Size of Elastic Single-Chain Nanoparticles in Solution and on Surfaces

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ABSTRACT: We introduce herein a simple model of covalent-bonded single-chain nanoparticles (SCNPs) by considering these intrachain crosslinked nano-objects as elastic unimolecular networks. According to this model, each SCNPs is regarded as a network of elastic strands connected by crosslinks. The free energy of the SCNPs is decomposed in two contributions: i) an elastic free energy due to the presence of these strands, and ii) an excluded volume contribution arising from the balance of monomer-monomer and monomer-solvent interactions. We provide scaling law expressions for the size (R), diffusion coefficient (D), apparent molar mass (M_{app}) and shrinking factor ($\langle G \rangle$) of elastic SCNPs in good, theta and bad solvents. Also we derive scaling laws for the height (H) of elastic SCNPs deposited on both low- and high-surface free energy substrates. A comparison of experimental results to model predictions for covalent-bonded polystyrene SCNPs in solution of narrow molecular weight distribution ($1.09 \leq D \leq 1.26$), different amount of reactive groups ($0.025 \leq x \leq 0.3$) and varying precursor molar mass ($44 \leq M \leq 235$ kDa) is performed based on literature data. For the same system, a similar comparison of experimental and theoretical H data corresponding to the case of SCNPs deposited on low- and high-energy substrates is also carried out. This simple elastic model provides a useful framework for connecting the amount of reactive groups and precursor molar mass with the size upon intrachain crosslinking for a variety of covalent-bonded SCNPs both in solvents of different quality and on substrates of different surface free energy.

1. INTRODUCTION

The folding of individual synthetic polymer chains (precursors) to single-chain polymer nanoparticles (SCNPs) is reminiscent of protein folding to its functional, native state and is attracting significant interest in recent years.¹⁻³ The self-folding process can be driven by different intramolecular interactions such as non-covalent, covalent and dynamic covalent bonds, by using well-defined precursors decorated with appropriate functional groups and experimental conditions such that inter-chain coupling events are minimized to a large extent.⁴⁻¹³ The resulting SCNPs are useful soft nano-objects with potential applications in nanomedicine,¹⁴⁻¹⁷ biosensing^{18,19} and catalysis,²⁰⁻²³ among other different fields.²⁴⁻⁴⁰ Theoretical models providing a connection between both the amount of functional groups, x , and precursor molar mass, M , with the size of the resulting SCNPs are of great interest to establish useful structure-property relationships. In particular, relevant parameters for end-use applications such as surface area, intrinsic viscosity and diffusion coefficient do depend on SCNP size.

Recently, a model providing the expected size reduction upon folding single chains of size R_0 to SCNPs of size R by means of reversible interactions (non-covalent bonds) as a function of x and M has been introduced.⁴¹ This simple model affords a valuable *a priori* estimation of the size reduction upon folding single chains to conventional SCNPs *via* reversible interactions (~7 % of average deviation of calculated data from experimental ones; 72 SCNPs and 22 reversible interactions analyzed). In spite of such advance in providing useful structure-property relationships for developing practical applications of responsive SCNPs, to the best of our knowledge no equivalent model has been yet reported for the case of covalent-bonded SCNPs.

When compared to reversible SCNPs, the structure of covalent-bonded SCNPs includes permanent intramolecular crosslinks that affect to a large extent the final compaction degree achieved^{41,42} and provide them with superior thermal stability. Hence, covalent-bonded SCNPs in solution with similar nature, M and x than reversible SCNPs do display, on average, a higher level of chain compaction.⁴¹ In this work, we introduce a simple model of covalent-bonded SCNPs by considering these intrachain crosslinked nano-objects as elastic unimolecular networks composed of elastic strands connected by crosslinks.⁴³ We derive scaling law expressions for the size, diffusion coefficient, apparent molar mass and shrinking factor of elastic SCNPs in good, theta and bad solvents. Also we obtain scaling laws for the height of elastic SCNPs deposited on both low- and high-surface free energy substrates.

The article is organized as follows. In Section 2 we introduce the model of elastic SCNPs and derive scaling law expressions for elastic SCNPs in solution and on solid substrates. A comparison of the model predictions to experimental results is provided in Section 3, for the case of covalent-bonded polystyrene (PS)-SCNPs in good solvent (tetrahydrofuran, THF) and on low-surface energy (silanized wafer) and high-surface energy (mica) substrates. Force *versus* stretching curves for PS-SCNPs of different intramolecular crosslinking degree are predicted. Comparison of model predictions to experimental data for other covalent-bonded SCNPs is also performed. Concluding remarks are given in Section 4.

