Characterization of Commercial and Experimental Sodium Caseinates by Multiangle Laser Light Scattering and Size-Exclusion Chromatography

John A. Lucey,† Magesh Srinivasan,‡ Harjinder Singh,* and Peter A. Munro

Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand

A range of sodium caseinate samples were characterized by a multiangle laser light scattering (MALLS) system or by the use of MALLS as an on-line detector with size-exclusion chromatography (SEC). Sodium caseinate solutions, analyzed using a MALLS system alone, gave weight-average molar mass (M_w) values in the range 1200−4700 kDa and *z*-average root-mean-square radius (R_g) values ranged from ∼50 to 120 nm. When these solutions were ultracentrifuged at 90000*g* for 1 h, a cloudy top layer was formed; the subnatant was carefully removed and analyzed by SEC-MALLS. The M_w values were found to be in the range ∼30−575 kDa, and R_g values ranged from ∼22 to 49 nm. During SEC, the MALLS system detected some very large-sized material that eluted close to the void volume; this material was hardly detected by the concentration detectors, i.e., ultra-violet (UV) and differential refractive index (DRI). The intensity of the light scattering (LS) signal from this very large sized material was greatly reduced in the subnatant. SEC of sodium caseinate samples revealed two main peaks with M_w of ∼420−750 kDa and 39−69 kDa, respectively. The R_g values were very large for a protein molecule, and initial calculations suggested that the shape of caseinate molecules was likely to be highly elongated.

Keywords: *Sodium caseinate; size-exclusion chromatography; light scattering; molar mass*

INTRODUCTION

Sodium caseinate is a widely used food ingredient because of its excellent functional and nutritional properties. The manufacture, properties, and uses of casein and caseinate have been reviewed (Southward, 1989; Mulvihill, 1992), although much less has been reported on the size or shape of caseinates. Manufacturing processes for sodium caseinate can vary from producer to producer. Differences in the chemical composition have been found between batches of sodium caseinate from the same and different manufacturers (Dalgleish and Law, 1988). Lynch et al. (1997) reported some differences in the profiles obtained by size-exclusion chromatography (SEC) on different batches of sodium caseinate. Little has been published on the weight-average molar mass (M_w) and size distribution of samples of caseinate produced by different processes; this information could be useful in understanding the functional behavior of this ingredient.

Casein constitutes the main protein component in milk and forms stable colloidal particles of approximately spherical shape known as casein micelles. The structure and properties of casein micelle has been extensively reviewed (Schmidt, 1982; Walstra, 1990; Holt, 1992). Four major proteins (α_{s1}-, α_{s2}-, β-, and κ-casein) are present in micelles of bovine milk. The self-association behavior of the individual casein components in aqueous solution has been extensively studied (Schmidt, 1982; Holt, 1992; Rollema, 1992; Farrell et al., 1996). Caseins are very prone to association due to their high hydrophobicity and peculiar charge distribution (Rollema, 1992). It is also known that the caseins not only exhibit self-association but also interact with each other to form associated structures. The particle size of purified individual caseins depends on factors such as protein concentration, presence of reducing agents, calcium concentration, ionic strength, temperature, and pH (Farrell et al., 1996). There is still considerable controversy surrounding the structure of casein micelles and their possible subunits (submicelles); however, considerable progress has been made (Holt, 1992; Horne, 1998). Horne (1998) has suggested that the bonding between individual self-associating caseins was probably similar to that prevailing in industrial casein preparations.

SEC is widely used to characterize the molecular weight of food materials such as proteins and polysaccharides as well as aggregates of these materials. SEC has been used to investigate the aggregation state of casein (Pepper and Farrell, 1982). Using molecular standards, the approximate molecular weight of individual peaks can be determined. Light scattering (LS) has long been used as an absolute method to determine the M_w, shape, and conformation of polymers (Billingham, 1977; Wyatt, 1993). However, LS techniques are very sensitive to large-sized materials (as they scatter a lot of light), and to obtain information about the conformation of the polymer a number of angles must be used, which is often time-consuming. The incorporation of a multiangle laser light scattering (MALLS) detector into an SEC system is a powerful new develop-

* To whom correspondence should be addressed. Tel: + 64 6 350 4401. Fax: +64 6 350 5655. E-mail: H.Singh@massey.ac.nz.

† Current address: Department of Food Science, University of Wisconsin−Madison, 1605 Linden Drive, Madison, WI 53706-1565.

