Cross-linked polymer microparticles: characterization by size exclusion chromatography using universal calibration

Sébastien Rouzeau, Françoise Méchin, Jean-Pierre Pascault, Benoît Magny

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Sébastien Rouzeau [a)], Françoise Méchin [a)], Jean-Pierre Pascault [a)], Benoît Magny [b)]

[a)] *Laboratoire des Matériaux Macromoléculaires / IMP – UMR CNRS 5627, Bât. Jules Verne, INSA Lyon*
20, avenue Albert Einstein - F-69621 Villeurbanne Cedex - France
Fax : 33 4 72 43 85 27 ; fmechin@insa-lyon.fr

[b)] *Cray Valley – Total Chimie, Centre de Recherche de l'Oise, APAC*
Parc Technologique ALATA - BP 22
F-60550 Verneuil-en-Halatte - France

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Abstract

Cross-linked Polymer Microparticles (CPMs), commonly known as microgels, have been prepared and characterized by Size Exclusion Chromatography (SEC) using the principle of universal calibration (UC). Not only has SEC-UC proved itself a very useful method to determine molar masses of CPMs, but it also allowed an on-line measurement of their exponent *a* in Mark-Houwink-Sakurada (MHS) equation, i.e. $[\eta]=KM^a$. As cross-linked particles, CPMs keep their spherical shape in solution and show values of exponent *a* lower than 0.5. On the contrary, homologous linear macromolecules display higher values of *a*, about 0.6-0.8. SEC-UC also provides values for the radius of gyration, R_z, and is much more sensitive to the presence of low molar mass species as compared to SEC using Multi Angle Laser Light Scattering (MALLS) detector.

Keywords: cross-linked polymer microparticle; microgels; size exclusion chromatography; universal calibration

Introduction

Cross-linked Polymer Microparticles (CPMs), are defined as intramolecularly cross-linked macromolecules. They have been commonly known as microgels for many years, and can be viewed as a new, fourth class of polymers (besides linear, branched macromolecules and macroscopic polymer networks), on the border between molecules and particles [1,2]. Such macromolecular objects are based on at least one monomer with a functionality greater than 2, as a cross-linking agent. Microgels can be found for example in natural rubber, but numerous synthetic methods are also available, among which emulsion or microemulsion free radical polymerization, dispersion polyaddition, highly dilute, semi-dilute solution or bulk polymerization [1-5]. They now find applications in several practical and/or industrial fields:

i) Coatings: because of their small size and globular shape, microgels can form colloidal solutions with high solid contents and low viscosities; therefore they are often incorporated as components of the binder for organic coatings, with the aim of adjusting the rheological behavior and preventing sagging, controlling the surface properties and reducing shrinkage, or filling pores and irregularities of some substrates (wood) [2].

ii) When sufficiently small (diameter below 100 nm), microgels can also be used as nanometric carriers for numerous dyes, pharmaceutical or biochemical compounds, and therefore play an essential role for therapeutic and diagnostic applications. Proteins and enzymes can be covalently linked to the microgel surface either directly or through a spacer in order to develop immuno assays and controlled drug delivery systems [3], especially as some microgels (based on poly(acrylic acid) or poly(N-alkylacrylamide)) can display pH-and temperature-sensitive swelling/deswelling behavior in aqueous media [6-8]. Moreover, other microgels can also be molecularly imprinted towards various chemical probes [9]. Bigger microgels can also exhibit high ion-exchange capacity for cationic hydrophilic drugs [10]. Finally when doped with specific nanoparticles (semi-conducting, superparamagnetic…), hybrid submicronic microgels have promising potential applications in photonics, catalysis or separation technologies [11].

iii) Microgels can finally be incorporated as high performance fillers in plastics, thermosetting polymers or coatings [12-13], in order to improve shock or abrasion resistance for instance.

