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Dilute solution properties of lactosylated polyamidoamine dendrimers and their structural characteristics

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Abstract

Six generations of lactodendrimers based on poly(amidoamine) were studied in dilute solutions of 0.2 M NaCl by methods of molecular hydrodynamics (velocity sedimentation and viscometry) and optics (static and dynamic light scattering). Molecular weights, sedimentation and diffusion coefficients, and intrinsic viscosity values were measured. These data were analysed and compared with previous results for lactodendrimers. The values of hydrodynamics invariant and average substance density in a dendrimer molecule are discussed. The volume occupied by lactogroups was evaluated and it was shown that lactogroups do not experience strong steric hidrances in lactodendrimer molecules. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Carbohydrate dendrimers; Molecular and hydrodynamic properties; Polyamidoamine dendrimers

1. Introduction

In the last decades dendrimer molecules (molecular trees) became the subject of extensive studies [1–6]. Their regular molecular structure suggests that they can be applied in various fields of supramolecular chemistry, catalysis, surface phenomena, study of life and materials technology. In addition to the study of regular dendrimer molecules, hyper-branched dendrite systems obtained by single-stage synthesis have also been investigated [2–3]. The natural representatives of the latter are amylopectin, glycogen and lignin.

Chemical modifications of initial dendrimer molecules are being intensively studied [5–8]. Functional groups are bound to the core of the dendrimer molecules and fix the desired properties in a limited volume. The chemical nature of these groups usually differs from that of the core. In this way hybrid molecules are obtained; the analogues among linear macromolecules may be copolymers. These molecules may exhibit specific properties which have also been subjected to detailed investigations [5,6]. Glucodendrimers also draw considerable attention [7,8] because they make it possible to simulate and study glycoprotein interactions. The aim of these studies is to establish the role of sugar containing molecules in living systems. Glycodendrimers also extend the class of hydrophilic molecular systems which can be used to prepare or modify pharmacologic preparations.

Dendrimers are also an interesting object of studies in molecular physics. However, real dendrimers and their properties have not been studied in detail experimentally.

In this work new experimental data obtained by methods of molecular hydrodynamics (velocity sedimentation and viscometry) and optics (static and dynamic light scattering) are presented on the molecular parameters of lactodendrimers based on poly(amidoamines) (PAMAM). These data are discussed and compared with previously obtained results for lactodendrimers [9–12].

2. Methods

Lactosylated dendrimers are a new type of neoglycoconjugates of considerable interest in the study of multivalent carbohydrate–protein interactions [7,13]. Using commercially available Starburst PAMAM dendrimers containing amine residues as surface functionality, it was possible to synthesise a wide range of sugar-based 'glycodendrimers' using unprotected *p*-isothiocyanatophenyl glycosides in aqueous media. The synthesis of lactosylated starburst

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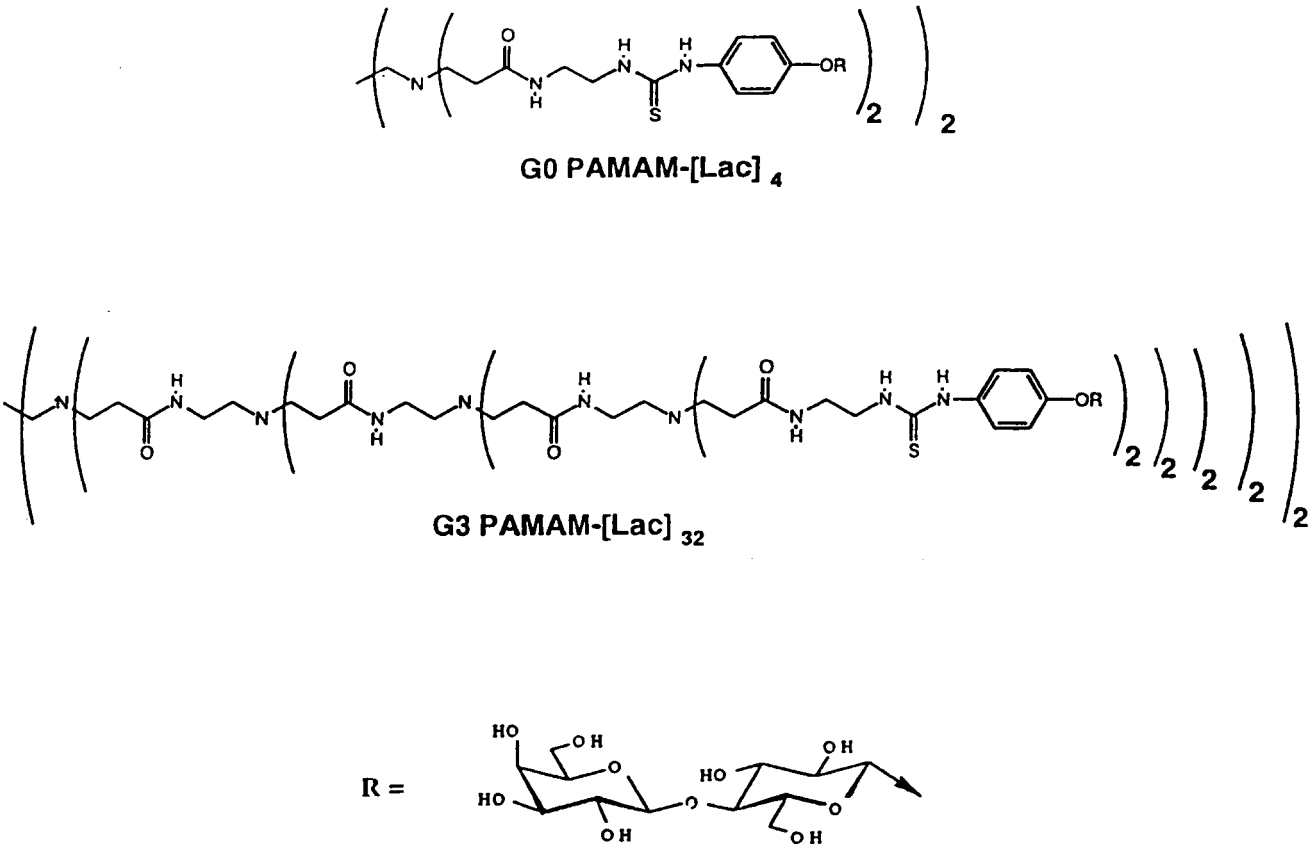

