Colloid Polym Sci (2008) 286:1049–1059
DOI 10.1007/s00396-008-1868-0

ORIGINAL CONTRIBUTION

Grafting characterization of natural rubber latex particles: wet-STEM imaging contributions

**A. Bogner · A. Guimarães · R. C. O. Guimarães ·
A. M. Santos · G. Thollet · P. H. Jouneau · C. Gauthier**

Received: 30 October 2007 / Revised: 8 February 2008 / Accepted: 13 March 2008 / Published online: 17 April 2008

Abstract Natural rubber latexes investigated in this study have been chemically modified by seeded emulsion polymerization. Depending on the water affinity of the monomer involved (MMA or DMAEMA), the expected result was the grafting of the corresponding polymer inside or on the surface of the latex particles. The present article focuses on the grafting characterization of these modified natural rubber latexes. In this purpose, non-imaging classical experimental methods such as dynamic light scattering and nuclear magnetic resonance have been completed by microscopy techniques, including transmission imaging in scanning electron microscopy (SEM) and a recent imaging mode called wet-STEM. It consists in transmission imaging in an Environmental SEM operating in the wet-mode, allowing transmission observations of particles suspended in a liquid layer with good resolution and contrast. In the present study, we have adopted a comparative characterization approach between a non-grafted natural rubber latex and two grafted ones. Such an approach indeed contributes to highlight the particles morphology resulting from chemical modification using either MMA or DMAEMA. Transmission images in SEM of thin foils are relatively well interpreted and are completed with wet-STEM images of latexes in their native state, bringing important contributions for grafting characterization.

Keywords Natural rubber latex · Seeded emulsion polymerization · Grafting · ESEM · Wet-STEM

Introduction

Natural rubber (NR) is a polydisperse latex that is rarely used in its native state: its industrial applications often require reinforcing by fillers such as carbon black or silica [1], physical blends with other polymer particles, or modification of the particles morphology, through polymer grafting for example. Thus, the chemically modified particles display different morphologies like core-shell, inverted nucleus raspberry-like, fruitcake, and hairy-layer types. Grafting can also be a way to improve the dispersion of the latex particles or of the added fillers, using two methods: (1) by chemical modification of the natural rubber itself [2–8], (2) by fillers surface modification.

In the present study, chemical modification of NR has been performed by seeded emulsion polymerization varying (1) the water affinity of the monomer to be polymerized and grafted, which directly influences the polymerization site, and (2) the monomer concentration in relation with the latex amount [9, 10]. The obtained hydrated samples can be characterized in their native state, using not straightforward techniques: dynamic light scattering giving a mean diameter of the nano-objects in suspension with a limit of detection in size of a few nanometers, nuclear magnetic resonance allowing chemical composition analysis. Otherwise, samples are characterized in a fixed state, allowing them to be more

A. Bogner · G. Thollet · P. H. Jouneau · C. Gauthier (✉)
INSA-Lyon, MATEIS, UMR CNRS 5510, Université de Lyon,
bât B. Pascal,
69621 Villeurbanne cedex, France
e-mail: catherine.gauthier@insa-lyon.fr

A. Guimarães
Indústrias Químicas Taubaté S/A–IQT,
Rua Irmãos Albernaz, 300,
12050-190 Taubaté, São Paulo, Brazil

R. C. O. Guimarães · A. M. Santos (✉)
Departamento de Engenharia Química, DEQUI–EEL–USP,
Estrada Municipal do Campinho, S/N°,
12.602.810 Lorena, São Paulo, Brazil
e-mail: amsantos@dequi.eel.usp.br

stable under the restricted observation conditions imposed by electron microscopy; this state is in a way optimized, i.e., specially modified to be adapted to the imaging mode (for example a selective staining of a compound can be performed with heavy atoms for atomic contrast imaging modes). This paper presents results using all these characterization techniques including electron microscopy techniques like transmission imaging of thin foils in SEM (scanning transmission electron microscopy, STEM). It insists on the contributions of wet-STEM imaging, which allows the observation of such samples in their native state, serving the puzzling task of grafting evaluation.

Materials and methods

Materials

The present article concerns the characterization of the grafting performed by seeded emulsion polymerization on natural rubber latex particles, using two different monomers. The monomers methyl methacrylate (MMA, 99%) and dimethylaminoethylmethacrylate (DMAEMA, 98%), the initiators cumene hydroperoxide (CHP, 80%) and tetraethylenepentamine (TEPA, 99%), and the surfactant sodium lauryl sulfate (SLS) were purchased from Sigma-Aldrich. All the reagents were used as received. Deionized water was used throughout the work. The natural rubber latex (Hevea Brasiliensis from Quimional Ltda) has a solid content of 60% and exhibits particle sizes from 50 nm to 2 μm. Note that in the following, the monomer content in the different samples will be given in phr (x phr means x grams of monomer per hundred grams of rubber).

Grafting of NR with MMA

Modification of NR was made by using the hydrophobic monomer MMA at different concentrations (15, 30, 40, and 50 phr). Seeded emulsion polymerization was carried out in a 1,000 ml jacketed glass reactor at 50 °C under a nitrogen atmosphere. The pH was maintained at 9.0 by adding 1.5 vol% of ammonium hydroxide solution. The mixture was continually stirred at 400 rpm with an anchorlike impeller. The polymerization procedure was as follows. The NR was diluted with distilled water and NH_4OH solution. SLS was added to stabilize the NR particles: the adapted concentration was calculated to avoid the MMA homopolymerization in the water phase. The MMA monomer was then added, and this system was maintained by 12 h at 25 °C, for swelling the NR particles with this monomer. Its polymerization started when adding a couple of radical generating initiators: cumene hydroperoxide (CHP) and tetraethylenepentamine (TEPA). CHP was introduced first, and after 30 min the temperature of the reactor was adjusted to 50 °C and TEPA solution was added at a rate of 0.04 ml min^{-1} over 6 h. The reactions were allowed to proceed under agitation for two more hours. The final conversion rates are given in Table 1.

Natural rubber/PMMA latexes: physical blends

As a comparative system for natural rubber grafted with MMA, natural rubber/PMMA physical blends were also prepared from native natural rubber and PMMA latexes. PMMA latex was prepared by batch emulsion polymerization under a nitrogen atmosphere at 60 °C in a three-necked glass reactor equipped with a condenser and a mechanical agitator stirring at 270 rpm. The experiment was performed as follows: the reactor was first loaded with water, SLS and MMA. The reaction medium was purged with nitrogen during 20 min, at room temperature and then heated to 60 °C. Reaction was started by injecting 2 g of a 0.5% (*w*/*w*) aqueous solution of ammonium persulphate, previously degassed with nitrogen during 20 min. Samples were occasionally withdrawn to follow the conversion by gravimetry and measure the average particle size. Final PMMA particle diameters were estimated to 90 nm by dynamic light scattering.