The “microgels” described above can have real nanometric sizes, but can also be rather micrometric particles, somewhat different from the definition given by Funke et al. [2]. In contrast, this paper is especially devoted to the characterization of CPMs or microgels with very small diameters (below 50 nm). It describes a new, reliable method to measure molar masses of CPMs with Size Exclusion Chromatography (SEC) using universal calibration (UC). In this study, CPMs and their homologous linear polymers (LP), prepared by copolymerizing lauryl acrylate (LA), butyl acrylate (BA) and cardura acrylate (CA), with hexanediol diacrylate (HDDA) as a cross-linking agent in the case of CPMs, were characterized by SEC-UC method. The synthesis of the CPMs we have used is described in other papers [14].

Background

Size Exclusion Chromatography

As individual polymer particles, CPMs have the particularity to keep their globular shape in solution, although they remain soluble in convenient solvents, just like linear macromolecules [2]. This solubility allows the analysis of CPMs by Size Exclusion Chromatography, SEC. Indeed, determination of CPMs’ molar mass is a critical issue, due to the fact that they are non linear polymers and also mostly copolymers.

SEC is based on the separation of macromolecules according to their hydrodynamic volumes, partially or totally swelled, in the eluting solvent. In most cases, it is processed using just a concentration detector (e.g. refractometer). For accurate measurements, columns must be calibrated with a set of well-defined polymer standards having a chemical nature and a structure identical with the polymer to be analyzed. In practice, such polymer standards are usually not available, except for a few polymers

like poly(methyl methacrylate) (PMMA) and polystyrene (PS). Conventional calibration with PS standards is widely used, but in the case of CPMs, molar masses obtained in this way can differ from the true ones by a factor 5 [1,15,16]. This is due to the different chemical nature and also to the compact structure of CPMs as compared to similar linear macromolecules.

SEC-MALLS

In order to determine an accurate molar mass for any polymer, a so-called absolute technique can be used: SEC coupled with an on-line Multi Angle Laser Light Scattering (MALLS) detector [17]. The light scattering signal is directly proportional to the molar mass of the polymer. Consequently, there is no need to calibrate columns. Molar masses are calculated from Eq. (1):

$$\frac{KC}{R_0} = \frac{1}{M_w} + 2A_2C \tag{1}$$

where R_0 is the Rayleigh ratio at zero angle scattering (obtained by extrapolating data from other angles to zero angle), C is the concentration of the solution, K is an optical constant proportional to the square of the refractive index increment $(dn/dc)^2$, and A_2 is the second virial coefficient.

In SEC-MALLS, the LS signal intensity is proportional to $(dn/dc)^2$ and concentration, and M_w is also inversely proportional to $(dn/dc)^2$. This parameter must thus be determined very precisely if accurate molar masses are requested. For polymers with a very low dn/dc, like in our case PBA in THF at 25°C (dn/dc=0.06) [18], the concentration has to be about 10 times as high as for PS in THF at 25°C (dn/dc≈0.185) [19] to obtain a similar LS signal. But as in contrast, eq. (1) is only valid at very low concentrations, the adjustment of the concentration of the injected samples is quite delicate.

In addition to molar mass, SEC-MALLS can also provide another very important parameter: the z-average radius of gyration R_z of the particles, provided they are not smaller than 15nm. SEC-MALLS has been widely used in the determination of molar mass and size of hyperbranched polymers [20,21] and in CPM characterization [3,14-16,22-24].

SEC with Universal Calibration

Another method to get accurate values of molar masses is SEC with universal calibration (SEC-UC). Unlike conventional calibration, UC is supposed to be valid for all polymers [17,25]. It is based on Fox-Flory's law [26], which states that the hydrodynamic volume of a macromolecule in solution is proportional to the product of its intrinsic viscosity, $[\eta]$ and its molar mass, M (eq. 2):

$$[\eta]M = \Phi \langle r_g^2 \rangle^{3/2} \propto R_h^3 \tag{2}$$

When applying this relationship to a given set of SEC columns, there is a direct relationship with the elution volume V (eq. 3):

$$V \propto [\eta]M \tag{3}$$

This relation is valid for any polymer. Column calibration can thus be obtained from any set of well-defined polymer standards, e.g. polystyrene, and the calibration curve is given by $\log([\eta]M) = f(V)$.