Fig. 1. Structural formula of thiolactosyl modified polyamidoamine dendrimers for generations 0 and 3.

poly(amidoamine) dendrimers (LPAMAMD) up to generation five possessing symmetrical structures (Fig. 1) with an unprotected lactoside derivative [14] followed the strategy described for mannosylated and sialylated dendrimers [15,16].

Dynamic light scattering of solutions was studied by using a DynaPro-801TC, Protein Solutions, Inc., photometer which measures at a fixed angle (90°). It is possible to measure with it the translational diffusion coefficients of globular proteins and molecules the shape of which is close to a sphere [17]. The scattered photons are counted by using a system of cascade photodiode. The wavelength of the laser source λ is 780 nm. The membrane filter with Whatman Anotop 10 attachment and the pore diameter of 0.02 μm was used and 150 μl of solution was injected. Solution concentrations were approximately 10^{-2} g/cm^3.

The signal accumulation time for one measurement depends on the molecule size and the difference between refractive indices of the polymer and the solvent and ranges from 2 to 25 min. It was found that lactodendrimers solutions in 0.019 M (0.165%) NaCl single measurement can be carried out only for a sample of the highest generation. This probably related to the effect of incompletely screened charges and the appearance of short living clusters from dendrimer molecules. The increase in the concentration of the low molecular weight salts to 1.169% (≈ 0.2 M) enabled us to avoid this difficulty and measure diffusion coefficients for all generations (Fig. 2). In all further studies, 0.2 M NaCl was used as solvent. It had the following characteristics: $\rho_0 = 1.002$ g/cm^3, $\eta_0 = 0.914$ cp at 25°C.

Dendrimers of different generations were also studied by GPC with the aid of three-detector recording [18] (Fig. 3). These three recording procedures were: (1) refractometric recording with the aid of an interference refractometer of Optilab 901/Wyatt Technology, Santa Barbara, CA, USA; (2) scattered light recording by using a detector of static scattering of the He–Ne laser light of a 5 mw at $\lambda = 632.28$ nm (Wyatt Technology, Santa Barbara, CA, USA); and (3) viscometric recording with a viscometric detector of the hydrodynamic bridge type (similar to the Wheatstone bridge) [19,20]. The viscometric detector makes it possible to detect different viscosities of the solution and the solvent at minimal concentrations when $(\eta - \eta_0)/\eta_0 c$ (or $\ln \eta_r/c$) can be assumed to be the value of intrinsic viscosity. The injected volume (100 μl) passes from three TSK G6000PW, G5000PW and G4000PW (Anachem, Luton, UK) analytical columns connected in series. They make it possible to separate polyethylene oxide molecules at molecular weights ranging from 1×10^3 to 2×10^6 g/mol. The eluent was injected at a rate

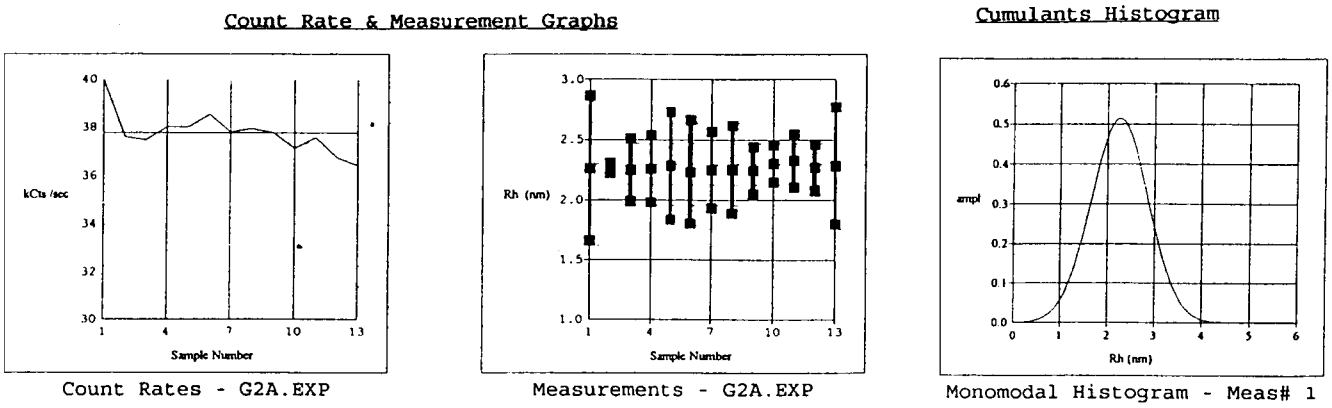

Fig. 2. Stokes' radii (nm) of lactodendrimer (generation 2) obtained from dynamic light scattering.