Grafting of NR with DMAEMA

In this case, the grafting is carried out to obtain more hydrophilic polymer chains on the natural rubber latex particles surface [10]. Modified NR was prepared by seeded emulsion polymerization using the hydrophilic monomer DMAEMA at different concentrations (5, 15, and 30 phr). As reported in [11], because of the hydrophilic nature of the monomer, the grafting is expected on the surface of the latex particles leading to a hairy-layer like core-shell morphology. The PDMAEMA is indeed a tertiary amine that can be protonated in acid conditions. In this way, although the modified latex displays a poor colloidal stability at pH∼8, it shows a strong improvement in colloidal stability in acid conditions (pH∼2). This stability is attributed to the electrostatic stabilizer role of the grafted hydrophilic polymer. The modification reactions were

Table 1 Solid contents and final conversion for the chemical modification of NR by MMA

	[MMA], phr			
	15	30	40	50
Solids contents (%)	22.4	24.2	25.4	26.3
Final conversion (%)[a]	98.1	96.7	95.2	93.8

[a] Reaction time=6 h

carried out in a 1,000 ml jacketed glass reactor at 2 °C under a nitrogen atmosphere. A low temperature was chosen to reduce branching and cross-linking. The pH was maintained at 9.0 by adding 1.5 vol% of ammonium hydroxide solution. The mixture was continually stirred at 400 rpm with an anchorlike impeller. The polymerization procedure was as follows. The NR was diluted with distilled water and NH_4OH solution at 10 °C. DMAEMA was then added followed by the CHP. After 30 min, the reaction medium was cooled to 2 °C, and TEPA was added at a rate of 0.04 ml min^{-1} over 6 h. The reactions were allowed to proceed under agitation for two more hours. The final conversion rates are given in Table 2.

In the following sections, the terminology adopted is: "NR-g-Xmon" for natural rubber grafted with x phr of monomer, and "NR-xPOL" for natural rubber mixed with x phr of polymer.

Experimental methods

The native and modified natural rubber latexes were characterized with a series of experimental techniques: dynamic light scattering, nuclear magnetic resonance and different microscopy techniques, including wet-STEM imaging.

Dynamic light scattering

Latex particle sizes were assessed by dynamic light scattering on a Beckman Coulter apparatus, Mod. LS230. This method uses the real-time random (Brownian) motion of particles in solution, which gives rise to a Doppler effect, and so the scattered light possesses a range of frequencies shifted from the frequency of the incident light. These frequency shifts are related to the dynamics of the particles, directly providing information about the particles size [12]. In order to avoid the interference phenomena typical from the light scattering technique, the samples were highly diluted. The equipment indicates if the dilution rate is appropriate (Ratemeter). The pH of the samples was fixed at 6 for all measurements.

Table 2 Solid contents and final conversion of NR modified by DMAEMA

	[DMAEMA], phr		
	5	15	30
Solids contents (%)	21.0	21.8	24.4
Final conversion (%)[a]	97.7	96.4	97.3

[a] Reaction time=6 h

Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (^{1}H NMR) experiments were performed to evaluate the grafting efficiency after chemical modification. High-resolution liquid NMR spectroscopy was carried out with a Bruker DRX 400 spectrometer operating at 400 MHz. 1D spectra were obtained with a 5 mm QNP probe.

To quantify the PMMA grafted inside the natural rubber latex particles, a Soxhlet extraction was carried out for a period of 24 h at 56 °C, using acetone as solvent. The gel fraction was then characterized by NMR.

In the case of NR-g-xPDMAEMA, the grafting degree is extremely hard to determine because of the difficulty of removing all ungrafted hydrophilic polymer chains. For this purpose, the purification method was divided in two steps: firstly the modified material was submitted to a Soxhlet extraction during 24 h at 80 °C, using petroleum ether as a solvent for NR. The second extraction step was performed on the resulting gel fraction, for a period of 3 days at 40 °C, in a mixture of acetone and MEK (1:1; *v*/*v*). In this ratio, the solvent mixture exhibits a boiling point close to 80 °C. The material resulting from the second step (gel fraction) was dried and characterized by NMR using chloroform as solvent. It is important to point out that these gel fractions were analyzed in the swollen state.

Unmodified NR latex was submitted to the same extraction conditions for NMR analysis.

Electron microscopy techniques

The environmental scanning electron microscope (ESEM) is a specific scanning electron microscope, where it is possible to keep liquid water and still form a high-resolution image [13]. The key development lays in the use of a differential pumping system in the column: a series of pressure-limiting apertures (PLA) is present below the gun, delimiting different pressure zones. In this way, it is possible to keep the electron gun under high vacuum, and the sample in its wet state due to the low vacuum—up to 12 torr—in the microscope chamber.

First, provided that the distance from the last PLA to the sample is short, the scattering of the electron beam as it passes through a region containing gas molecules is not excessive. The second requirement to keep a good resolution is the detector capability to operate in low vacuum. For imaging in the SE mode, Danilatos has designed a specific detector known as the "environmental secondary electron detector" [14]. Electrons emitted from the sample undergo collisions with gas molecules, including ionizing collisions which lead to a cascade amplification process: many more electrons are detected than were emitted. Positive ions are also produced during this cascade, drift back to the surface of the sample, and can thus compensate the usual build-up of charges.

STEM-in-SEM investigations Transmission mode can be performed in a SEM, and can be designated with the acronym “STEM-in-SEM”. Such an imaging mode was performed in a FEI FEG XL 30 ESEM operating at 30 kV in low vacuum mode, to observe thin foils prepared by ultramicrotomy. The detector usually used for backscattered electrons (BSE) collection is placed just below the sample, and in such a way that it collects scattered electrons with both of the diodes, resulting in a kind of annular dark-field mode. Schematically, it corresponds to brighter areas for high atomic number elements and high thickness, i.e., an inverse mass–thickness contrast.