2. THEORETICAL SECTION

2.1 Model of Elastic Single-Chain Nanoparticles

We consider a SCNP precursor of total degree of polymerization N and root-mean-square size R_0 containing two different monomers distributed randomly along the chain: reactive monomers of type A, and unreactive monomers of type B. The number fraction of A monomers in the precursor is x . Let us assume that the precursor follows a scaling law such as⁴³

$$R_0 \approx a_0 N^{\nu_0} \quad (1)$$

where a_0 is proportional to the monomer size, and ν_0 is the corresponding scaling exponent. For the precursor in good solvent conditions, $\nu_0 = \nu_F = 3/5 = 0.6$ (0.588 according to renormalization group theory).⁴³

Upon SCNP formation in a solvent at high dilution *via* intrachain covalent bonds, the A monomers are expected to dimerize to produce crosslinks of type A-A. By working at very high dilution, the nature of the cross-linking should be intramolecular due to isolation of each individual chain under such conditions. Each resulting SCNP can be regarded as a network of elastic strands connected by crosslinks (see Figure 1).

The free energy (F) of such SCNP of size R is decomposed in two contributions: i) an elastic free energy (F_{el}) arising from the presence of the elastic strands, and ii) an excluded volume contribution (F_{ex}) from the balance of monomer-monomer and monomer-solvent interactions, such as

$$F = F_{el} + F_{ex} \quad (2).$$

The elastic free energy can be expressed as

Figure 1. Schematic illustration of an elastic single-chain nanoparticle (SCNP) regarded as a network of elastic strands connected by crosslinks.

$$\frac{F_{el}}{k_B T} \approx \frac{3}{2} \frac{R^2}{R_0^2} + KR^2 = \left(\frac{3}{2R_0^2} + Ax \right) R^2 \quad (3)$$

where k_B is the Boltzmann constant, T the absolute temperature and K an effective constant related to the elasticity of the unimolecular network. We assume that K is proportional to x where A is the corresponding proportionality constant. When $x \rightarrow 0$, eq. 3 reduces to the Flory expression for a linear chain.⁴³ For elastic SCNPs eq. 3 becomes

$$\frac{F_{el}}{k_B T} \approx KR^2 = AxR^2 \quad (4)$$

by assuming $K \gg \frac{3}{2R_0^2}$, which is a good approximation (see section 3.4). An expression similar to eq. 4 was originally introduced by Vilgis⁴⁴ for the case of soft microgels in the presence of excluded volume interactions.

The excluded volume contribution to the free energy can be expressed as a virial expansion of ρ ⁴⁵

$$\frac{F_{ex}}{k_B T} \approx \frac{N}{2} (\rho B + \rho^2 C + \dots) \quad (5)$$

where $\rho \propto \left(\frac{N}{R^3}\right)$ and $B, C, \dots$ are effective second, third, ... virial coefficients of the monomer interaction. The first term in eq. 5 corresponds to the excluded volume coefficient ($B > 0$ in a good solvent, $B = 0$ in a θ -solvent and $B < 0$ in a bad solvent). The second term in eq. 5 accounts for three-body interactions ($C > 0$).

The equilibrium configuration of the elastic SCNP is obtained by minimizing eq. 2 with respect to R , so

$$2AxR - 3 \left(\frac{BN^2}{2R^4} + \frac{CN^3}{R^7} \right) = 0 \quad (6).$$

Eq. 6 will be used in next section to derive useful expressions relating the relative amount of reactive monomers, x , and precursor molecular weight, M , with the size, R , and diffusion coefficient, D , of elastic SCNPs in solvents of different quality.

2.2 Size, Diffusion Coefficient, Apparent Molar Mass and Shrinking Factor of Elastic Single-Chain Nanoparticles in Solution

A. Good Solvent Conditions

Size: In good solvent ($B > 0$) the third term in eq. 6 can be neglected, so we obtain the following scaling law for the size of the elastic SCNP

$$R = a_1 x^{-1/5} N^{2/5} = b_1 x^{-1/5} M^{2/5} = b_1 x^{\nu_1} M^{\nu_1} \quad (7)$$

where $a_1 \equiv \left(\frac{3B}{4A}\right)^{1/5}$, $b_1 \equiv \left(\frac{3B}{4Am_0^2}\right)^{1/5}$, $m_0 = \frac{M}{N}$ and M is the molar mass. Consequently,

the elastic SCNP has a scaling exponent ($\nu_1 = 2/5 = 0.4$) smaller than that of a linear chain in a good solvent ($\nu_0 \approx 3/5 = 0.6$). The same value of scaling exponent was previously derived for the case of soft microgels in the presence of excluded volume interactions.⁴⁴ A ratio of radius of gyration (R_G) to hydrodynamic radius (R_H) very close to 1 is expected for chains having $\nu = 0.4$ (see Appendix A, Figure A1). Hence, eq. 7 is expected to be valid for R_G as well as for R_H data. It is worth of mention that the size of elastic SCNPs scales with the relative amount of reactive monomers, x , with an exponent $\omega_1 = -1/5 = -0.2$. According to eq. 7 the size of the elastic SCNP depends on the ratio of the excluded volume coefficient B to the effective elastic constant A , with a value of scaling exponent of $1/5$.