With an on-line viscosity detector, the elution volume and intrinsic viscosity of any unknown polymer sample are measured directly, and molar masses can be deduced from the calibration curve. This method also allows the determination of molar masses of any type of copolymer, which cannot be achieved with conventional SEC. Therefore it has been widely used to characterize linear copolymers, graft copolymers, hyper-branched polymers [20,27-30], dendrimers and proteins, all of which are complex systems that cannot be studied with conventional SEC. Even a few studies on CPMs by Ishizu *et al.* resorted to SEC-UC to determine molar masses [31-33], but in this case viscosity measurements were not made on-line.

With on-line measured intrinsic viscosities, it is possible to study the relationship between $[\eta]$ and M for the sample, referring to the well-known Mark-Houwink-Sakurada (MHS) equation (eq. 4):

$$[\eta] = K \cdot M^a \tag{4}$$

The exponent a can assume values varying between 0 and 2, corresponding to spherical and rod-like macromolecules, respectively [34]. For non-ionic macromolecules with linear flexible chains and random coil conformation, a can vary between 0.5 in theta-solvents and 0.8 in thermodynamically very good solvents. For a given polymer, a depends on the "quality" of the solvent and on the

architecture of the macromolecules: the more branching on the polymer chains, the lower the value of *a*. Several recent studies on hyperbranched polymers showed their exponent *a* was lower than 0.5 in good solvents [20,21,29,35]. Finally CPMs also keep a rather globular shape in solution, and as a result of this compact structure, their viscosity in solution is much lower than that of similar linear macromolecules with the same molar mass. Thus, MHS exponent *a* is also smaller than 0.5 for CPMs [2].
Finally, SEC-UC also provides radii of gyration of polymers, using the Fox-Flory relation for polymer coils (eq. 5) [26,36,37]:

$$R_g^{\,3} = \frac{[\eta]M}{6^{3/2}\Phi_0} \tag{5}$$

Contrarily to SEC-MALLS, this method allows the determination of the radius of gyration even for small macromolecules, due to their better detection through viscosity measurement than using MALLS detection.

Experimental

Materials
Monomers are presented in Table 1 and were used as supplied.
Polystyrene (PS) standards were obtained from Sigma-Aldrich. The solvent used for SEC was HPLC grade THF from SDS.

Syntheses of LP and CPMs
LP and CPMs were synthesized by free-radical polymerization in an organic solvent (heptane, methyl ethyl ketone or heptane/isopropanol mixture) (initial monomer concentration: 25wt.-%). The initiator was 2,2'-AzobisMethylButyroNitrile (AMBN) at 10 mmol/L. The completion of the reaction was achieved within 6 hours at 70°C.
Polymerization conditions are detailed in other papers [14].

Characterization
SEC-UC method was processed with three Styragel HR 5E columns (mixed bed: extended range of porosity) from Waters in series, heated at 35°C. The detectors used for refractive index (RI) and intrinsic viscosity (IV) were respectively VE 3580 and T60A from Viscotek.. Polymer solutions were prepared with concentrations from 1 to 4 mg/mL in THF. 100 µL of solution was injected onto the columns (1 mL/min) for each measurement. The refractometer and viscosimeter were calibrated with different PS standards of known concentrations and viscosities.
SEC-MALLS was processed using a Dawn detector from Wyatt Technology, with laser light tuned on 632.8 nm.
The eluent was THF at 1.0 mL/min.

Results and discussion

Molar mass determination with SEC-UC
A linear copolymer and its corresponding CPM were characterized by SEC-UC and SEC-MALLS in order to validate the universal calibration method. The molar composition of the copolymer was LA/BA/CA/HDDA with 10/70-x/20/x, x=0 and 5 mol-% for linear copolymer and CPM, respectively. Table 2 shows values obtained for molar masses with both SEC methods. Results slightly differ, by about 15 to 20%. This probably comes from the fact that, in SEC-MALLS, the refractive index increment dn/dc of the sample has to be known very precisely. The value that was used here was that of pure poly(butyl acrylate) in THF at 25°C (d*n*/d*c*=0.06), available from the literature [18]. This is a good approximation, due to the high content in BA of the copolymers, but it is certainly not precise enough to allow the exact calculation of the molar masses.