of 0.8 ml/min at room temperature. The refractive index increments dn/dc were measured in independent experiments with the Optilab 901 interference refractometer.

Sedimentation coefficients were determined on a Beckman XLI analytical ultracentrifuge in the absorbtion regime at $\lambda = 280$ nm (Fig. 4). Concentration of dendrimer solutions did not exceed $c = 0.01 \times 10^{-2}$ g/cm^3, which makes it possible to assume that the obtained sedimentation coefficients are values extrapolated to zero concentration ($c[\eta] < 0.0004$). Sedimentation coefficients were calculated from the time displacement of the middle point of the tangent to the sedimentation curve. This curve is limited by straight lines corresponding to $c = 0$ and $c = c_p$ where c_p is the plateau concentration. It is evident that for symmetric curves this point is the inflection point and corresponds to the maximum in the following coordinate system: distance from the rotation axis—concentration gradient.

Experimental data at 25°C are given in Table 1.

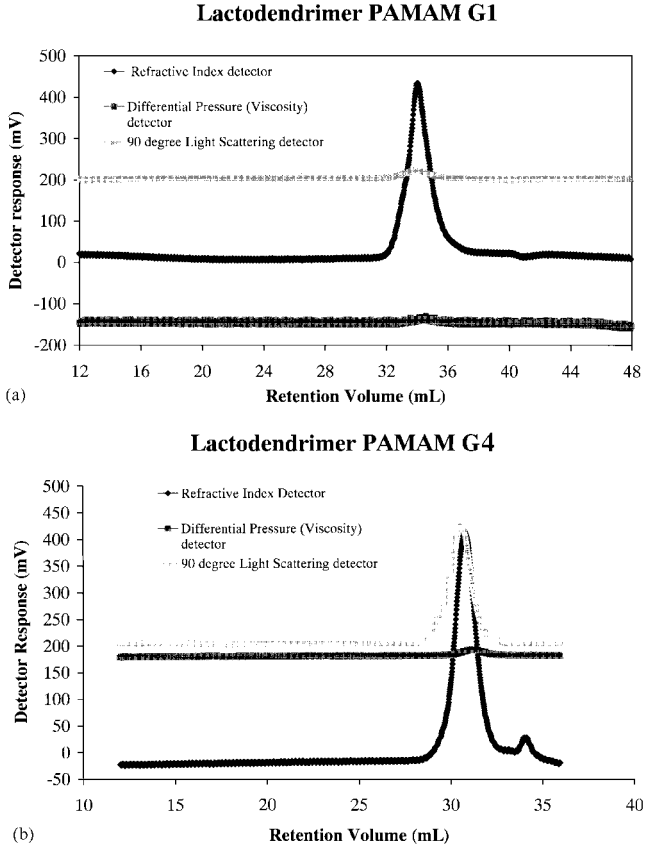

Fig. 3. Three-detector chromatogram of lactodendrimers (generations 4(a) and (b)): (1) signal of refractometric detector; (2) signal of light scattering detector; (3) signal of viscometric detector.