‡ Current address: Dairy Product Technology Center, California Polytechnic State University, San Luis Obispo, CA 93407.

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ment in this field. In combination with concentration detectors such as UV and differential refractive index (DRI), this system can calculate the M_w, *z*-average root-mean-square radius (R_g), and concentration of material eluting in small, individual slices of the SEC chromatogram. Alternatively, the MALLS detector can be used in the stand-alone (microbatch) mode to give information on the overall M_w and R_g of an unfractionated sample. SEC-MALLS has been used to characterize the M_w and size distribution of particles in food systems such as starch and heated β-lactoglobulin solutions (Fishman et al., 1996; Hoffman et al., 1997).

The aim of this study was to characterize the M_w and size distribution of commercial sodium caseinates using SEC and MALLS and compare these samples with pilot-plant and laboratory prepared caseinates.

MATERIALS AND METHODS

Materials. A range of commercial and experimental sodium caseinates was obtained from the New Zealand Dairy Board, Wellington, and the New Zealand Dairy Research Institute, respectively. Sodium caseinate was also prepared in the laboratory from unheated milk obtained from the Massey University dairy farm. The milk was skimmed by centrifugation at 2000*g* for 20 min at 20 °C, and this step was repeated. The skim milk was acidified to pH 4.6 at 20 °C with 2 M HCl, and the curd was washed twice with distilled water and dewatered using cheesecloth. The washed curd was dispersed in a 1:1 mixture with distilled water, and the pH was raised with gentle stirring to pH ~6.8 with 2 M NaOH. This sodium caseinate slurry was lyophilized or used directly for MALLS experiments. All the chemicals used were of analytical grade obtained from either BDH (BDH Ltd, Poole, England) or Sigma (St. Louis, MO).

Sample Preparation. Sodium caseinate solutions were prepared in water that was prefiltered through a 0.025-μm filter (Millipore Corp.). Samples were left overnight at 4 °C and then warmed to ~22 °C before analysis. Sodium caseinate solutions (~20 g) were ultracentrifuged at 90000*g* for 60 min at 20 °C in a temperature-controlled centrifuge (Sorvall RC5C centrifuge, DuPont), and about 5 mL of the subnatant was collected carefully. The subnatant was then filtered through a 0.22-μm filter (Millipore Corp.) prior to injection into the SEC column.

For microbatch MALLS experiments, a series of caseinate concentrations (e.g., 5×10^{-5} to 2.5×10^{-4} g/mL) were prepared from the stock sodium caseinate solution by mixing with a buffer containing 20 mM imidazole and 50 mM NaCl at pH 7.0 (prefiltered through 0.025-μm filter). Samples were injected into the detector cell with a syringe pump (Razel, model A-99, Razel Scientific Instruments Inc., Stanford, CA) at a flow rate of 0.4 mL/min. Samples were filtered in-line with a 0.22-μm filter.

Size-Exclusion Chromatography. Separation of sodium caseinate solutions and their subnatants was carried out by SEC on a Superose 6HR 10/30 column (Pharmacia, Uppsala, Sweden) attached to a GBC HPLC system (GBC Scientific Equipment Ltd, Victoria, Australia). A solution containing 20 mM imidazole and 50 mM NaCl at pH 7.0 was used as an elutant buffer. All the samples were filtered through a 0.22-μm filter. Sample injection volume was 50 μL, and nominal flow rate was 0.4 mL/min.

Multiangle Laser Light Scattering (MALLS). The chromatography system consisted of a Superose 6HR 10/30 column, a UV absorbance detector (GBC Scientific Equipment Ltd, Victoria, Australia) operating at 280 nm, a DAWN-DSP MALLS photometer (Wyatt Technology, Santa Barbara, CA) fitted with a helium−neon laser ($\lambda = 632.8$ nm) and a K-5 flow cell, and a DRI detector (Waters, model R401, Milford, MA).

The electronic outputs of the UV, DRI, and MALLS were sent to a 486 personal computer. The data were processed with ASTRA (version 4.0) software. The DRI response factor was measured by injecting a series of known NaCl concentrations into the detector with the syringe pump. This response factor was obtained from the slope of the linear plot between NaCl concentration and DRI response. The factor to correct the Rayleigh ratio to 90° for instrument geometry was obtained by measuring the LS intensity of filtered (0.025-μm) HPLC-quality toluene at 90°. The responses to LS intensity of the photodiodes arrayed around the scattering cell were normalized to the diode at 90° with a bovine serum albumin (BSA) sample (monomeric BSA with a nominal molecular weight of 66 kDa).