Under our experimental conditions, SEC-UC can thus be considered as a convenient method to measure molar masses of CPMs, which is not possible with conventional SEC. Though it does not seem to be a better method than SEC-MALLS, we will show how useful it can be to characterize CPMs' compactness and to determine their radius of gyration.

Determination of Mark-Houwink-Sakurada exponent, a

For each slice of the chromatogram obtained by SEC-UC, the intrinsic viscosity, $[\eta]$, and the molar mass, M, of the corresponding polymer are known. It is thus possible to plot $\log[\eta]$ versus $\log M$. Mark-Houwink-Sakurada (MHS) equation can be re-written as follows (eq. 4bis):

$$\log[\eta] = a \log M + \log K \qquad (4bis)$$

As a result, the value of exponent a is given by the slope of the linear plot $\log[\eta]$ versus $\log M$.

Curves were plotted for the linear copolymer and the CPM previously described (Figure 1).

The value of the MHS exponent a is 0.62 for the linear copolymer. First of all, a is greater than 0.5, which proves that THF at 35°C is a relatively good solvent for the copolymers we intend to characterize. For further discussion, Table 3 shows values of exponent a for homo-PBA and copolymers of BA with CA and LA.

The value obtained for exponent a of homo-PBA is practically equal to 0.8 and is consistent with the value reported in the literature for a good solvent [16]. One can note a strong decrease in a for copolymers containing CA, or both CA and LA. This is probably due to the presence of long dangling aliphatic chains coming from the co-monomers LA and CA (C_{12} and C_9 dangling chains), which make THF at 35°C a poorer solvent [38]. No value of a for homo-poly(cardura acrylate) seems to be available from the literature, but it has been reported to be 0.58 for homo-poly(lauryl acrylate) in THF at 25°C [16]. This is consistent with values observed for copolymers in this study.

For the CPM, the value of a is 0.37, which is definitely lower than 0.5, and characteristic from dense particles in solution in a good solvent. Unlike the corresponding linear copolymer, the CPM keeps its globular shape in THF at 35°C. The only difference between the two macromolecules is the presence of 5 wt.-% HDDA as a cross-linking agent in the case of CPM. It can thus be concluded that the CPM is really an intramolecularly cross-linked, or at least highly branched macromolecule. Furthermore, the difference in slope between CPMs and linear copolymers (0.37 vs 0.62) is much more pronounced than what was observed for example for a hyperbranched polyurethane (based on an *in situ*-generated 3,5-bis(hydroxyethoxy)phenyl isocyanate) and its linear analog (prepared from diethylene glycol and toluene 2,4-diisocyanate), i.e. 0.41 vs 0.47 in THF [39]. This could confirm the real densely crosslinked character of our particles.

Another point which is important in the case of CPMs raises from the fact that the curve $\log[\eta] = f(\log M)$ is a straight line over the entire range of molar mass. It implies that the value of a is the same for molecules with low or high molar masses. This shows that all the macromolecules have the same degree of crosslinking for their structure, whatever their size. The experimental conditions used for the synthesis lead only to a definite type of CPM molecules, and not to a mixture of linear, branched copolymers and cross-linked macromolecules. Even though the molar mass distribution is broad (PDI=7.5), the density of the particles is homogeneous.

Particles size – Radius of gyration

After these first results, the relation between the radius of gyration and the molar mass was determined, both for LP and CPMs. Therefore LP and CPMs with a wide range of molar masses were synthesized by changing the experimental conditions, and their radii (R_z) were measured by SEC-UC. Plots of R_z against molar mass are shown in Figure 2.

In both cases, the log-log plot of radius of gyration versus molar mass is a straight line. The slopes are ν=0.59 and ν=0.53, respectively for LP and CPM, where ν is defined as the excluded volume index. Thus, the radius of gyration increases faster with respect to molar mass in the case of linear copolymers. This is consistent with differences in compacity between linear and cross-linked macromolecules. The density of the latter is higher than that of a linear macromolecule with a similar molar mass.