Thin foils preparation The latex colloidal dispersions have been mixed with glutaraldehyde to react with the proteins on the particle surface (cross-linking) and avoid damage during the sample slice step. It has been fixed with OsO_4 (this molecule reacts with the double bond of the natural rubber resulting in an improved contrast between natural rubber and grafted polymers), dehydrated with anhydrous ethanol and finally mixed with a resin until the pellet state. It has then been sliced at ambient temperature with an ultramicrotome Reichelt Ultracut S equipped with a diamond knife at a cutting rate of 1 mm/s, resulting in thin foils from 50 to 70 nm in thickness. Additional information concerning such sample preparation methods can be found in the literature [15]. Using this type of samples, resulting STEM-in-SEM images are rather close to usual conventional TEM imaging of latex particles.

Wet-STEM imaging The wet-STEM device is described in Fig. 1. The same electron detection conditions as for STEM-in-SEM described above have been used for wet-STEM imaging (BSE annular detector placed below, i.e., in dark-field imaging conditions). Wet-STEM is a recent imaging mode developed in Environmental SEM: operating with the STEM mode in ESEM enables the observation of samples in water in its liquid state in transmission imaging conditions. This new imaging mode is described in details in [16]. Pressures allowed in ESEM specimen chamber, and temperature available due to a Peltier cooling stage allow to maintain the equilibrium for the liquid state of water, or other compounds depending on their saturated vapor pressure.

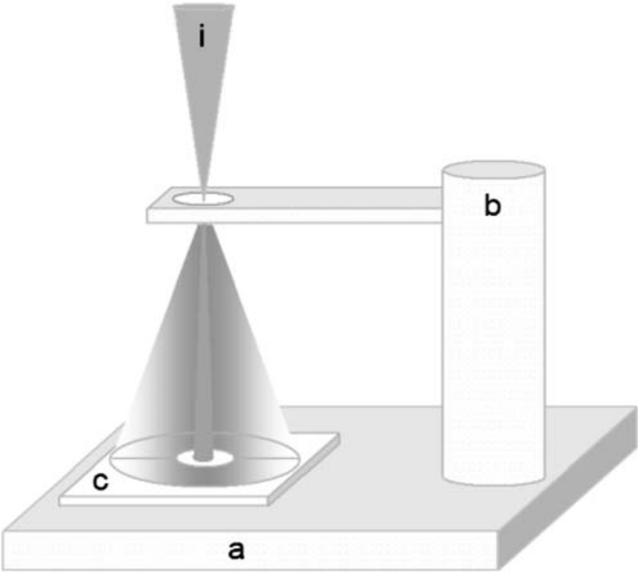

Fig. 1 Wet-STEM imaging mode in Environmental SEM *a* Peltier stage; *b* SEM mount supporting the latex droplet on a TEM grid; *c* solid state detector for annular dark-field collection; ***i***: incident convergent electron beam

Thin liquid films preparation Samples for wet-STEM imaging have been subjected to a dilution step: this is actually the only step for preparation, i.e., the latex is observed in its native state. A small amount of the diluted latex is dropped on a TEM grid fixed above the Peltier cooling stage, and a controlled pumpdown procedure is performed to minimize evaporation of and condensation on the sample before imaging [17]. During imaging, the chamber pressure is controlled and the sample temperature as well, due to the Peltier stage. The sample thickness can then be adjusted in situ if required, to obtain a sample thin enough to allow electrons transmission.

Dialysis process

To check the hypothesis of homopolymerization of MMA in the aqueous phase in the case of NR modification, the NR modified with 15 phr of MMA was submitted to a dialysis process. The membrane used in the dialysis was the Spectra/Por (MWCO 12–14 kDa) purchased from Spectrum Laboratories, Inc. The water recovered during dialysis was characterized by wet-STEM to check the presence of small nodules of PMMA.

Results and discussions

Unmodified NR latex

First, the unmodified natural rubber latex was characterized, in order to constitute reference data for a comparative study with grafted latexes. Pure NR latex particle diameters evaluated by dynamic light scattering are presented in Table 3. Natural rubber latex particles exhibit an important dispersion in size: a major part of particles exhibits diameter around 170 nm, and 10% around 500 nm.

Table 3 Percentages in number and particle size for unmodified NR, NR-g-15MMA and NR-g-30DMAEMA according to dynamic light scattering measurements

Composition	Percent in number	Particle size (nm)
NR	90	173
	10	495
NR-g-15MMA	83.5	190
	16.5	630
NR-g-30DMAEMA	88	225
	12	683

Two annular dark-field STEM-in-SEM images of thin foils prepared with NR are presented in Fig. 2. The wide dispersion of latex particle sizes is confirmed: 50 nm to several µm, with an average diameter of 650 nm estimated by image analysis. As the analysis is performed on a micrograph with a given magnification adapted to consider a large number of particles, the amount of small particles may be underestimated.

From the STEM-in-SEM images, it is also possible to observe the structure of the particles, which seems to be homogeneous, and their geometrical stability: they are frequently deformed and look more elliptical than circular. However, it should be noted that samples observed are in fact slices of particles embedded in a resin: it differs from the native state of the latex (particles colloidal dispersion in water). Moreover, one should be aware that thin foils preparation sometimes induces artifacts: particles deformation could also be linked to this preparation step.

To complete these observations, natural rubber latex was also observed in its native state, using wet-STEM imaging: two images are presented in Fig. 3. An average particle diameter was estimated by image analysis and is about 650 nm. As noted from the precedent thin foils observations, the wide particles size distribution, the homogeneous morphology and the geometrical instability are confirmed. However, in the present case, no sample preparation was performed except dilution (no staining, embedding, or slicing): it constitutes an advantage in terms of time consumption, and by the way avoids artifacts as no sample feature modification is induced by preparation.

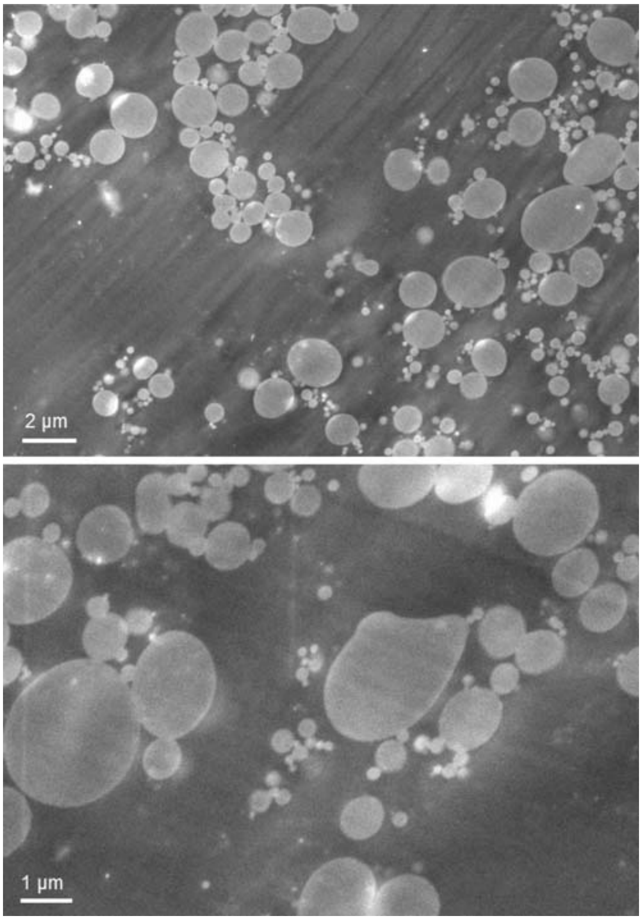

Fig. 2 Annular dark-field STEM-in-SEM images of native NR latex particles, after double staining (glutaraldehyde and OsO_4), embedding in a resin and ultramicrotomed section