Data Treatment. The DAWN DSP MALLS detector simultaneously provides up to 16 LS chromatograms, each at a different scattering angle, for a polymer solute as it emerges from the SEC column (Wyatt, 1993). Additional chromatographs can be obtained from the concentration detectors. The LS data was processed using the DRI concentration detector, and the M_w and R_g of material eluting in each slice were calculated with a first-order Debye fit (higher order polynomials were also tried but gave a poorer fit to the data), using a specific refractive index increment (dn/dc) value of 0.190 cm^3/g (for both caseinate and BSA) (Huglin, 1972) and a second virial coefficient (A_2) of zero. Wyatt (1993) reviewed both the theoretical and practical aspects of the MALLS technique. With the ASTRA software, it was also possible to realign the SEC profiles from the different detectors caused by volume delays between units such as the UV and DRI detectors. In the microbatch mode the angular and concentration dependence of sodium caseinate samples were used to prepare a Zimm plot using ASTRA software. The coefficient of variation between replicate M_w measurements was typically <3%; within a single run the ASTRA software calculated averages and standard deviations of the M_w and R_g for each peak.

RESULTS AND DISCUSSION

Microbatch MALLS Experiments. A range of sodium caseinates was analyzed in microbatch mode (i.e., direct injection of samples into the MALLS detector without SEC). In this technique, sodium caseinate solutions of different concentrations were injected into the MALLS cell and the LS data analyzed using the Zimm plot technique, which provided information on the M_w and R_g of the unfractionated solution. An example of a Zimm plot obtained for a commercial sodium caseinate is shown in Figure 1a and a Debye plot calculated using data from one of the concentrations used in the Zimm analysis procedure shown in Figure 1b. In a Debye plot, $K^*c/R(\theta)$ versus $\sin^2 \theta/2$ is plotted, and this yields a curve whose intercept gives M_w and whose slope at low angles gives R_g (Wyatt, 1993). Although Zimm plots are notoriously difficult for aqueous solvents, excellent plots were obtained for all samples.

A summary of the M_w and R_g values for unfractionated sodium caseinate (derived from Zimm plots) is shown in Table 1. The M_w and R_g values for unfractionated sodium caseinate samples ranged from 1228 to 4746 kDa and 54 to 123 nm, respectively (Table 1). Standard commercial sodium caseinate and laboratory-made (never dried) caseinate had similar M_w and R_g values, while the pilot-scale sodium caseinate had much larger M_w and R_g values. When caseinate samples were ultracentrifuged at 90000*g* for 60 min some residual lipid material formed a "cloudy" supernatant layer. It was considered that this very large-sized material could influence the MALLS results so a comparison was made of uncentrifuged and ultracentrifuged caseinates (Table 1). Ultracentrifugation of all caseinate samples, prior to analysis, resulted in a large reduction in both M_w and R_g values, which ranged from 335 to 575 kDa and 22 to