Attempts to measure radii of gyration were also made using SEC-MALLS measurements. However, SEC-MALLS is unable to measure reliable radii smaller than 15nm, which is the case for most of our

LP and CPMs. It is all the more critical, as d*n*/d*c* is very low for our polymers; this increases incertitude concerning small macromolecules. Results could only be obtained for CPMs, and are compared to those from SEC-UC in Table 4.
It can be seen that radii of gyration measured by SEC-MALLS are larger than those obtained from SEC-UC. However, the difference is bigger for smaller molar masses. For CPMs with $M_w=10^3$ kg/mol, identical values of R_z are obtained from both methods. SEC-MALLS obviously overestimates radii of gyration of small-sized particles.
SEC-UC is thus more convenient to obtain accurate values, and exponent *a* is directly given by $[\eta]$-M_w relationship.

Conclusion

Size exclusion chromatography with universal calibration (SEC-UC) has proved to be a very useful method to characterize cross-linked polymer particles (CPMs). Not only does it give access to molar masses, but also to the radius of gyration and to the value of Mark-Houwink-Sakurada exponent *a*. Radii of gyration obtained in this way are much more accurate than those measured by SEC-MALLS for small CPMs (with R_z<15nm and M_w<150 kg/mol). The MHS exponent *a* is directly characteristic of the crosslinking density of macromolecules in the solvent. In this study, it was shown that a polymer containing 5mol-% of cross-linking agent has an exponent *a* lower than 0.5, whereas it is higher than 0.5 for a linear polymer of the otherwise same composition. SEC-UC is thus an efficient method to prove CPMs are intramolecularly cross-linked, or at least very highly branched macromolecules.

References

[1] **Graham, N.B., Cameron, A.,** "Nanogels and microgels: the new polymeric materials playground", *Pure Appl. Chem*., **70** (1998), 1271-1275.

[2] **Funke, W.E., Okay, O., Joos-Müller, B.,** "Microgels. Intramolecularly crosslinked macromolecules with a globular structure", *Adv. Polym. Sci.,* **136** (1998), 139-234.

[3] **de Groot, J.H., Zurutuza, A., Moran, C.R., Graham, N.B., Hodd, K.A., Norrby, S.,** "Water-soluble microgels made by radical polymerization in solution", *Colloid Polym. Sci.,* **279** (2001), 1219-1224.

[4] **Radhakrishnan, B., Cramail, H., Cloutet, E.,** "Synthesis of uniform polyurethane particles by step-growth polymerization in a dispersed medium", *Colloid Polym. Sci.,* **280** (2002), 1122-1130.

[5] **Ramanathan, L.S., Baskaran, D., Shukla, P.G., Sivaram, S.,** "Preparation of polyurethane microspheres via dispersion polycondensation using poly(1,4-isoprene)-block-poly(ethylene oxide) as steric stabilizer", *Macromol. Chem. Phys.,* **203** (2002), 998-1002.

[6] **Alvarez-Lorenzo, C., Concheiro, A.,** "Reversible adsorption by a pH- and temperature-sensitive acrylic hydrogel", *J. Control. Release,* **80** (2002), 247-257.

[7] **Soppimath, K.S., Kulkarni, A.R., Aminabhavi, T.M.,** "Chemically modified polyacrylamide-g-guar gum-based crosslinked anionic microgels as pH-sensitive drug delivery systems: preparation and characterization", *J. Control. Release,* **75** (2001), 331-345.

[8] **Kazakov, S., Kaholek, M., Teraoka, I., Levon, K.,** "UV-induced gelation on nanometer scale using liposome reactor", *Macromolecules,* 35 (2002), 1911-1920.

[9] **Ye, L., Cormack, P.A.G., Mosbach, K.,** "Molecular imprinting on microgel spheres", *Anal. Chim. Acta,* **435** (2001), 187-196.

[10] **Bromberg, L., Temchenko, M., Hatton, T.A.,** "Smart microgel studies. Polyelectrolyte and drug-absorbing properties of microgels from polyether-modified poly(acrylic acid)", *Langmuir,* **19** (2003), 8675-8684.

[11] **Zhang, J., Xu, S., Kumacheva, E.,** "Polymer microgels: reactors for semiconductor, metal, and magnetic nanoparticles", *J. Am. Chem. Soc.,* **126** (2004), 7908-7914.[12] **Valette L., Pascault J.P., Magny B.,** "Use of acrylic functionalized (meth)acrylic crosslinked polymer

microparticles in photopolymerized acrylic films", *Macromol. Mater. Eng.,* **288** (2003), 642-657.