Diffusion Coefficient: By assuming $R_G / R_H \approx 1$, we use the Stokes-Einstein equation⁴³ to obtain an expression for the diffusion coefficient (D) of elastic SCNPs in good solvent as a function of x and M , such as

$$D = \frac{k_B T}{6\pi\eta R} = d_1 x^{1/5} N^{-2/5} = e_1 x^{1/5} M^{-2/5} \quad (8)$$

where $d_1 \equiv \left(\frac{k_B T}{6\pi\eta a_1}\right)$, $e_1 \equiv \left(\frac{k_B T}{6\pi\eta b_1}\right)$ and η is the viscosity of the solvent.

Apparent Molar Mass: Size exclusion chromatography (SEC) allows determining the apparent molar mass of SCNPs, referred to the molar mass of linear polymer standards. In SEC experiments, macromolecules are separated according to their respective

hydrodynamic radius.⁴⁶ The hydrodynamic size of a linear polymer standard is related to its molar mass according to

$$R_H \approx c_0 M^{\nu_0} \quad (9)$$

where c_0 is a constant. According to eq. 7 and 9, an elastic SCNP of size R prepared from a precursor of molar mass M and relative amount of reactive monomers x , will have a SEC apparent molecular weight (M_{app}) given by⁴⁷

$$b_1 x^{\omega_1} M^{\nu_1} \approx c_0 M_{app}^{\nu_0} \quad (10)$$

and hence

$$M_{app} = f_1 x^{\alpha_1} M^{\beta_1} \quad (11)$$

where $f_1 \equiv \left(\frac{b_1}{c_0} \right)^{1/\nu_0}$, $\alpha_1 = \omega_1 / \nu_0$ and $\beta_1 = \nu_1 / \nu_0$. For $\nu_0 = 3/5$, we obtain

$\alpha_1 = -1/3 \approx -0.33$ and $\beta_1 = 2/3 \approx 0.67$ so eq. 11 becomes

$$M_{app} = f_1 x^{-1/3} M^{2/3} \quad (12).$$

Shrinking Factor: The shrinking factor is defined as $\langle G \rangle = M_{app} / M$. Consequently, by using eq. 11 we obtain

$$\langle G \rangle = f_1 x^{\alpha_1} M^{\beta_1 - 1} \quad (13).$$

For $\nu_0 = 3/5$ eq. 13 becomes

$$\langle G \rangle = f_1 x^{-1/3} M^{-1/3} \quad (14).$$

B. Theta Solvent Conditions

Size: In a theta solvent, the second term in eq. 6 vanishes ($B = 0$) so we obtain the following expression for the size of the elastic SCNP

$$R = a_2 x^{-1/8} N^{3/8} = b_2 x^{-1/8} M^{3/8} = b_2 x^{\omega_2} M^{\nu_2} \quad (15)$$

where $a_2 \equiv \left(\frac{3C}{2A} \right)^{1/8}$ and $b_2 \equiv \left(\frac{3C}{2Am_0^3} \right)^{1/8}$. As expected, a smaller scaling law exponent

($\nu_2 = 3/8 = 0.375$) is obtained for the elastic SCNP in theta solvent than that in good solvent. In this case, the size of elastic SCNPs scales with x with a smaller exponent $\omega_2 = -1/8 = -0.125$. Eq. 15 should be also valid for R_G as well as for R_H data (see Appendix A, Figure A1). In this case, the size of the elastic SCNP depends on the ratio of the coefficient of three-body interactions C to the effective elastic constant A , with a value of scaling exponent of $1/8$.

Diffusion Coefficient: By assuming $R_G / R_H \approx 1$, the diffusion coefficient of an elastic SCNP in theta solvent is given by

$$D = \frac{k_B T}{6\pi\eta R} = d_2 x^{1/8} N^{-3/8} = e_1 x^{1/8} M^{-3/8} \quad (16)$$

where $d_2 \equiv \left(\frac{k_B T}{6\pi\eta a_2} \right)$ and $e_1 \equiv \left(\frac{k_B T}{6\pi\eta b_2} \right)$.