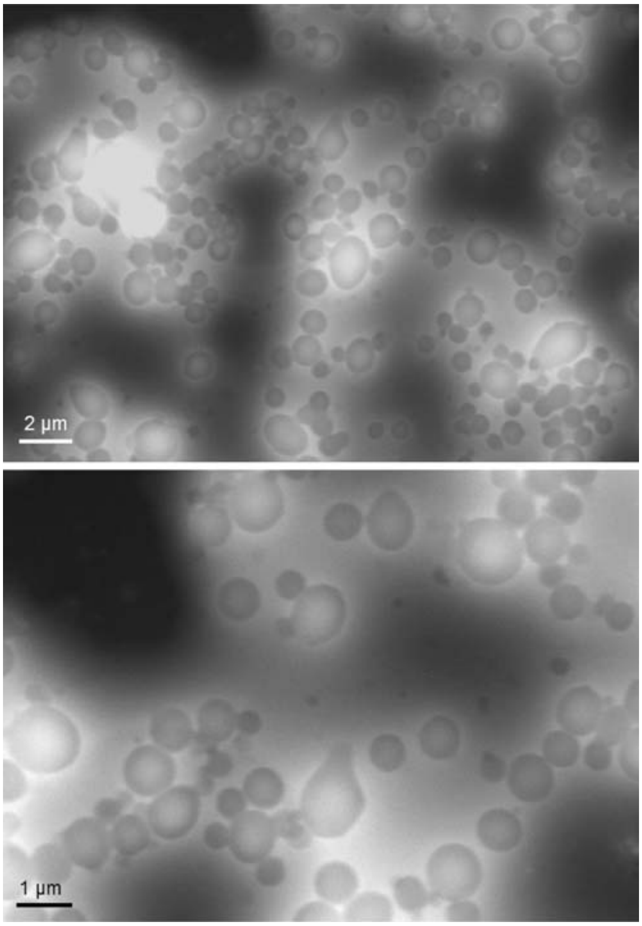

Fig. 3 Annular dark-field wet-STEM images of native NR latex particles

NR-PMMA physical blends

For the puzzling task of grafting characterization, it is also interesting to compare wet-STEM images obtained on grafted latexes or physical mixture latexes.

Concerning the NR/PMMA physical blends (Fig. 4), poly-dispersed NR particles similar to that observed in pure NR are easily recognized. In addition, monodispersed PMMA particles with sizes around 90 nm are also observed. Most of these particles are suspended in the water phase, and some are present at the surface of the NR particles. PMMA particles exhibit very important contrast on wet-STEM images without any staining of the samples and are very sensitive to the electron beam. Actually, contrasts on wet-STEM images resulting from large collection angles and low voltage of the electron beam (i.e., high interaction cross sections) are very strong [18]. In addition, PMMA is known to be a very electron beam sensitive material. Thus, PMMA particles in the blends are enhanced by the electron beam impact and easily recognized.

NR latex grafted with the more hydrophobic monomer MMA

As evocated before, the water affinity of the monomer directly determined the polymerization site, i.e., the grafting site. In the case of NR chemically modified with MMA, because of the more hydrophobic nature of the monomer and the process conditions, the grafting is expected to take place inside the latex particles, resulting in a fruitcake-like morphology.

Comments about classical characterization

Table 3 presents the distribution of particles diameter measured by dynamic light scattering. The chemical modification of NR with MMA results in an increase of the particles diameter: approximately 10% for the predominant particle class (170–230 nm) and 30% for the other particle class (500–700 nm). This indicates that a part of the polymerized PMMA is inside or on the surface of NR particles (either adsorbed or grafted).

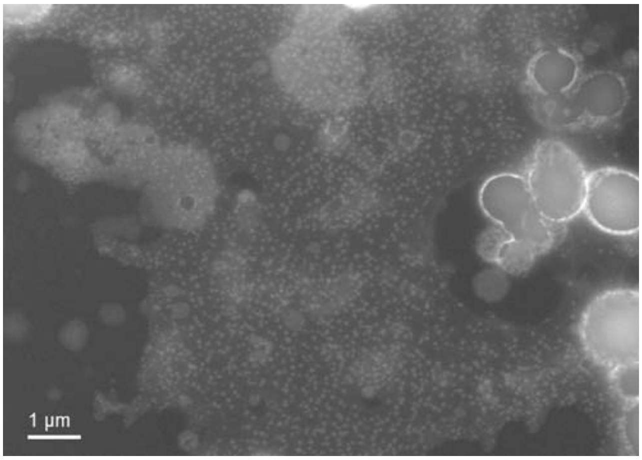

Fig. 4 Annular dark-field wet-STEM images of physical mixture NR-40PMMA latex particles, in their native state

To go further, the degree of grafting has been estimated by analyzing gel fractions after Soxhlet extraction for the NR-g-xMMA series. NMR spectra of unmodified NR and NR-g-15MMA are presented on Figs. 5 and 6, respectively. The shift of the different peaks are used to assign the nature of the corresponding chemical units, and their amplitude to quantify the grafting density. On both spectra, a peak was detected at $\delta=5.1$ corresponding to the C–H bonds of poly-*cis*-isoprene. In the spectrum of the NR-g-15MMA gel fraction, another peak was observed at $\delta=3.6$, attributed to the C–H bonds of the $-OCH_3$ groups of PMMA. We must consider that a part of the MMA polymerized is present inside the particles but not necessarily grafted onto the poly-*cis*-isoprene backbones. In this case, the free PMMA is removed from the sol phase during the Soxhlet extraction [10]. The grafting process can occur either through abstraction of an allylic hydrogen or addition across the double bonds of the backbone of polydiene substrates. Use of relatively bulky oxygen-centered radicals, such as the cumyloxyl radical, will favor abstraction over addition. Moreover, more nucleophilic radicals such as cumyloxyl radicals have been seen to favor the hydrogen abstraction reaction over addition to double bonds [10, 19].