[13] **Valette L., Pascault J.P., Magny B.,** "Use of functional (meth)acrylic crosslinked polymer microparticles as toughening agents for epoxy/diamine thermosets", *Macromol. Mater. Eng.,* **288** (2003), 867-874.

[14] **[14a] Valette, L., Pascault, J.P., Magny, B.,** "Meth)acrylic crosslinked polymer microparticles: synthesis by dispersion polymerization and particle characterization", *Macromol. Mater. Eng.,* **287** (2002), 31-40; **[14b] Rouzeau, S., Méchin, F., Pascault**, J.P., to appear.

[15] **Pille, L., Solomon, D.H.,** "Studies on microgels. I. Microgel formation studied by gel-permeation chromatography with an on-line light scattering detector", *Macromol. Chem. Phys.,* **195** (1994), 2477-2489.

[16] **Pille, L., Jhingran, A.G., Capareda, E.P., Solomon, D.H.,** "Studies on microgels. II. Analysis of the reaction between "living" poly(4-t-butylstyrene) and dimethacrylates by size exclusion chromatography coupled with DRI, UV and MALLS detectors", *Polymer,* **37** (1996), 2459-2464.

[17] **Kostanski, L.K., Keller, D.M., Hamielec, A.E.,** "Size exclusion chromatography - a review of calibration methodologies", *J. Biochem. Biophys. Methods,* **58** (2004), 159-186.

[18] **Penzel, E., Goetz, N.,** "Solution properties of polyacrylic esters. I. Light scattering and viscosity measurements in tetrahydrofuran", *Angew. Makromol. Chem.,* **178** (1990), 191-200.

[19] **Huglin, M.B.,** "Specific Refractive Index Increments of Polymers in Dilute Solution", in: *Polymer Handbook*, 3dr edition, J. Brandrup, I.H. Immergut, Eds., John Wiley & Sons, New York 1989, p. VII/409 ff.

[20] **Simon, P.F.W., Müller, A.H.E., Pakula, T.,** "Characterization of highly branched poly(methyl methacrylate) by solution viscosity and viscoelastic spectroscopy", *Macromolecules,* **34** (2001), 1677-1684.

[21] **Baskaran, D.,** "Hyperbranched polymers from divinylbenzene and 1,3-diisopropenylbenzene through anionic self-condensing vinyl polymerization", *Polymer,* **44** (2003), 2213-2220.

[22] **Matsumoto, A., Ohashi, T., Oe, H., Aota, H., Ikeda, J.I.,** "Amphiphilic polymethacrylates as crosslinked polymer precursors obtained by free-radical monomethacrylate/dimethacrylate copolymerizations", *J. Polym. Sci., Part A: Polym. Chem.,* **38** (2000), 4396-4402.

[23] **Abrol, S., Solomon, D.H.,** "Studies on microgels. IV. The effect of solvent on the synthesis of t-butylstyrene-divinylbenzene microgels by anionic polymerization", *Polymer,* **40** (1999), 6583-6589.

[24] **Matsumoto, A., Kodama, K., Aota, K., Capek, I.,** "Kinetics of emulsion crosslinking polymerization and copolymerization of allyl methacrylate", *Eur. Polym. J.,* **35** (1999), 1509-1517.

[25] **Grubisic, Z., Rempp, P., Benoît, H.,** "A universal calibration for gel permeation chromatography", *J. Polym. Sci., Part B: Polym. Phys.,* **5** (1967), 753-759.

[26] **Flory, P.J., Fox, Jr, T.G.,** "Treatment of intrinsic viscosities", *J. Am. Chem. Soc.,* **73** (1951), 1904-1908.

[27] **Ambler, M.R., McIntyre, D.,** "Randomly branched styrene/divinylbenzene copolymers. I. Preparation, molecular weight characterization, and GPC analysis", *J. Appl. Polym. Sci.,* **21** (1977), 3237-3250.

