; Steels and Haynes, 1998. The curvatures observed in the lnK-MW plots are in fact predicted by the numerical model, as shown in Figure 5.6. It is likely that the better agreement with experiment is due to the fact that in this model the chains are more realistically considered as attached to the surface with one end. By comparison, in the mean field approach, the grafted layer is considered a polymer solution, where the grafted chains are free to assume more conformations because both
ends of the chain are free. Considering contact with low molecular weight solutes, the difference between the two models is not significant. However, when a large molecule attempts to enter the brush, it becomes important if the chain is immobilized on the surface with just one free end or if it can move away in solution. The number of possible conformations is lower in the first case. The mean field model overestimates the loss in entropy upon contact of the brush with a large solute particle. The SCF model predicts therefore that the selectivity of the column packed with grafted beads is maximum in the low molecular weight range. The model also predicts that exclusion will be higher at higher density of the grafted chains or when longer tethers are present.

Experiments at low ionic strength were done on column H3GR5, using insulin, myoglobin, trypsin inhibitor, carbonic anhydrase, β-lactoglobulin, ovalbumin and BSA. The elution times didn’t differ by more than 10% compared those at high ionic strength. The myoglobin peak was broader at low ionic strength, suggesting a weak adsorption effect. It is concluded that the grafted chains shielded the positively charged surface, but not as efficiently as in the case of grafting on aldehyde groups, due probably to lower density of the grafted chains.

5.3.4. Comparison between the grafted beads and traditional SEC packings

The chromatography packings synthesized by grafting flexible neutral polymers on non-porous beads represent a new approach to SEC, based only on chain mixing. The experimental results show that pores are not necessary in order to obtain size-exclusion effects.
Figure 5.6: Calculated partition coefficients as a function of protein molecular weight

Calculations are based on SCF in a cylindrical geometry (Steels and Haynes, 1998; reproduced with permission) for surface graft density of 10% (approximate tether coverage 6 groups/Å²). Flory interaction parameters: $\chi_{12} = \chi_{13} = 0.4$ and $\chi_{23} = 0$.

The non-porous packing has better mechanical strength than traditional stationary phases used in SEC. Also, the separation is faster because the analyte doesn’t require time to diffuse in and out of the pores. A disadvantage of the non-porous packing is the high back pressure, which restricts usage to small columns for analytical purposes at present. As predicted by the SCF theoretical model, the best application would be for chromatography of low molecular weight biomolecules. This is the range in which traditional SEC packings show the lowest selectivity. The experimental conditions of the grafting reaction can be adjusted to tailor the tethered surface according to the intended application, using the theoretical predictions as a guide to optimize density and length of the chains.
5.4. CONCLUSIONS

The experimental data confirmed qualitatively the predictions of the mean field model with respect to the influence of the protein molecular weight and the volume fraction of the grafted polymer. In the case of the aldehyde beads, the data were not detailed enough to prove separate effects of the density and length of the tethers upon the exclusion qualities of the surface. Using the data for the hydroxylated beads, it was found that the model overestimated the loss in entropy when the solute enters the brush due to the assumption that the protein is a freely jointed polymer chain. A correction parameter was introduced, with the value of $1.8 \times 10^{-3}$. A crude interpretation of this parameter is that it characterizes the fraction of the freely jointed polymer chain conformations actually available for the protein molecule in solution. Modeled data using this parameter gave correct estimates of the partition coefficients of proteins up to 30,000 Da. At higher molecular weight, the model fails to predict the curvature observed on the experimental plots. The discrepancy is likely due to the fact that the grafted chains are modeled as being free to move in solution. The SCF model, on the other hand, considers the tethers attached to the surface with one end. The trend in the variation of the partition coefficients with the molecular weight of the solute is correctly predicted by the SCF model.
CHAPTER 6: Concluding remarks and suggestions for further study

In this thesis a method has been developed for synthesizing poly(styrene) latex of sufficient size to act as a chromatographic phase. Two approaches to grafting neutral chains on their surface were taken, the aldehyde route probably producing more dense chains but the hydroxylated surface providing more information on the properties of the grafted layer. Both types of surfaces were shown to provide useful SEC results with proteins.

The novelty of this work consists of the following:

- Experimental conditions for producing surfactant-free cationic poly(styrene) particles in the range 2-3 μm using ABA.2HCl as initiator were determined.

- A method of synthesis of aldehyde coated latex in the above size range was developed.

- Aldehyde groups were used for Ce(IV) initiated polymerization of MEA.

- SEC was performed on a non-porous support.

The protein excluding surfaces synthesized in this work have applications as new size exclusion chromatography media. The experimental conditions for the grafting reaction can be adjusted to tailor the tethered surface, using the theoretical predictions as a guide to optimize density and length of the chains. The grafting efficiency can be improved by crosslinking the copolymer shell with an oil-soluble compound (e.g. divinyl benzene). This would minimize the oligomer leakage and MEA solution polymerization. By using
methods like XPS, contact angle measurements and ATF (atomic force microscopy), more information about the grafted layer in principle can be achieved.