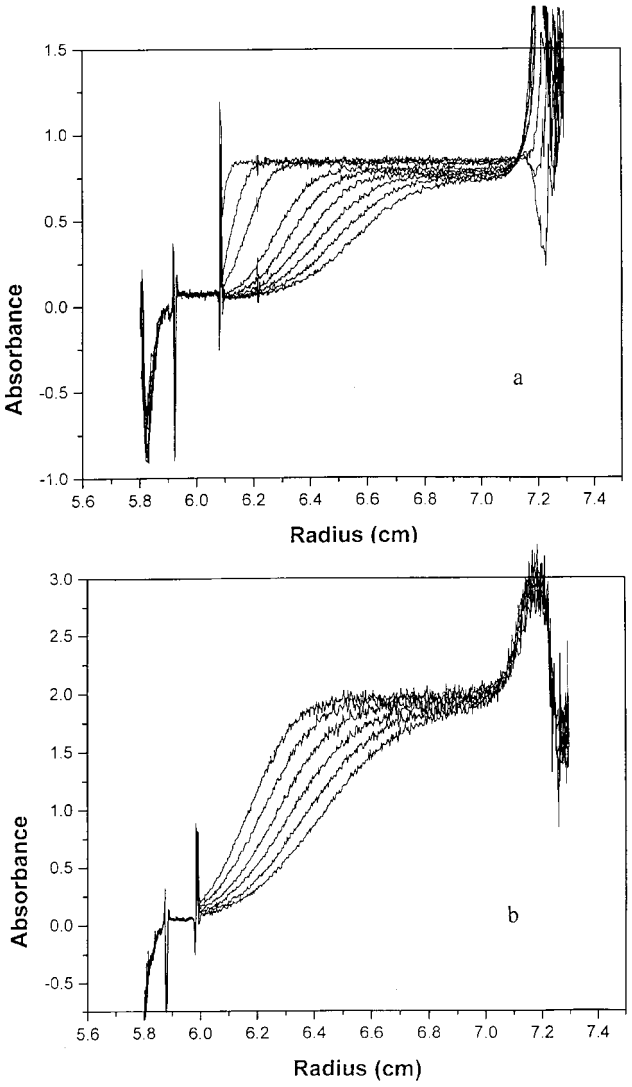

Fig. 4. Absorption sedimentogram obtained for the sedimentation of the lactodendrimers (generations 3(a) and 2(b)) at $c = 0.01 \times 10^{-2}$ g/cm^3. The time intervals between scanning are 20 min (a) and 30 min (b), $n = 50\,000$ rpm.

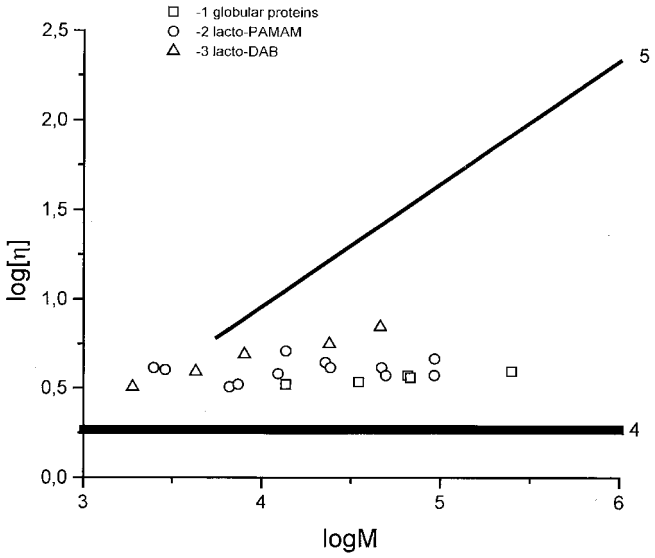

Fig. 5. Double logarithmic dependence of $[\eta]$ on M: (1) globular proteins [19,20]; (2) lactodendrimer based on polyamidoamine according to data in Table 1 and Refs. [11,12]; (3) lactodendrimer based on polypropylenimine according to Refs. [9,10]; (4) limiting $[\eta]$ value calculated for the model of a rigid impermeable sphere; and (5) dependence for a linear polymer (polyvinylpyrrolidone) according to Refs. [21].

3. Discussion

3.1. Comparison of hydrodynamic characteristics

Comparison of hydrodynamic and molecular characteristics in Table 1 with those obtained previously for the same dendrimers by using other devices and procedures shows that they are in good agreement. The values of M_w in Table 1 and the values of M_{sD} obtained in Refs. [11] and [12] virtually coincide. Moreover, the results obtained by using GPC demonstrate very slight sample polydispersity ($M_w/M_n < 1.07$).

Further comparison of hydrodynamic values will be carried out in terms of the corresponding double logarithmic dependences shown in Figs. 5 and 6. Fig. 5 shows the dependences of $\log[\eta]$ on $\log M$ according to the data in Table 1 and in [11,12]. The same figure shows data for lactodendrimers based on poly(propylenimine) [9,10] and for globular proteins [23,24]. The lower zone implies the limiting theoretical value of $[\eta]$ which can be calculated for rigid impermeable spheres by using experimental values of specific partial volume, υ ($[\eta]_{theor} = 2.5\upsilon$). The comparison of viscometric data indicates that: (1) the intrinsic viscosity of lactodendrimers is virtually independent of M; (2) it is also virtually independent of the core nature for the two structures being compared; and (3) it is close to the corresponding value for globular coils. At the same time the values of $[\eta]$ slightly exceed the low theoretical limit for rigid impermeable spheres. Fig. 5 also shows for comparison the dependence of $[\eta]$ on M for a linear water-soluble polyvinylpyrrolidone [25]. A similar comparison is shown in Fig. 6 for data on velocity sedimentation coefficient and demonstrates the high sensitivity of the sedimentation coefficient or the translational friction coefficient to the molecular weight of dendrimer molecules.

Fig. 7 shows generalised data obtained in the study of translational friction of lactodendrimers in 0.019 M and 0.2 M NaCl. The data are presented in the coordinates of dependence of $[f]$ on molecular weight where $[f] \equiv f/\eta_0 = k/[D] = M/[s]N_A$, k is the Boltzman constant and N_A is the Avogadro's number and $[D] \equiv D_0\eta_0/T$.