[28] **Kaivez, A., Gallez, X.A., Daoust, D., Devaux, J., Godard, P.,** "Molecular structure characterization of linear and branched polystyrene blends by size exclusion chromatography coupled with viscosimetry", *Polymer,* **43** (2002), 3181-3190.

[29] **Geladé, E.T.F, Goderis, B., de Koster, C.G., Maijerink, N., van Benthem, R.A.T.M., Fokkens, R., Nibbering, N.M.M., Mortensen, K.,** "Molecular structure characterization of hyperbranched polyesteramides", *Macromolecules,* **34** (2001), 3552-3558.

[30] **Ye, Z., Zhu, S.,** "Newtonian flow behavior of hyperbranched high-molecular-weight polyethylenes produced with a Pd-diimine catalyst and its dependence on chain topology", *Macromolecules,* **36** (2003), 2194-2197.

[31] **Chen, H., Ishizu, K., Fukutomi, T., Kakurai, T.,** "Preparation of soluble microgel by the copolymerization of methyl methacrylate with p-divinylbenzene in the presence of tetrabromomethane", *J. Polym. Sci.*, *Part A: Polym. Chem.,* **22** (1984), 2123-2130.

[32] **Ishizu, K., Kuwabara, S., Chen, H., Mizuno, H., Fukutomi, T.,** "Kinetics of crosslinking reactions. I. Reaction rate of intramolecular crosslinkings by using a soluble microgel", *J. Polym. Sci.*, *Part A: Polym. Chem.,* **24** (1986), 1735-1746.

[33] **Ishizu, K., Tsubaki, K., Mori, A., Uchida, S.,** "Architecture of nanostructured polymers", *Prog. Polym. Sci*., **28** (2003), 27-54.

[34] **Netopilik, M., Kratochvil, P.,** "Polystyrene-equivalent molecular weight versus true molecular weight in size-exclusion chromatography", *Polymer,* **44** (2003), 3431-3436.

[35] **Kharchenko, S.B., Kannan, R.M., Cernohous, J.J., Venkataramani, S.,** "Role of architecture on the conformation, rheology, and orientation behavior of linear, star, and hyperbranched polymer melts. I. Synthesis and molecular characterization", *Macromolecules,* **36** (2003), 399-406.

[36] **Jackson, C., Chen, Y.J., Mays, J.W.,** "Size exclusion chromatography with multiple detectors: solution properties of linear chains of varying flexibility in tetrahydrofuran", *J. Appl. Polym. Sci.,* **61** (1996), 865-874.

[37] **Jackson, C.,** "On the accuracy of molecular weight and radius of gyration measured by right-angle light scattering with SEC-viscometry", *Int. J. Polym. Anal. & Charac.,* **2** (1996), 425-437.

[38] **Bohdanecký, M., Netopilik, M.,** "The Mark-Houwink-Kuhn-Sakurada exponent of polymers with long side groups: is $a_0 = 1/2$ a reliable criterion of the theta state?", *Polymer,* **36** (1995), 3377-3384.

[39] **Hong, L., Wang, X., Tang, X.,** "Studies on hyperbranched polyurethane by multidetector size exclusion chromatography", *J. Appl. Polym. Sci.,* **85** (2002), 2445-2450.

Table 1. ***Monomers used in this study***

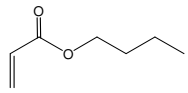
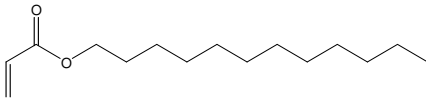
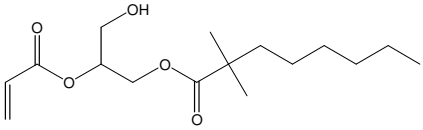
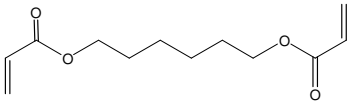
Name	Designation	Structure	*M* (g/mol)	Supplier
butyl acrylate	BA		128	Aldrich
lauryl acrylate	LA		240	Aldrich
cardura acrylate	CA		300	Cray Valley
hexanediol diacrylate	HDDA		226	Aldrich

Table 2. ***Determination of molar masses for a linear polymer, LP, and its cross-linked polymer particle counterpart, CPM, with SEC-UC and SEC-MALLS. PDI=polydispersity index.***