Apparent Molar Mass: For an elastic SCNP in theta solvent, M_{app} is given by

$$M_{app} = f_2 x^{\alpha_2} M^{\beta_2} \quad (17)$$

where $f_2 \equiv \left(\frac{b_2}{c_0}\right)^{1/v_0}$, $\alpha_2 = \omega_2/v_0$ and $\beta_2 = v_2/v_0$. For $v_0 = 3/5$, we obtain

$\alpha_2 = -5/24 \approx -0.21$ and $\beta_2 = 5/8 \approx 0.63$ so eq. 17 becomes

$$M_{app} = f_2 x^{-5/24} M^{5/8} \quad (18).$$

Shrinking Factor: The shrinking factor of an elastic SCNP in theta solvent is given by

$$\langle G \rangle = f_2 x^{\alpha_2} M^{\beta_2-1} \quad (19).$$

For $v_0 = 3/5$ eq. 19 becomes

$$\langle G \rangle = f_2 x^{-5/24} M^{-3/8} \quad (20).$$

C. Bad Solvent Conditions

Size: In bad solvent $B < 0$, so the size of the elastic SCNP can be approximated by

$$R = a_3 N^{1/3} = b_3 M^{1/3} = b_3 M^{v_3} \quad (21)$$

where $a_3 \equiv \left(\frac{3C}{|B|}\right)^{1/3}$ and $b_3 \equiv \left(\frac{3C}{|B|m_0}\right)^{1/3}$. In this case, the size of elastic SCNPs scales

with N with the expected exponent for globules ($v_3 = 1/3 \approx 0.33$) and no dependence of R on x is expected.

Diffusion Coefficient: It is well-known that for compact globules $R_G/R_H = \sqrt{3/5} \approx 0.775$, so the diffusion coefficient of an elastic SCNP in bad solvent becomes

$$D = \frac{k_B T}{6\pi\eta R} = d_3 N^{-1/3} = e_3 M^{-1/3} \quad (22)$$

where $d_3 \equiv \left(\frac{\sqrt{3}k_B T}{6\sqrt{5}\pi\eta a_3} \right)$ and $e_3 \equiv \left(\frac{\sqrt{3}k_B T}{6\sqrt{5}\pi\eta b_3} \right)$.

Apparent Molar Mass: For an elastic SCNP in bad solvent, M_{app} is given by

$$M_{app} = f_3 M^{\beta_3} \quad (23)$$

where $f_3 \equiv \left(\frac{b_3}{c_0} \right)^{1/\nu_0}$ and $\beta_3 = \nu_3 / \nu_0$. For $\nu_0 = 3/5$, we obtain $\beta_3 = 5/9 \approx 0.56$ so eq.

23 becomes

$$M_{app} = f_3 M^{5/9} \quad (24).$$

Shrinking Factor: The shrinking factor of an elastic SCNP in bad solvent is given by

$$\langle G \rangle = f_3 M^{\beta_3 - 1} \quad (25).$$

For $\nu_0 = 3/5$ eq. 25 becomes

$$\langle G \rangle = f_3 M^{-4/9} \quad (26)$$

2.3 Size of Elastic Single-Chain Nanoparticles on Surfaces

A. Low-Surface Free Energy Substrate

Let us consider the case of an elastic SCNP deposited onto a very low-surface free energy substrate. The SCNP is expected to take a compact, globular conformation onto

this surface which allows minimizing the contact between the SCNP and the low-surface energy substrate. The volume of the SCNP should be

$$V = \frac{M}{\rho_0 N_A} = \frac{4}{3} \pi R^3 \quad (27)$$

where ρ_0 is the bulk density. Consequently, the height ($H = 2R$) of the SCNP will be given by⁴⁸

$$H = b_4 M^{1/3} \quad (28)$$

where $b_4 = \left(\frac{6}{\pi \rho_0 N_A} \right)^{1/3}$.

B. High-Surface Free Energy Substrate

When compared to the previous case, now the elastic SCNP is expected to spread to some extent over the surface. Let us assume that the SCNP over the substrate adopt a conformation equivalent to that in the melt.^{49,50} We obtain an estimation of the lateral spreading by using eq. 15 in two dimensions ($d = 2$, see Appendix B) such as

$$R_{||} \propto x^{-1/(2d+2)} M^{3/(2d+2)} \propto x^{-1/6} M^{3/6} \quad (29)$$

Since the volume of the elastic SCNP in the high-surface free energy substrate, $V \approx H(\pi R_{||}^2)$, should be equal to the volume of the SCNP in a low-surface free energy substrate (eq. 27) we obtain

$$H = g_1 x^{1/3} \quad (30)$$

where $g_1 \equiv \left[\frac{m_0}{\pi \rho_0 N_A} \left(\frac{C}{A} \right)^{-1/3} \right]$ is a constant.