All data fall on a single dependence which corresponds to the following scaling equation:

$$[f] = 1.94 \times 10^{-7} M^{0.329 \pm 0.014}, \; r = 0.9807,$$

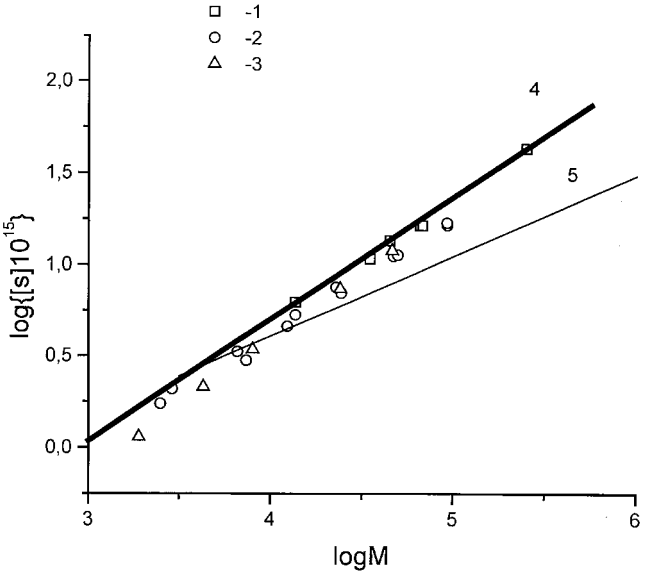

Fig. 6. Double logarithmic dependence of [s] on M. 1–5 are the same as in Fig. 4.

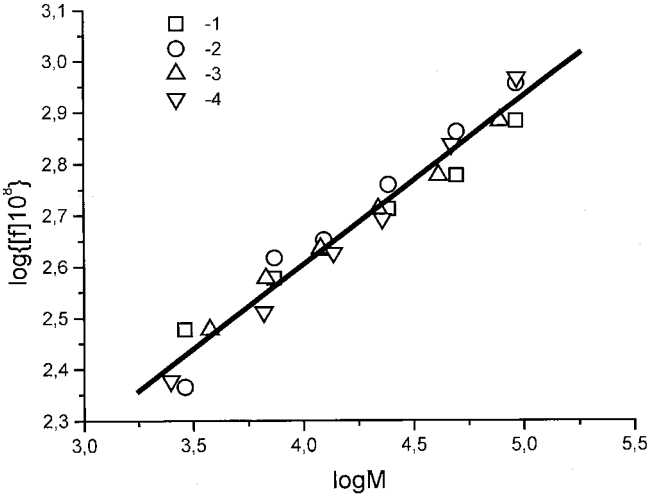

Fig. 7. Characteristic coefficient of translational friction [f] vs. M on double logarithmic scale: (1) $[f]_D$ on M_w in 0.2 M NaCl; (2) $[f]_s$ on M_w in 0.2 M NaCl; (3) $[f]_D$ on M_{sD} in 0.2 M NaCl; and (4) $[f]_D$ on M_{sD} in 0.019 M NaCl.

where r is linear correlation coefficient of the dependence of [f] on M on double logarithmic scale.

This empirical equation was obtained as a result of direct and independent determinations of characteristics related to the translational friction of molecules and to molecular weights. The scaling index coincides with that for rigid impermeable spheres (0.333) within experimental error. This fact indicates that the impermeable sphere model can adequately describe the behaviour of lactodendrimers investigated at least on the level of scaling indices.

The comparison of results in Fig. 5–7 and the previous results [9,11] show that the values determined by translational friction of molecules are the most sensitive to molecular weight changes in dendrimer molecules. In this connection it is very topical to develop the theory of translational friction coefficient for regulary branched molecules.

3.2. Hydrodynamic invariant

This system of molecular and hydrodynamic characteristics makes it possible to analyse the value of hydrodynamic invariant A_0, widely used in the molecular physics of polymers [21,22]. In this case the value of A_0 can be calculated by three methods:

$$A_{01} = [D](M_w[\eta])^{1/3}$$

$$A_{02} = \mathrm{R}[s][\eta]M_w^{-2/3}$$

$$A_{03} = (\mathrm{R}[s][D]^2[\eta])^{1/3},$$

where R is the universal gas constant, $[s] \equiv s_0\eta_0/(1 - \upsilon\rho_0)$.

The values of hydrodynamic invariant calculated by these methods are given in Table 2. (It is evident that $A_{01}^2A_{02} \equiv A_{03}^3$) Table 2 also lists the values of A_{03} obtained previously for the same samples in a solvent containing 0.019 M NaCl. The average value of A_0 obtained in 0.2 M NaCl is $A_0 = (2.47 \pm 0.11) \times 10^{-10}$ cm^2 s^{-2} K^{-1} mol$^{-1/3}$. The corresponding average value in 0.019 M NaCl is $A_0 = (2.61 \pm 0.16) \times 10^{-10}$ [11,12]. The total average value for all data is $A_0 = (2.51 \pm 0.08) \times 10^{-10}$. The value of A_0 differs from the theoretical value $A_0 = 2.914 \times 10^{-10}$ obtained for rigid impermeable spheres. This fact implies, in particular,

Table 1
Molecular and hydrodynamic characteristics of lactoPAMAM dendrimers

N[a]	s 10^{13} (s)	D10^7 (cm^2/s)	$[\eta]$ (cm^3/g)	dn/dc (cm^3/g)	M_w (10^{-3})	M_w/M_n	M_{sD}[b] (10^{-3})	M_{theor} (10^{-3})
4	0.77	15.0	4.0	0.177	2 900	1.05	2 500	2 420
8	1.1	11.9	3.3	0.196	7 400	1.00	6 600	5 230
16	1.7	10.4	3.8	–	12 400	1.02	13 700	10 840
32	2.6	8.7	4.1	0.162	24 300	1.03	22 800	22 120
64	4.2	7.5	3.7	–	49 700	1.07	47 000	44 640
128	6.3	5.9	3.7	0.183	92 400	1.07	93 000	89 690

[a] Number of end groups.
[b] From [11,12].