Sample	**d*n*/d*c***	M_w **(kg/mol)**		**PDI**
		SEC-MALLS	**SEC-UC**	
LP	0.06	14	16.5	1.7
CPM	0.06	106	124	6.5

Table 3. ***Influence of comonomers on MHS exponent a in copolymers based on BA. PDI=polydispersity index.***

Composition		M_w **(kg/mol)**	**PDI**	***a***
LP	BA	156	3	0.79
	CA/BA (20/80)	16.5	1.7	0.67
	LA/BA/CA (10/70/20)	16.5	1.7	0.62
CPM	LA/BA/CA/HDDA (10/65/20/5)	124	7.5	0.37

Table 4. ***Radii of gyration of CPMs obtained from SEC-MALLS and SEC-UC***

M_w (kg/mol)	R_z from SEC-UC (nm)	R_z from SEC-MALLS (nm)
950	34	34
565	24.5	29
165	13	22
100	9.5	17
45	7	-

Figure captions

Figure 1. Plot of log([η]) versus log(M) for a linear polymer, LP, and its corresponding cross-linked polymer particle CPM ([η] in dL/g and M in g/mol).
Figure 2. Plot of radius of gyration versus molar mass for linear polymers, LP, and their corresponding cross-linked polymer particles, CPMs (R_z in nm and M_w in g/mol).

Figure 1. Plot of log([η]) versus log(M) for a linear polymer, LP, and its corresponding cross-linked polymer particle CPM ([η] in dL/g and M in g/mol).

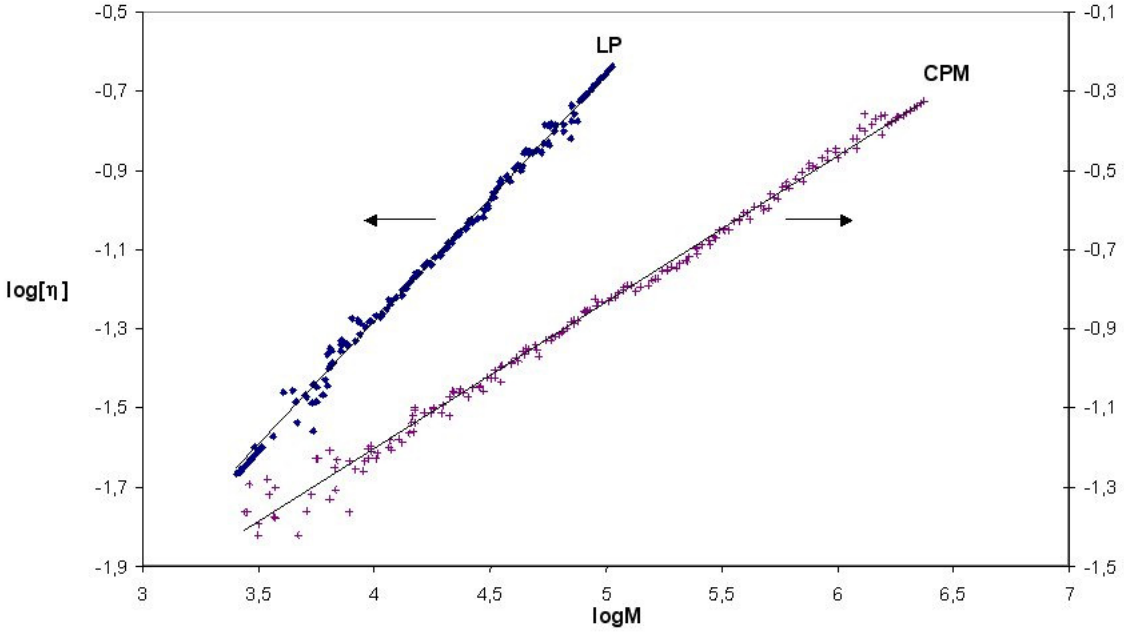


Figure 2. Plot of radius of gyration versus molar mass for linear polymers, LP, and their corresponding cross-linked polymer particles, CPMs (R_z in nm and M_w in g/mol).