C. Single-Chain Nanoparticle Stretching

From Eq. 4 we obtain the force (f) *versus* stretching (z) equation of elastic SCNPs such as

$$f = \frac{\partial F_{el}}{\partial z} \approx (2k_B T A x) z = K_s z \quad (31)$$

where K_s is the effective (x -dependent) spring coefficient. Eq. 31 could be used to determine the constant A from experimental f vs. z curves by AFM of covalent-bonded SCNPs with different amount of reactive groups, x . To the best of our knowledge, such experiments have not been performed yet. For the case of a linear chain ($x \rightarrow 0$), the classical result corresponding to the freely jointed chain (FJC) model⁵¹ is obtained from eq. 3:

$$f = \frac{\partial F_{el}}{\partial z} \approx \left(\frac{3k_B T}{R_0^2} \right) z \quad (32)$$

2.4 Summary of Scaling Laws for Elastic Single-Chain Nanoparticles

A summary of the scaling law expressions for elastic SCNP in solution and on solid substrates is given in Table 1, as derived by assuming $\nu_0 = 3/5$.

Table 1. Summary of Scaling Law Expressions for Elastic SCNPs (see text).

Magnitude	Solution		
	Good solvent	Theta solvent	Bad solvent
Size	$R \propto \chi^{-1/5} M^{2/5}$	$R \propto \chi^{-1/8} M^{3/8}$	$R \propto M^{1/3}$
Diffusion coefficient	$D \propto \chi^{1/5} M^{-2/5}$	$D \propto \chi^{1/8} M^{-3/8}$	$D \propto M^{-1/3}$
Apparent molar mass	$M_{app} \propto \chi^{-1/3} M^{2/3}$	$M_{app} \propto \chi^{-5/24} M^{5/8}$	$M_{app} \propto M^{5/9}$
Shrinking factor	$\langle G \rangle \propto \chi^{-1/3} M^{-1/3}$	$\langle G \rangle \propto \chi^{-5/24} M^{-3/8}$	$\langle G \rangle \propto M^{-4/9}$
Height	Solid Substrate		
	Low Surface Free Energy		High Surface Free Energy
	$H \propto M^{1/3}$		$H \propto \chi^{1/3}$
Spring Coefficient	Nanoparticle Stretching		
	$K_s \propto \chi$		

3. RESULTS AND DISCUSSION

3.1 Comparison to Experimental Results

The most accurate compilation of size data for covalent-bonded SCNPs is due to Hawker and coworkers^{48,52} corresponding to polystyrene (PS)-SCNPs (see Appendix C,

Table C1 and C2). The intrachain crosslinking process was carried out at high temperature (250 °C) by using precursors with highly reactive benzocyclobutene functional groups. In particular, a large set of size data in solution (THF as solvent) was reported corresponding to precursors of narrow molecular weight distribution ($1.09 \leq \bar{D} \leq 1.26$), different amount of reactive groups ($0.025 \leq x \leq 0.3$) and varying precursor molar mass ($44 \leq M \leq 235$ kDa).⁵² Size characterization of the resulting covalent-bonded PS-SCNPs was carried out by SEC in THF. Moreover, the absolute molar mass by static light scattering (SLS) of the PS-SCNPs of higher molar mass ($M = 230$ kDa) was found to be in agreement, within experimental error, with the molar mass by SEC of the corresponding linear precursor ($M = 234$ kDa) supporting the formation of nanoparticles each composed of a single chain. Concerning size data on surfaces (mica and silanized wafer substrates), they cover data from precursors of $\bar{D}$ between 1.04 and 1.91, molar mass between 33.0 and 211 kDa and amount of reactive groups between 0.025 and 0.60.⁴⁸

In section 3.2 we perform a comparison of the experimental data from Table C1 with model predictions corresponding to elastic SCNPs in good, theta and bad solvent conditions. For the same system, a similar comparison of experimental and theoretical H data corresponding to SCNPs deposited on low- and high-energy substrates (Table C2) is carried out in section 3.3. Comparison of model predictions to experimental data for SCNPs different from those given in Table C1 and C2 is performed in section 3.5. Relevant information about the conformation of SCNPs in solution and on solid substrates can be found in references 42 and 48, respectively.