If we assume that all the nongrafted PMMA has been concentrated in the sol phase during extraction, the PMMA remaining in the gel fraction is the grafted one. The $\delta=3.6$ amplitude allows the determination of the degree of grafting of PMMA. Results in Table 4 show that the grafting degree is maximum and practically equal when using 15 and 30 phr of MMA (~75% of grafting, i.e., 75% of the PMMA present is grafted on the NR). It is important to point out that the MMA conversion in the grafting reactions was always around 96%, that means 96% of the initial MMA has been polymerized and is now PMMA. Considering the saturation on the degree of grafting, it can be proposed that some of the MMA may have been polymerized in the aqueous phase. In addition, the grafting percentage decreases with the increase of the MMA content from 40 to 50 phr. This can be explained by MMA saturation in the natural rubber particles after the monomer swelling step [20]. It must be pointed out that the amount of initiators CHP and TEPA (redox couple) was the same for all the reactions, leading to the same number of radicals in the reaction media. Therefore, this fact may also contribute to the limitation of the grafting degree. Nakason et al. [21] have observed the same behavior for MMA grafting, even when using different reaction conditions. Considering all

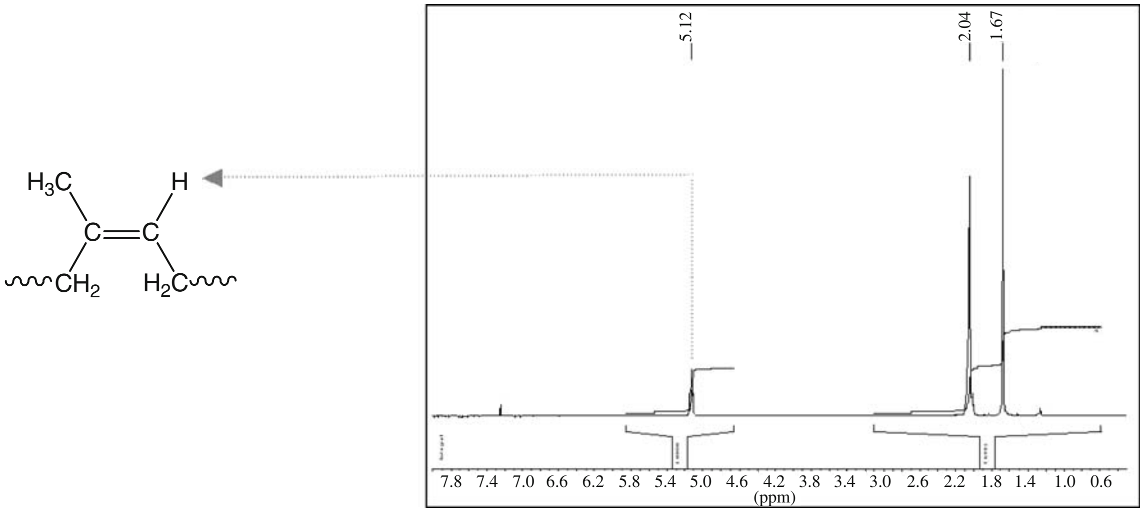

Fig. 5 ^{1}H NMR spectrum of unmodified NR latex

the statements above, it can be concluded that the probability of formation of PMMA particles in the water phase is higher for the reaction carried out with increasing content of MMA.

Electron microscopy investigation

The same STEM/wet-STEM characterization strategy was followed on the grafted latex modified by MMA and is illustrated in the case of NR-g-40MMA. Thin foils STEM-in-SEM images are presented on Fig. 7, and wet-STEM observations on Fig. 8.

After grafting with MMA, the latex still looks polydisperse, but the particles geometry seems more stable than in the case of unmodified NR, as highlighted by the more spherical aspect of the particles. The internal structure of the particles has been evidenced through thin foils observations. It corresponds to fruitcake morphology, small nodules of PMMA being grafted inside each NR latex particle. The average size of these nodules is about 25–30 nm, and the distance between nodules ranges from 20 to 100 nm. Note that the contrast in these dark-field micrographs is important but artificial due to the staining procedure. The amount of nodules in the particles is found to increase with the monomer content.

Both STEM-in-SEM and wet-STEM images show the presence of nodules on the surface of the latex particles. However, only wet-STEM imaging allows the observation of “free nodules”, i.e., nodules that are not grafted inside the NR latex particles but free in the continuous phase of the latex. In the physical blends characterization, we previously showed that PMMA particles contrast is

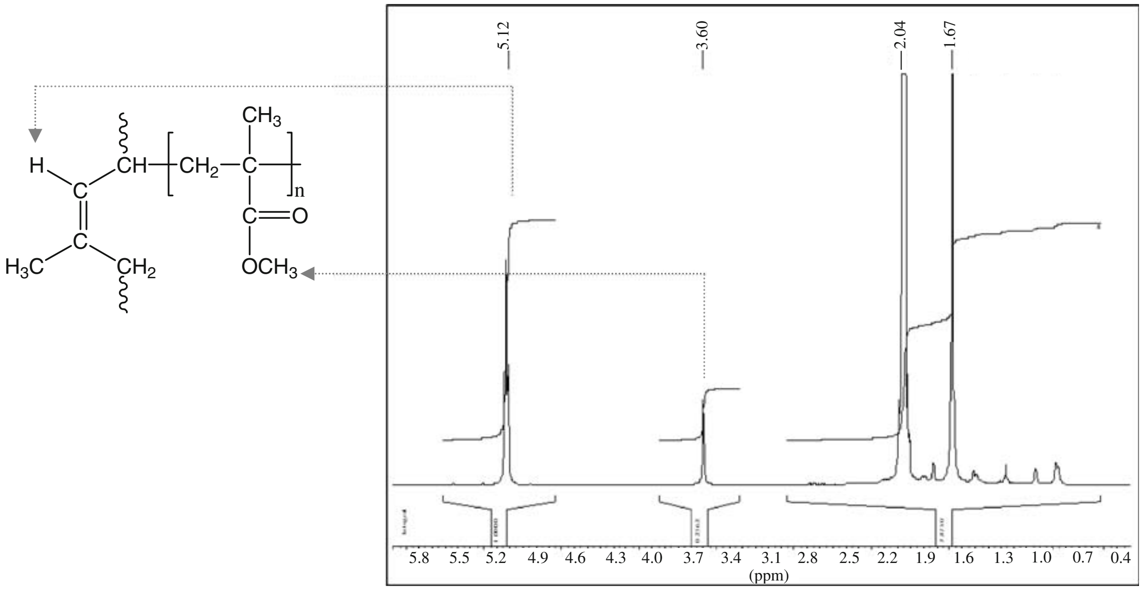

Fig. 6 ^{1}H NMR spectrum of NR-g-15MMA after extraction with acetone

Table 4 Grafting percentage as a function of the MMA concentration

[MMA], (phr)	[MMA], (% weight)	Grafting (%)
15	9.8	75.1
30	17.5	75.8
40	17.7	62.0
50	13.0	39.0