Table 2
Values of the hydrodynamic invariant A_0 (g cm^2 s^{-2} K^{-1} mol$^{-1/3}$) and density of dendrimer molecules ρ (g cm^{-3}) of lacto PAMAM dendrimers

G	A_{01} 10^{10}	A_{02} 10^{10}	A_{03} 10^{10}	A_{03}[a] 10^{10}	A_0^{aver} 10^{10}	ρ_f[b]	ρ_η
0	2.24	2.91	2.45	2.68	2.57 ± 0.23	0.42 ± 0.13	0.63
1	2.28	2.09	2.21	2.50	2.27 ± 0.12	0.32 ± 0.03	0.76
2	2.48	2.40	2.45	2.85	2.55 ± 0.16	0.39 ± 0.02	0.66
3	2.67	2.40	2.57	2.76	2.60 ± 0.12	0.41 ± 0.05	0.61
4	2.82	2.32	2.64	2.46	2.56 ± 0.17	0.49 ± 0.10	0.68
5	2.73	2.31	2.59	2.39	2.51 ± 0.16	0.45 ± 0.08	0.68
Average values	2.54 ± 0.20	2.41 ± 0.17	2.49 ± 0.12	2.61 ± 0.16	2.51 ± 0.08	0.41 ± 0.04	0.67 ± 0.04

[a] From Refs. [11,12].
[b] Average value $\rho_f = (\rho_{sD} + \rho_D + \rho_s)/3$.

that the values of $M_{D_\eta} = A_0^3([D]^3[\eta])^{-1}$ calculated by using the theoretical value of A_0 can be too high (in this case approximately 1.6 times), whereas the value of $M_{s_\eta} = (R^3[\eta][s]^3/A_0^3)^{1/2}$ will be 1.3 times too low.

3.3. Average density of dendrimer substance in the volume limited by the dendrimer molecule

The average density of the dendrimer substance in the volume limited by the dendrimer molecule can be calculated on the basis of hydrodynamic and molecular characteristics. It can be easily shown that by using a spherical model when data on translational friction are available, density can be calculated by three procedures:

$$\rho_{sD} = 3^4 2\pi^2 k^{-2}[s][D]^2$$

$$\rho_D = 3^4 2\pi^2 N_A^{-1} k^{-3} M_w [D]^3$$

$$\rho_s = 3^4 \pi^2 N_A^2 [s]^3 / M_w^2$$

On the basis of viscometric data, density can be calculated from the ratio

$$\rho_\eta = 2.5/[\eta].$$

The values of density ρ_η and the average values $\rho_f = (\rho_{sD} + \rho_D + \rho_s)/3$ are given in Table 2.

These data do not show any dependences of ρ values on generation number (or M) obtained on the basis of both translational friction and viscometry. The value of density obtained from viscometric data is approximately 1.5 times greater than those obtained from translational friction data. This is the reflection of incomplete adequacy of the rigid sphere model for describing the hydrodynamic behaviour of dendrimer molecules.

Fig. 8 shows the comparison of density values for dendrimer and polymer substances in the volume occupied by a dendrimer molecule and a linear polymer molecule. A flexible-chain linear polysaccharide, polymaltotriose (pullulan) is considered as the polymer object for comparison [26]. In this case the polymer coil density was calculated in the Gaussian approximation according to the equations:

$$\rho_{sD} = P^3 k^{-2} (0.36)^{-1} [s][D]^2$$

$$\rho_\eta = \Phi / N_A 0.36[\eta]$$

where P and Φ are the Flory hydrodynamic parameters [22].

For dendrimer molecules of the highest generations the density exceeds that of the polymer substance in a coil formed by a linear molecule by one order of magnitude or greater. (A polymer substance density in a volume limited by a rigid-chain molecule will be even one more order of magnitude lower.) In the case of lactodendrimers, density is virtually independent of M_w, whereas for linear molecules it decreases by one order on passing to the range of high M. These evaluations of the averaged character do not make it possible to determine substance distribution on passing from the centre of the dendrimer molecule to its periphery.