3.2 Size, Diffusion Coefficient, Apparent Molar Mass and Shrinking Factor of Polystyrene Single-Chain Nanoparticles in Tetrahydrofuran

We investigate the ability of the elastic SCNP model corresponding to good, theta and bad solvent conditions (eq. 11, 17 and 23, respectively) to reproduce the M_{app} data from Table C1 as a function of x and M , by using the values in eq. 9 corresponding to PS standards in THF ($c_0 = 1.44 \times 10^{-2}$, $v_0 = 0.561$).⁵³ Best fitting of the M_{app} data to eq. 11 corresponding to elastic SCNPs in good solvent gives $f_1 = 5.95$, $b_1 = c_0 f_1^{v_0} = 3.92 \times 10^{-2}$ and $\frac{B}{A} = \frac{4}{3} (b_1^5 m_0^2) = 1.33 \times 10^{-3}$. Average deviation of data calculated from $M_{app} = 5.95 x^{-0.357} M^{0.713}$ and experimental M_{app} data was only 4.2 % (see Figure 2A). Conversely, best-fitting the M_{app} data to eq. 17 and 23, corresponding to theta and bad solvent conditions, gives average deviation of calculated and experimental M_{app} data of 13.0 % and 33.4 %, respectively (Figure 2B and 2C). Hence, as illustrated in Figure 2, the observed experimental trend is only reproduced with reasonable accuracy by the elastic SCNP model for good solvent conditions, as it is expected for the case of THF solutions. The description is fairly achieved in terms of a single model parameter (the ratio of the excluded volume coefficient B to the effective elastic constant A).

Figure 2. M_{app} data of PS-SCNPs in THF (solid green circles: $M = 232$ kDa, solid red squares: $M = 111$ kDa, and solid blue circles: $M = 44$ kDa).⁵² The same data are described by the elastic SCNPs model (solid green, red and blue lines, respectively) assuming the case of good solvent conditions (A), theta solvent conditions (B) and bad solvent conditions (C). The experimental trend is only reproduced by the elastic SCNP model for good solvent conditions in terms of a single model parameter (see text).

Figure 3 provides a comparison of calculated size, diffusion coefficient and shrinking factor to experimental data reported in Table C1 according to the elastic SCNP model for good solvent conditions.

Figure 3. Comparison of calculated size (R_H), diffusion coefficient (D) and shrinking factor ($\langle G \rangle$) from the elastic SCNP model corresponding to good solvent conditions to experimental data from Table C1.⁵² Closed symbols and continuous lines have the same meaning as in Figure 2. Open symbols correspond to data from dynamic light scattering (DLS) experiments.

3.3 Size of Polystyrene Single-Chain Nanoparticles on Silanized Wafer and Mica Substrates

A comparison of predictions from the elastic SCNP model and experimental size data for PS-SCNPs⁴⁸ deposited onto substrates of different surface free energy is illustrated in Figure 4. On a low-free energy substrate such as the silanized wafer surface, PS-SCNPs with $x = 0.2$ and 0.6 behave as solid globules, a result previously reported by Dukette *et al.*⁴⁸ It is worth of mention that departure from the globular conformation is observed for PS-SCNPs with very low amount of reactive functional groups ($x = 0.025$).

Figure 4. Comparison of theoretical predictions from the elastic SCNP model to experimental size data of PS-SCNPs⁴⁸ (solid blue circles: $x = 0.025$, solid red squares: $x = 0.20$, and solid green circles: $x = 0.60$) on (A) low- and (B) high-surface free energy

substrates (see text and Table C2). Solid green, red and blue lines are the predicted behavior for $x = 0.025$, 0.20 and 0.60 , respectively.

Figure 4B shows the ability of the elastic SCNP model to reproduce the experimental behavior of PS-SCNPs deposited onto a high-surface energy substrate such as mica⁴⁸ in terms of a single parameter (related to the ratio of the third virial coefficient C to the effective elastic constant A). Best fitting of the data in Figure 4B to eq. 30 gives

$$g_1 = 5.81, \quad \frac{C}{A} = 7.3 \times 10^{-7} \quad \text{and} \quad C \approx 3.47 \times 10^{-5} \quad \text{by using} \quad A \approx 47.6 \quad (\text{see Section 3.4}).$$

3.4 Stretching of Polystyrene Single-Chain Nanoparticles

Here we use the elastic SCNP model to estimate the force (f) *versus* stretching (z) curves for PS-SCNPs at low values of z . By using $\frac{B}{A} = 1.33 \times 10^{-3}$ (see Section 3.2) and

$B \approx 6.33 \times 10^{-2}$ (see Appendix D), the estimated value of A is 47.6 which guarantees the condition $K = Ax \gg \frac{3}{2R_0^2}$ (see eq. 4) even for the lower values of M and x of Table C1

and C2.

Figure 5. Predicted force (f) versus stretching (z) curves for PS-SCNPs of different values of x at low deformation.

Figure 5 shows the predicted force (f) *versus* stretching (z) curves for PS-SCNPs of different values of x in the regime of low deformations according to eq. 31. The corresponding values of the effective spring constant are $K_s = 9.8, 19.6, 39.2, 117.5$ and 235.0 for $x = 0.025, 0.05, 0.10, 0.30$ and 0.60 , respectively.