enhanced by the electron beam impact. This is a pertinent remark as it helps us to understand the MMA grafting mechanism. On wet-STEM images of NR-g-MMA, it is possible to highlight the presence of a large number of small PMMA particles in the continuous phase, probably formed by homogeneous nucleation. To confirm the chemical nature of the nodules, wet-STEM observations and NMR analysis have been performed on the water coming from the dialysis process. These experiments confirm with no doubt that the small nodules in the wet-STEM images are PMMA, resulting from the homopolymerization of MMA in the water phase. These nodules may correspond to the missing percents of PMMA in the grafting efficiency estimation performed on NMR spectra.

This homogeneous nucleation was not avoided even if the grafting reaction was performed using an enough amount of SLS to stabilize the NR particles, avoiding micellar nucleation. The critical micellar concentration of SLS in the presence of the NR particles was yet determined by conductivimetry. In spite of the conditions mentioned above for the grafting reactions, the possibility of the MMA polymerization in the aqueous phase is not a surprising result considering the important solubility of MMA in this phase.

These free nodules could not be detected in thin foils because of the exchange of the continuous phase (water to anhydrous ethanol) that takes place during the embedding step. Indeed in the latter case, objects eventually present in the latex phase are agglomerated on the surface of the particles, or removed from the sample and replaced by the embedding resin.

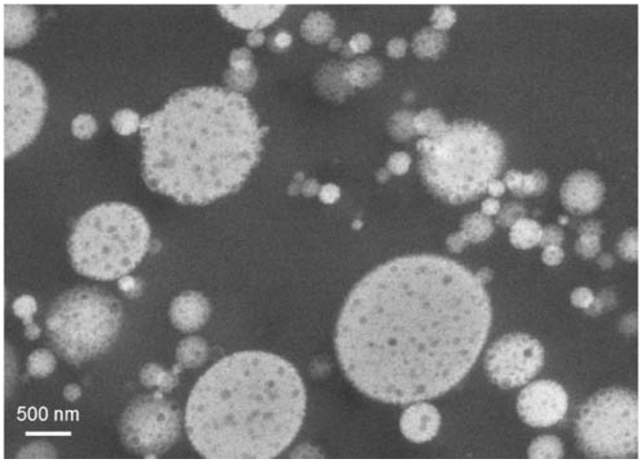

Fig. 7 Annular dark-field STEM-in-SEM image of grafted NR-g-40MMA latex particles, after double staining (glutaraldehyde and OsO_4), embedding in a resin and ultramicrotomed section

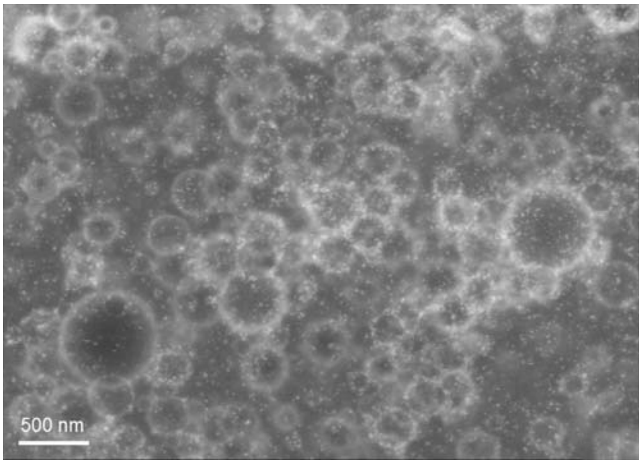

Fig. 8 Annular dark-field wet-STEM image of grafted NR-g-40MMA latex particles, in their native state

NR latex grafted with hydrophilic monomer DMAEMA

Comments about classical characterization

Table 3 presents the particles diameter distribution measured by dynamic light scattering. The chemical modification of NR with DMAEMA also results in an increase in the particles diameter: 88% for the predominant particle class of 225 nm and 12% for the other particle class of 683 nm. From these data, it seems that smaller NR particles are more grafted than larger ones.

Moreover, the grafting amount has been estimated by analyzing NMR spectrum of the gel phase after extraction. As presented for NR-g-30DMAEMA on Fig. 9, the NMR spectrum shows two peaks at $\delta=4$ and $\delta=5.1$, corresponding to the C–H bonds of O–CH_2, and C–H groups of PDMAEMA and NR, respectively. For such system, also it can also be assumed that all the nongrafted PDMAEMA was concentrated in the sol fraction after extraction and in this case, the amplitude of the last two peaks allows the determination of the grafting degree of PDMAEMA. From Table 5, it is possible to verify a decrease in the grafting efficiency with the increase of DMAEMA content. This decrease may be related to a point of saturation concerning the number of grafting sites onto the surface of the NR latex particles. Considering that the same NR latex was used for all the experiments, i.e., same solid content and particle size distribution, it is possible to suggest that the whole surface of NR particles is coated when using only 15 phr of DMAEMA. For a higher monomer content (i.e., 30 phr), a significant reduction of the grafting degree is observed.