3.4. Volume occupied by lactose groups in lactodendrimers

In the lactodendrimers investigated, up to 80% of the mass is concentrated in the lactose units. Let us calculate the additional volume occupied by lactodendrimer molecules

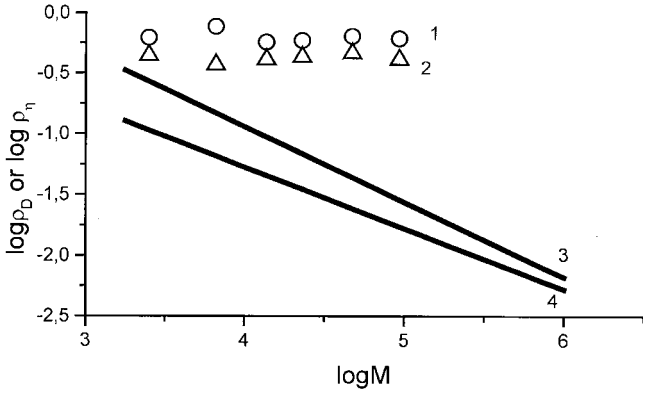

Fig. 8. Dendrimer substance density ρ vs M on double logarithmic scale: (1) density was calculated from data on rotational friction; (2) density was calculated from data on translational friction; (3) density of linear molecule of pullulan polysaccharide, calculated from data in Ref. [22] on rotational friction; and (4) density of linear molecule of pullulan polysaccharide, calculated from data in Ref. [22] on translational friction.

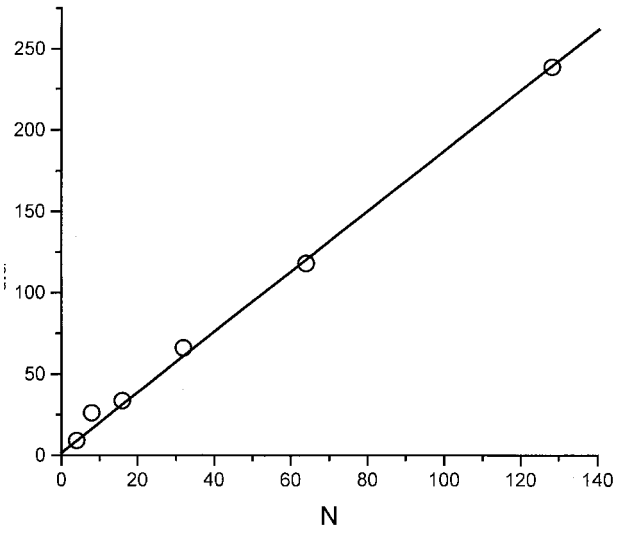

Fig. 9. Excess volume of lactodendrimer molecules vs. number of lactose groups in the polyamidoamine based lactodendrimers.

as compared to that of the initial dendrimer and compare this volume with the number of end groups in each generation. This volume will be evaluated as that of a spherical layer $\Delta V = V_{\mathrm{LPAMAM}} - V_{\mathrm{PAMAM}} = 4\pi/3(R^3_{\mathrm{LPAMAM}} - R^3_{\mathrm{PAMAM}})$, where V_{LPAMAM} is the volume of the lactodendrimer molecule and V_{PAMAM} is the volume of the initial PAMAM-dendrimers. The values of V_{PAMAM} were calculated by using radii obtained for the initial PAMAM dendrimer molecules [1,27]. For all lactodendrimer generations, the following ratio between the sizes is observed $R_{\mathrm{lacto}} > R_{\mathrm{core}}$. Hence, lactodendrimer molecules have a greater volume than the initial dendrimers. This may be caused by either the contribution of the end lactogroups themselves and/or by a possible increase in core volume. However, it should be borne in mind that dendrimers based on polyamidimine are short-chain dendrimers, for which the distance between two neighbouring branching points is small (they may also be called rigid-chain dendrimers because $l/A < 1$ where l is the contour length of a chain part between two neighbouring branching points and A is the Kuhn segment length for this chain). In this case [10] it is necessary to take into account a possible increase in equilibrium rigidity A, caused by the interaction between side chains in the dendron and by electrostatic interaction, which can lead to $l/A \ll 1$. This implies that the increase in lactodendrimer size due to core swelling is unlikely and that to a first approximation all changes can be considered to be caused by the contribution of lactogroups.

Fig. 9 shows the dependence of ΔV (calculated as the mean value of the data on translational and rotational friction) on the number of lactose groups N in dendrimers of the corresponding generations. It follows from Fig. 9 that the values of ΔV are directly proportional to the number of lactose end groups in a dendrimer molecule. This implies that the volume per lactose group which is approximately $\Delta V/N \approx 1900 \times 10^{-24}$ cm^3 virtually does not change on passing from low to high generations. Let us compare this volume with the volume of the lactose molecule, which we can evaluate from the diffusion coefficient of lactose measured in H_2O. We obtained the value $D_{\mathrm{lactose}} = (44.0 \pm 0.5) \times 10^{-7}$ cm^2/s and consequently $R_{\mathrm{D}} = 5.7 \times 10^{-8}$ cm and so $V_{\mathrm{lactose}} = 776 \times 10^{-24}$ cm^3. The volume per lactose group in lactodendrimer molecules exceeds in average 2.4 times its own volume. It is also easy to compare the volume per end group in lactodendrimer molecules with the volume which, a free lactose can exclude for its unbonded neighbours in solution. In fact, the excluded volume due to pair interactions of independent particles is proportional to their own volume V [23,28,29]: $V_{\mathrm{excl}} = 8\nu V$ where the proportional coefficient ν reflects the effects of shape asymmetry of the colliding particles. It ranges from $\nu = 1$ for a sphere to $\nu = 0.25(l/d)$ for a rod ($l \gg d$ where l is the rod length and d is the rod diameter). In the intermediate region for a cylinder of length l and diameter d, the coefficient ν is calculated from the equation [28]