Protein adsorption studies as a function of the density and length of the grafted chains can be used to design surfaces with optimal excluding properties. The size of the beads can be adjusted to obtain maximum chromatographic separation effects with a minimum back pressure.

Other areas of application of the synthetic method include modification of surfaces to improve their biocompatibility. For this purpose, the grafting reaction should be attempted on solid polymer surfaces normally used for medical implants. In principle, by using the halo-isocyanate method (Bamford et al., 1987), acrolein can be polymerized on any synthetic surface which contains hydroxyl, amino, carboxyl, or amido groups. Some examples of supports therefore include: poly(urethane), poly(amides) and poly(esters). After the aldehyde groups have been introduced on the surface, the Ce(IV) initiated grafting can proceed according to the method described in this work.
References


Appendix
<table>
<thead>
<tr>
<th>Batch #</th>
<th>% solids</th>
<th>A.B.A. 2HCl moles/l</th>
<th>NaCl moles/l</th>
<th>Temperature °C</th>
<th>Reaction time hours</th>
<th>Swelling time hours</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>9G1</td>
<td>1</td>
<td>2.2 x 10^{-3}</td>
<td>1.2 x 10^{3}</td>
<td>45</td>
<td>5</td>
<td>24</td>
<td>50</td>
</tr>
<tr>
<td>9G11</td>
<td>1.5</td>
<td>2.2 x 10^{-3}</td>
<td>1.2 x 10^{3}</td>
<td>45</td>
<td>5</td>
<td>24</td>
<td>50</td>
</tr>
<tr>
<td>9G111</td>
<td>3.15</td>
<td>2.2 x 10^{-3}</td>
<td>2.4 x 10^{3}</td>
<td>45</td>
<td>15</td>
<td>24</td>
<td>50</td>
</tr>
<tr>
<td>9G1111</td>
<td>3</td>
<td>2.28 x 10^{-3}</td>
<td>2.4 x 10^{3}</td>
<td>45</td>
<td>12</td>
<td>22</td>
<td>50</td>
</tr>
<tr>
<td>9G1112</td>
<td>4</td>
<td>2.2 x 10^{-3}</td>
<td>6.6 x 10^{3}</td>
<td>45</td>
<td>12</td>
<td>24</td>
<td>50</td>
</tr>
<tr>
<td>9G112</td>
<td>3.17</td>
<td>2.2 x 10^{-3}</td>
<td>6.6 x 10^{3}</td>
<td>45</td>
<td>24</td>
<td>22</td>
<td>50</td>
</tr>
<tr>
<td>9G1122</td>
<td>4</td>
<td>2.2 x 10^{-3}</td>
<td>2.2 x 10^{3}</td>
<td>45</td>
<td>3</td>
<td>24</td>
<td>50</td>
</tr>
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<td>Temperature °C</td>
<td>Yield %</td>
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<td>Yield %</td>
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<td>Standard deviation</td>
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### TABLE A. 3: Second-stage latex growth

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<th>$\bar{p}$</th>
<th>$N_s$</th>
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<th>$r_{exp}$</th>
<th>Standard deviation</th>
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<td></td>
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<td>$\frac{1}{dm^3 s^{-1}}$</td>
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<td>$1.42 \times 10^{16}$</td>
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<td>$1.33 \times 10^{8}$</td>
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<td>NA</td>
</tr>
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<td>0.92</td>
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<td>$2.04 \times 10^{8}$</td>
<td>$0.79\pm0.4% \ n=136$</td>
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<td>$1.02 \times 10^{16}$</td>
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### TABLE A. 4: Third-stage latex growth

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<th>$N_i$ $dm^{-3}$</th>
<th>$N_i r_s$ $dm^{-2}$</th>
<th>$r_{exp}$ $\mu m$</th>
<th>Standard deviation</th>
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<td>5.92x10^{15}</td>
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<td>1.4x10^{13}</td>
<td>1.15x10^{8}</td>
<td>1.15±1% n=152</td>
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### TABLE A. 5: Recipes for styrene/acrolein copolymerization on the core latex

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<th>Acrolein moles/g latex</th>
<th>Styrene moles/g latex</th>
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### TABLE A. 6: Recipes for Ce(IV) initiated polymerization of MEA on aldehyde beads