3.5 Comparison of Model Predictions to Experimental Data for Other Covalent-Bonded SCNPs

A. SCNP Size in Good Solvent Conditions

We are interested to investigate the predictive possibilities of the model for covalent-bonded PS-SCNPs obtained from other different cross-linking techniques, and also for covalent-bonded SCNPs of other chemical composition (*e.g.*, methacrylate- and

acrylate-based SCNPs). Figure 7 provides a comparison of experimental R_H data and calculated ones from $R_H(nm) = 3.92 \times 10^{-2} x^{-1/5} M^{2/5}$ (see section 3.2) for SCNPs in good solvent conditions. Experimental R_H data were taken from ref. 42 corresponding to

and the effective elastic constant A could change depending on the specific chemical nature of the reactive functional groups and monomer repeat units (styrene vs (meth)acrylate), the overall chain rigidity, etc. When only PS-SCNPs are compared, the average standard deviation between calculated and experimental data reduces to 8.5 %.

B. SCNP Size on High-Surface Free Energy Substrates

A comparison of experimental H data from AFM experiments over mica substrate and calculated ones from $H(nm) = 5.81x^{1/3}$ (see section 3.3) is provided in Table 2. It is worth of mention that only a reduced number of covalent-bonded SCNPs have been investigated by AFM on mica substrate.^{16,48,54-57} In spite of the scarce data available, a reasonable agreement is found between predictions and experimental results for covalent-bonded PS-SCNPs obtained by crosslinking techniques different from benzocyclobutene dimerization,⁵⁴⁻⁵⁶ as well as for covalent-bonded poly(*tert*-butyl methacrylate) (PtBMA)⁵⁷ and poly(methacrylic acid) (PMAA)¹⁶ SCNPs.

Table 2. Comparison of Experimental H Data (H_{exp}) and Calculated Ones (H_{cal}) for Covalent-Bonded PS-SCNPs Obtained from Crosslinking Techniques Different from Benzocyclobutene Dimerization, as well as for Covalent-Bonded PtBMA- and PMAA-SCNPs

SCNP Type ^a	x	$H_{\text{exp}}(\text{nm})$	$H_{\text{cal}}(\text{nm})^b$	Ref.
PS	0.09	3.0	2.6	54
PS	0.10	1.6, 3.8	2.7	55
PS	0.10	1.7	2.7	56

PMAA	0.10	2.0	2.7	16
PtBMA	0.17	3.5	3.2	57

^a PtBMA = Poly(*tert*-butyl methacrylate); PMAA = Poly(methacrylic acid). ^b Calculated from: $H(nm) = 5.81x^{1/3}$ (see text).

4. CONCLUDING REMARKS

A simple model of covalent-bonded SCNPs has been introduced by considering these soft nano-objects as a network of strands, which behave as elastic springs, connected by crosslinks. Based on this model, scaling law expressions for the size, diffusion coefficient, apparent molar mass and shrinking factor of elastic SCNPs in good, theta and bad solvents have been derived, as well as scaling laws for the height of elastic SCNPs deposited on both low- and high-surface free energy substrates.

The elastic SCNPN model for good solvent conditions reproduces with reasonable accuracy the experimental size, diffusion coefficient, apparent molar mass and shrinking factor of PS-SCNPs in THF as a function of x and M , in terms of a single model parameter (the ratio of the effective excluded volume coefficient B to the effective elastic constant A). The predictions of the elastic SCNPN model are found in excellent agreement with i) the approximately constant height of PS-SCNPs of different M on high-surface energy substrates, and ii) the dependence of the height of PS-SCNPs on x , with a theoretical exponent of $1/3$ for the case of low-surface energy substrates. Force *versus* stretching curves for PS-SCNPs of different values of x at low deformation would allow determining experimentally the effective elastic constant A .

To summarize, the simple elastic SCNPN model introduced in this work provides a useful framework for connecting the amount of reactive groups and precursor molar

mass with the size upon intrachain crosslinking for a variety of covalent-bonded SCNPs both in solvents of different quality and on substrates of different surface free energy.

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Notes

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APPENDIX A: R_G / R_H Ratio as a Function of ν .

The R_G / R_H ratio as a function of ν can be estimated from:^{58,59}

$$R_G = \frac{b}{\sqrt{(2\nu+1)(2\nu+2)}} N^\nu \quad (\text{S1})$$

$$R_H = \frac{\pi(1-\nu)(2-\nu)b}{2\sqrt{6}\pi} N^\nu \quad (\text{S2})$$

$$\frac{R_G}{R_H} = \frac{2\sqrt{6}}{(1-\nu)(2-\nu)\sqrt{\pi(2\nu+1)(2\nu+2)}} \quad (\text{S3})$$

where b is the segment length. It is worth of mention that for compact globules ($\nu = 1/3$), the exact value of R_G / R_H is $\sqrt{3/5} \approx 0.775$.