$$\nu = (1/4P)[\pi/4 + (\pi + 3)P/2 + P2], \text{ where P} \equiv \text{l/d}.$$

In our case asymmetry of groups attached to the dendrimer core does not exceed $P < 2$. Hence, the volume which one independent lactose molecule excludes for other lactose molecules in solution will be about 6200×10^{-24} cm^3. Consequently the volume per lactose group in lactosylated polyamidoamine dendrimers is 2.4 times greater that the volume of the free lactose molecule but 3.2 times less than the excluded volume by this molecule. This result probably indicates that ends groups in lactodendrimer molecules exhibit some steric hindrances. It also indicates that the entire individual disaccharide has less overall mobility. In this case, presumably because of some hydrogen bonding with either the matrix or between sugar residues themselves, there is restricted 'conformational mobility'. This however does not automatically imply that the sugar residues are folded in toward the core. Since the volume of lactodendrimers as compared to that of the initial dendrimers increases proportionally to the number of end groups, this means, in our opinion, that lactogroups are predominantly located on the periphery of the molecules, thus ensuring this volume increase.

These results show that the experimental and calculated molecular weight are in good agreement, which confirms the fact that amine end groups in initial PAMAM dendrimers are completely replaced by lactose residues. This also means that amine end groups in initial PAMAM dendrimers should also be located on the periphery of the molecules.

References

[1] Tomalia DA, Naylor AM, Goddard WA. Angew Chem, Int Ed Engl 1990;29(2):138.

[2] Muzafarov AM, Rebrov EA, Papkov VS. Usp Khim (Adv Chem) 1991;60(7):1596.
[3] Bochkarev MN, Katkov MA. Usp Khim (Adv Chem) 1995;64(11):1106.
[4] Newkome GR, Moorefield CN, Voegtle F. Dendritic macromolecules. Concept, syntheses, perspectives. Weinheim: VCH, 1996.
[5] Voegtle F, editor. Dendrimers. Berlin: Springer, 1998.
[6] First Int. Dendrimer Symposium. Book of Abstracts. Frankfurt-Main, 1999.
[7] Roy R. Polym News 1996;21(7):226.
[8] Ashton PR, Boyds SE, Brown ChL, Nepogodiev SA, Meijer EW, Peerlings HWI, Stoddart JF. Chem Eur J 1997;3(6):974.
[9] Pavlov GM, Korneeva EV, Michailova NA, Roy R, Ortega PC, Perez MA. Vysokomol Soedin 1999;41A(11):1810.
[10] Pavlov GM, Korneeva EV, Nepogoiev SA, Jumel K, Harding SE. Vysokomol Soedin 1998;40A(12):2056.
[11] Pavlov GM, Korneeva EV, Jumel K, Harding SE, Meijer EW, Peerlings HWI, Stoddart JF, Nepogoiev SA. Carbohydr Polym 1999;38(1):195.
[12] Pavlov GM, Korneeva EV, Roy R, Michailova NA, Ortega PC, Perez MA. Prog Colloid Polym Sci 1999;113:150.
[13] Roy R. Top Curr Chem 1997;187:242.
[14] Andre S, Cejas Ortega PJ, Alamino Perez M, Roy R, Gabius H-J. Glycobiology 1999;9:1253.
[15] Page D, Roy R. Bioconjugate Chem 1997;8:714.
[16] Zanini D, Roy R. J Org Chem 1998;63:3486.
[17] Harding SE, Sattelle DB, Bloomfield VA, editors. Laser light scattering in biochemistry. Cambridge: Royal Chemical Society, 1992.
[18] Wyatt PJ. Anal Chim Acta 1993;272(1):1.
[19] Haney MA. Am Lab 1985;17(1):41.
[20] Harding SE. Prog Biophys Mol Biol 1997;68(2/3):207.
[21] Tsvetkov VN, Lavrenko PN, Bushin SV. J Polym Sci, Polym Chem 1984;22:3447.
[22] Tsvetkov VN. Rigid-chain polymers. New York, London: Consultants Bureau, 1989.
[23] Tanford Ch. Physical chemistry of macromolecules. New York: Wiley, 1961.
[24] Cantor ChR, Schimmel PR. Biophysical chemistry. San Francisco: Freeman, 1980.
[25] Pavlov G, Panarin E, Korneeva E, Kurochkin C, Baikov V, Ushakova V. Makromol Chem 1990;191:2889.
[26] Pavlov GM, Korneeva EV, Yevlampieva NP. Int J Biol Macromol 1994;16:318–23.
[27] Prosa TJ, Bauer BJ, Amis EJ, Tomalia DA, Scherrenberg R. J Polym Sci, Part B: Polym Phys 1997;35(10):2913.
[28] Isihara A. Statistical physics. New York: Academic, 1971.
[29] Magarik SYa, Pavlov GM, Fomin GA. Macromolecules 1978;11:294.