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<tr>
<th>Batch #</th>
<th>[CeIV] mole/l</th>
<th>[CH=O] mole/l</th>
<th>[MEA] mole/l</th>
<th>Reaction time hours</th>
<th>Solid content % by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2GR</td>
<td>5.14x10^{-2}</td>
<td>3.66x10^{-3}</td>
<td>0.648</td>
<td>40</td>
<td>19.91</td>
</tr>
<tr>
<td>A3GR</td>
<td>4.64x10^{-2}</td>
<td>2.60x10^{-3}</td>
<td>0.648</td>
<td>90</td>
<td>18.18</td>
</tr>
<tr>
<td>A8GR</td>
<td>5.14x10^{-2}</td>
<td>1.84x10^{-3}</td>
<td>0.648</td>
<td>64</td>
<td>18.18</td>
</tr>
<tr>
<td>A9GR</td>
<td>3.12x10^{-2}</td>
<td>0.67x10^{-3}</td>
<td>0.130</td>
<td>90</td>
<td>16.45</td>
</tr>
<tr>
<td>A10GR1</td>
<td>3.12x10^{-2}</td>
<td>1.28x10^{-3}</td>
<td>0.648</td>
<td>90</td>
<td>18.18</td>
</tr>
<tr>
<td>A10GR2</td>
<td>5.14x10^{-2}</td>
<td>2.89x10^{-3}</td>
<td>0.648</td>
<td>112</td>
<td>40.91</td>
</tr>
<tr>
<td>A10GR3</td>
<td>5.14x10^{-2}</td>
<td>1.28x10^{-3}</td>
<td>1.296</td>
<td>66</td>
<td>18.18</td>
</tr>
</tbody>
</table>
### TABLE A. 7: Recipes for styrene/HEA copolymerization on the core latex

<table>
<thead>
<tr>
<th>Batch #</th>
<th>Core latex</th>
<th>Solid conc. %</th>
<th>HEA mole/g latex</th>
<th>Styrene mole/g latex</th>
<th>ABA.2HCl mole/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>11</td>
<td>2.97</td>
<td>2x10⁻³</td>
<td>2x10⁻³</td>
<td>9.22x10⁻⁴</td>
</tr>
<tr>
<td>H2</td>
<td>14G1233</td>
<td>3.33</td>
<td>2x10⁻³</td>
<td>2x10⁻³</td>
<td>9.22x10⁻⁴</td>
</tr>
<tr>
<td>H3</td>
<td>14G1223</td>
<td>3.33</td>
<td>1.5x10⁻³</td>
<td>1.5x10⁻³</td>
<td>9.22x10⁻⁴</td>
</tr>
</tbody>
</table>

### TABLE A. 8: Recipes for Ce(IV) initiated polymerization of MEA on hydroxyl beads

<table>
<thead>
<tr>
<th>Batch #</th>
<th>[Ce(IV)] mole/l</th>
<th>[OH] mole/l</th>
<th>[MEA] mole/l</th>
<th>Reaction time (hours)</th>
<th>Solid content %</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2GR1</td>
<td>5.14x10⁻³</td>
<td>6.68x10⁻³</td>
<td>6.93x10⁻¹</td>
<td>68</td>
<td>24.36</td>
</tr>
<tr>
<td>H2GR2</td>
<td>1.03x10⁻²</td>
<td>6.68x10⁻³</td>
<td>6.93x10⁻¹</td>
<td>41</td>
<td>24.36</td>
</tr>
<tr>
<td>H3GR1</td>
<td>3.07x10⁻³</td>
<td>3.07x10⁻³</td>
<td>1.1</td>
<td>18</td>
<td>18.18</td>
</tr>
<tr>
<td>H3GR2</td>
<td>3.07x10⁻³</td>
<td>3.07x10⁻³</td>
<td>6.48x10⁻¹</td>
<td>18</td>
<td>18.18</td>
</tr>
<tr>
<td>H3GR3</td>
<td>1x10⁻²</td>
<td>3.07x10⁻³</td>
<td>6.48x10⁻¹</td>
<td>18</td>
<td>18.18</td>
</tr>
<tr>
<td>H3GR4</td>
<td>4.61x10⁻³</td>
<td>3.07x10⁻³</td>
<td>6.48x10⁻¹</td>
<td>18</td>
<td>18.18</td>
</tr>
<tr>
<td>H3GR5</td>
<td>1x10⁻²</td>
<td>3.07x10⁻³</td>
<td>1.30</td>
<td>18</td>
<td>18.18</td>
</tr>
</tbody>
</table>
Table A.9: Saponification conditions for latex H1

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Latex suspension 1) g</th>
<th>Solid latex g</th>
<th>[NaOH] 2) mole/l</th>
<th>Reaction time hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.9798</td>
<td>0.2435</td>
<td>0.083</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>2.2135</td>
<td>0.2734</td>
<td>0.167</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>2.1780</td>
<td>0.2690</td>
<td>0.333</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>2.0634</td>
<td>0.2515</td>
<td>0.67</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>1.9631</td>
<td>0.2424</td>
<td>1</td>
<td>72</td>
</tr>
</tbody>
</table>

1): To this amount, sodium hydroxide solution was added to give a final volume during saponification of 3 ml for all samples;
2): Concentration reported in the final volume during saponification (3 ml).
Formation of the complex between the aldehyde group on the surface and Ce$^{4+}$ ion

Formation of the complex between the hydroxyl group on the surface and Ce$^{4+}$ ion

Figure A.1: Possible configuration of the complex formed in the grafting initiation step between Ce$^{4+}$ and the reducing group on the surface