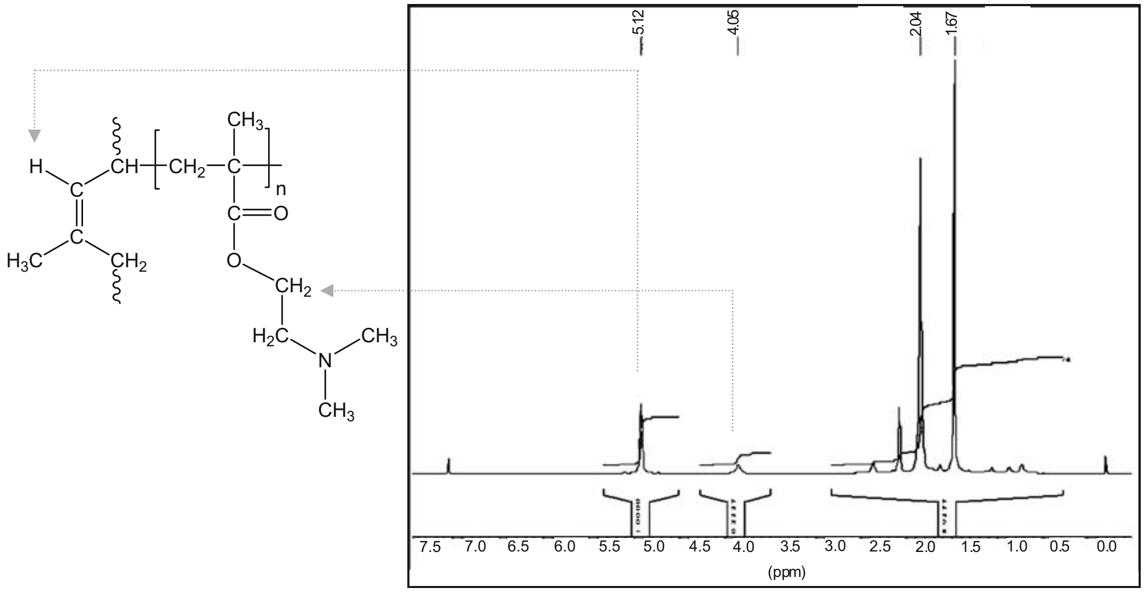

Fig. 9 ^{1}H NMR spectrum of NR-g-30DMAEMA after extraction with petroleum ether and a 1:1 (*v/v*) mixture of acetone/MEK

Similarly to what was observed in the case of MMA grafting reactions, higher conversions were obtained in grafting reactions using DMAEMA. These results allow to conclude that, in this case, polymerization of DMAEMA in the water phase must also be considered. This behavior was expected because of the hydrophilic nature of DMAEMA. For the same reason, in that case, it is expected that the grafting will preferentially take place on the latex surface and not inside the particles as observed in the case of MMA. To better understand the grafting mechanism when using DMAEMA, it will be very interesting to study further the partitioning of this hydrophilic monomer between aqueous and rubber particles.

Electron microscopy investigation

As for the grafted NR latex described in the precedent section, NR latex modified with DMAEMA was investigated by STEM-in-SEM and wet-STEM. The resulting micrographs are presented in Figs. 10, 11, and 12. Pure NR latex study described before is considered as a reference state, making easier the grafting characterization of NR-g-xDMAEMA.

As expected and observed for pure NR, NR-g-xDMAEMA particles size distribution is polydisperse with sizes ranging from several tens of nm to 3 µm, and a mean diameter of 950 nm assessed by image analysis. In addition, NR-g-xDMAEMA particles exhibit a better geometrical stability (i.e., they are more spherical) in comparison with non-grafted particles. This remark confirms the assumed stabilizing role of grafting. Another difference noted in comparison with NR is the aspect of the particles surface: STEM-in-SEM images show particles with a diffuse crown, which thickness is variable from one particle to another. In wet-STEM, grafted particles look more granular than smooth unmodified NR latex particles, and the granular area seems to be influenced by pH conditions: it seems more extended at pH 2. Note that due to different pH conditions particle sizes measured by wet-STEM could not be directly compared with DLS results.

Table 5 Grafting percentage as a function of DMAEMA concentration, obtained by NMR

[DMAEMA], (phr)	Grafting (%)
5	38.7
15	29.3
30	15.8

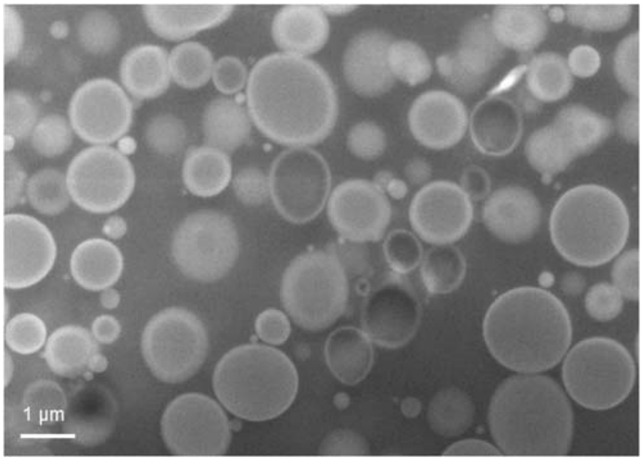

Fig. 10 Annular dark-field STEM-in-SEM image of grafted NR-g-30DMAEMA latex particles, after double staining (glutaraldehyde and OsO_4), embedding in a resin and ultramicrotomed section

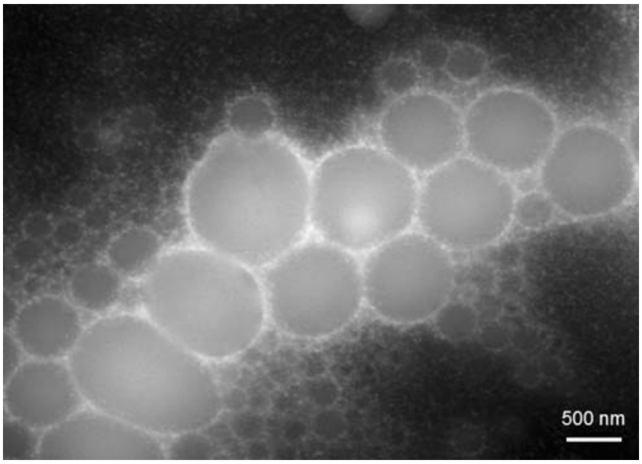

Fig. 11 Annular dark-field wet-STEM image of grafted NR-g-30DMAEMA latex particles, in their native state at pH=8

To conclude, the particles surface aspect observed is in agreement with the effective grafting and the hairy-layer particles morphology. Moreover, it allows understanding the increase in particles diameter detected by dynamic light scattering also consistent with the presence of particles with a hairy-layer like morphology.