Figure A1. R_G / R_H ratio as a function of the scaling exponent ν .

Figure A1 illustrates the values of R_G / R_H ratio for $\nu = 3/5$, $\nu = 1/2$ and $\nu = 1/3$. Both for $\nu = 2/5 = 0.4$ and $\nu = 3/8 = 0.375$ it is expected a value of R_G / R_H ratio very close to 1.

APPENDIX B: Generalization of the Free Energy Equation to d Dimensions for the Case of $B = 0$.

Generalization of eq. 2 to d dimensions for melt and theta solvent conditions gives

$$\frac{F}{k_B T} \approx AxR^2 + \frac{CN^3}{2R^{2d}} \quad (S4)$$

and by minimization of eq. S4 to

$$2AxR - \frac{dCN^3}{R^{2d+1}} = 0 \quad (S5).$$

Consequently,

$$R = \left(\frac{dC}{2A} \right)^{1/(2d+2)} x^{-1/(2d+2)} N^{3/(2d+2)} \quad (S6).$$

For the case of $d = 2$, eq. 29 in the main text results.

APPENDIX C: Summary of Size Data for PS-SCNPs

Table C1. Summary of Size Data for PS-SCNPs in THF⁵²

Precursors			SCNPs			
x	M (kDa)	$\bar{D}$	M_{app} (kDa)	$\langle G \rangle$	R (nm) ^a	D (nm ² /μs) ^b
0.025	44.0	1.09	41.7	0.95	5.6	85
0.050	44.5	1.08	38.2	0.86	5.4	88
0.10	45.5	1.09	29.1	0.64	4.6	103
0.15	43.5	1.10	20.7	0.48	3.8	125
0.20	44.0	1.07	18.5	0.42	3.6	132
0.0125	110.5	1.15	103.0	0.93	9.3	51
0.025	113.0	1.12	94.5	0.84	8.9	53

0.050	109.0	1.14	79.3	0.73	8.1	59
0.075	108.0	1.15	59.8	0.55	6.9	69
0.10	112.0	1.10	56.0	0.50	6.6 (6.2)	72 (77)
0.15	110.0	1.16	44.2	0.40	5.8	82
0.20	111.0	1.11	42.8	0.39	5.7	83
0.25	112.0	1.12	40.5	0.36	5.5	86
0.0125	231.0	1.23	189.8	0.82	13.2	36
0.025	235.0	1.25	174.0	0.74	12.5	38
0.050	228.0	1.22	109.0	0.48	9.6	49
0.075	230.0	1.19	98.0	0.43	9.1	52
0.10	233.0	1.26	91.5	0.39	8.7 (9.5)	55 (50)
0.125	231.0	1.24	81.0	0.35	8.2	58
0.15	235.0	1.23	80.3	0.34	8.1	59
0.20	230.0	1.21	66.0	0.29	7.3	65
0.25	229.0	1.24	62.0	0.27	7.0 (6.4)	68 (74)
0.3	234.0	1.23	63.5	0.27	7.1	32

^a Calculated from:⁵² $R_H (nm) = 1.44 \times 10^{-2} M^{0.561}$. Data from DLS are shown in

parenthesis. ^b Calculated from: $D = \frac{k_B T}{6\pi\eta R_H}$. Data from DLS are shown in parenthesis.

Table C2. Summary of Size Data for PS-SCNPs on Silanized Wafer and Mica substrates⁴⁸

PS-SCNPs			Silanized Wafer Substrate	Mica Substrate
<i>x</i>	<i>M</i> (kDa)	<i>Đ</i>	<i>H</i> (nm)	<i>H</i> (nm)
0.025	24.5	1.14	2.6	1.8
0.025	60.1	1.16	3.0	2.4
0.025	158	1.40	4.0	2.6
0.20	41.0	1.04	4.0	1.6
0.20	78.0	1.14	4.8	3.0
0.20	211	1.32	9.3	2.8
0.60	33.0	1.91	5.4	4.3

APPENDIX D: Estimation of B for PS in THF

From eq. 3 (with $x = 0$), eq. 5 ($B > 0$) and taking into account that $R_G / R_H \approx 1.86$ for $\nu = 3/5$ we obtain

$$B \approx \frac{2}{b^2} (1.86 \times c_0)^5 m_0^3 \quad (\text{S7})$$

where $b \approx 0.7$,⁶⁰ $c_0 = 1.44 \times 10^{-2}$ and $m_0 = 104$ so $B \approx 6.33 \times 10^{-2}$ for PS in THF.

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