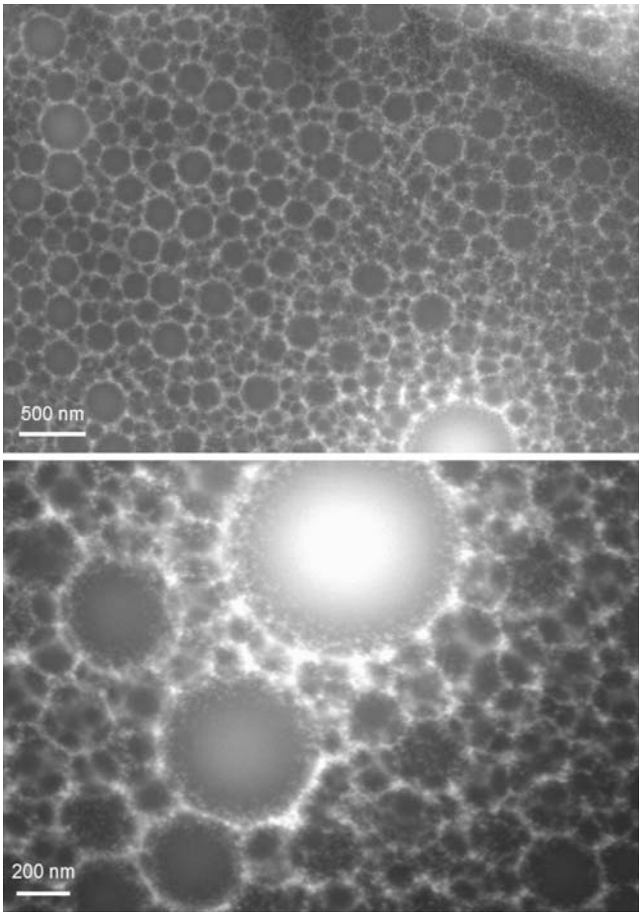

Fig. 12 Annular dark-field wet-STEM images of grafted NR-g-30DMAEMA latex particles, in their native state at pH=2

STEM-in-SEM images are closer to classical micrographs and lead to observations also highlighted on wet-STEM images: it is a way to valid the wet-STEM technique. In addition, wet-STEM observations allow us to achieve a more realistic view of the particles as the latex is in its native state. It strengthens the assumption of deformed unmodified NR particles and allows to characterize the grafted latex particles morphology as a function of pH conditions.

Conclusions

In the present article, as shown by electron microscopy observations, natural rubber latex consists in very polydisperse particles with a low geometrical stability and a homogeneous morphology. For both chemically modified NR latexes, the grafting was evaluated by NMR experiments and dynamic light scattering data, which showed an increase in the particles size after grafting. Electron microscopy observations highlighted an increase in the geometrical stability of particles after grafting. Moreover, microscopy characterization also allowed a more precise analysis of latexes: by imaging of the particles morphology, the grafting site of the polymer, the shape of this grafted polymer, the detection of nongrafted polymer nodules in the solution, or the influence of pH conditions.

Wet-STEM constitutes a straightforward technique for the characterization of suspension-type samples in their native state, completing results from other more usual characterization techniques. Due to its development in ESEM, it allows to maintain latexes in their hydrated state, without requiring any sample preparation. This constitutes a nonnegligible profit in time and allows to avoid possible artifacts during imaging, resulting from sample preparation. Furthermore, wet-STEM especially extends ESEM possibilities for latex characterization: observation of nanometric particles included in a liquid layer, due to the transmission mode, with a resolution reaching 5 nm, and enhanced contrast, thanks to the large collection angles and low voltages used in comparison with TEM.

Particularly, the characterization approach by both STEM-in-SEM and wet-STEM seems to be very relevant. First, the observation of thin foils, i.e., slices of stained particles embedded in a resin matrix, allows to consider details inside the particles. Second, wet-STEM imaging of latex droplets allows to observe details on the surface of the particles and inside the liquid surrounding the polymer particles. As a conclusion, electron microscopy characterization allows to check the efficiency and the homogeneity of the grafting and also the resulting structure of the particles.

Acknowledgement The Consortium Lyonnais de Microscopie Electronique (CLYME) is thanked for the access to the FEI XL 30 FEG ESEM. Karine Masseneli-Varlot is acknowledged for thin foils preparation. CAPES and FAPESP are thanked for financial support.

References

1. Boonstra BB (1979) Polymer 20:691
2. Yan M (2000) React Funct Polym 45:137
3. Subramaniam N, Balic R, Taylor JR, Griffiths M, Monteiro MJ, Gilbert RG, Ho CC, Abdullah I, Cacioli P (1997) J Nat Rubber Res 12:223
4. Subramaniam N, Balic R, Gilbert RG (1998) Modified Rubber Polymer Latex, PCT/AU98/00191
5. Sandardi F, Kadariah S (1984) J Appl Polym Sci 29:1515
6. Gilbert RG, Subramaniam N, Balic R, Taylor JR, Griffiths M, Monteiro MJ, Ho CC, Abdullah I, Cacioli P (1997) J Nat Rubber 12:223
7. Lee DY, Subramaniam N, Fellows CM, Gilbert RG (2002) J Polym Sci A: Polym Chem 40:809
8. Arayapranee W, Prasassarakich P, Rempel GL (2002) J Appl Polym Sci 83:2993
9. Guimarães RCO (2006) PhD thesis: Natural rubber-poly(methyl methacrylate) blends: influence of the chemical modification of the natural rubber, Engineering School of Lorena, University de São Paulo, Lorena/SP, Brazil
10. Oliveira PC, Guimarães A, Cavaillé JY, Chazeau L, Gilbert RG, Santos AM (2005) Polymer 46(4):1105
11. Lamb DJ, Anstey JF, Fellows CM, Monteiro MJ, Gilbert RG (2001) Biomacromol 2:518
12. Young RJ, Lovell PA (2000) Introduction to polymers, 2nd edn. CRC Press
13. Donald AM, He C, Royall CP, Sferrazza M, Stelmashenko NA, Thiel BL (2000) Colloids Surf A 174:37
14. Danilatos GD (1988) Adv Electron Electron Phys 71:109
15. Subramaniam N, Simpson A, Monteiro MJ, Shaffer O, Fellows CM, Gilbert RG (2004) Microsc Res Tech 63:111
16. Bogner A, Thollet G, Basset D, Jouneau PH, Gauthier C (2005) Ultramicroscopy 104:290
17. Cameron RE, Donald AM (1994) J Microsc 173:227
18. Bogner A, Jouneau PH, Thollet G, Basset D, Gauthier C (2007) Micron 38:390
19. Anstey JF, Subramaniam N, Pham BTT, Lu X, Monteiro MJ, Gilbert RG (2000) Macromol Symp 150/151:73
20. Perera MCS (1999) J Polym Sci B: Polym Phys 37:1141
21. Nakason C, Kaesaman A, Yimwan N (2003) J Appl Polym Sci 87:68