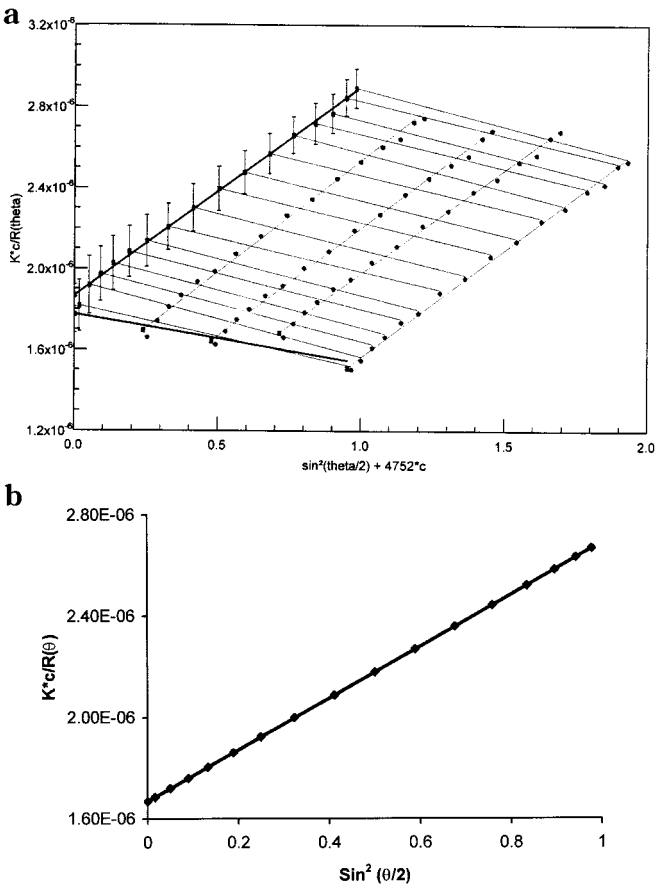

Figure 1. Zimm plot for a commercial sodium caseinate sample (ultracentrifuged), which had a *z*-average root-mean-square radius (R_g) of 48.7 ± 2.1 and weight-average molar mass (M_w) of (5.493 ± 0.334) × 10^5 (g/mol) (a) and an example of a Debye plot calculated from one of the concentrations used to make this Zimm plot, which yielded an R_g value of 51.2 ± 0.3 nm and a M_w of (5.993 ± 0.029) × 10^5 (g/mol) (b).

49 nm, respectively. The pilot-scale caseinate still had the highest M_w and R_g values, but the difference between it and the standard commercial caseinate was greatly reduced. The very high M_w and R_g values for pilot-scale sodium caseinate are probably related to the higher temperatures used in the pilot-scale process. Some of the larger caseinate particles may also have sedimented as a result of ultracentrifugation especially in the pilot-scale caseinate, which may also have contributed to the reduction in M_w and R_g values.

The R_g values for our ultracentrifuged laboratory-made sodium caseinate (22−27 nm) were higher than the R_g values for purified caseins (8 nm) reported by Farrell et al. (1996) that were made using small-angle X-ray scattering. The hydrodynamic radii for purified caseins was also reported by Farrell et al. (1996) and ranged from 7.7 to 9.4 nm. In many studies on purified proteins, caseins are often reduced, are extremely dilute, and extensively dialyzed against distilled water. In fact, many of the preparation steps in these purified studies are designed to get rid of any large aggregates or higher order structures. For example, in the study by Farrell et al. (1996) the following steps were taken: (1) ultracentrifugation at 100000*g*, (2) reduction or alkylation in 8 M urea, (3) extensive dialysis against water, (4) re-ultracentrifugation to remove any residual aggregated material, and (5) the use of high ionic strength buffers to inhibit ionic interactions. We suggest that the very different preparation procedures used by previous studies on purified caseins is probably responsible for the differences we report for R_g, M_w, and the structural conformation of the caseinate particles.

SEC-MALLS Experiments. During SEC, the MALLS system clearly detected large aggregated material that eluted close to the void volume (16−20 min) (Figure 2). In ultracentrifuged samples, the size of peak 1, which consisted of this very large-sized material, was greatly reduced, and peaks 2 and 3 were more clearly resolved. All subsequent SEC-MALLS experiments were performed on ultracentrifuged samples.

The SEC profiles for 2% sodium caseinate solution and the responses for the DRI, UV, and MALLS 90° LS detectors are shown in Figure 3. The SEC-DRI and UV profiles for sodium caseinate showed a very small peak near the void volume and two partly resolved peaks. Lynch et al. (1997) reported that SEC of sodium caseinate using a Superose 12 column resolved the caseins into two major peaks representing high and low molecular weight fractions. Both the UV and the DRI responses were similar. The MALLS system still detected large aggregated material that eluted close to the void volume, which was almost invisible to the UV and DRI detectors (as the concentration was very low).

A plot of M_w as a function of elution time for 2% sodium caseinate is shown in Figure 4. The M_w of the molecules eluting at the trailing end of the peak 3 was close to that for casein monomers (25−30 kDa), while very large aggregates were found near the start of peak 2. Clearly, there was a distribution of different sized casein complexes and aggregates across both peaks. The casein aggregate peak (peak 2) consisted predominantly of κ-casein polymers and some α_{s1}- and β-casein complexes (results not shown). Pepper and Farrell (1982) also found that κ-casein polymers eluted mainly at the leading edge of the first main peak during SEC of whole casein. Both Pepper and Farrell (1982) and Farrell et al. (1996) reported that in whole casein κ-casein exists in a wide size distribution of disulfide-bonded polymers. κ-Casein can form polymers of up to 30 monomers (~570 kDa) (Vreeman, 1979). Peak 3 had a substantial leading edge and contained casein complexes of various M_w (from ~200 to 45 kDa) and some monomers (~30 kDa) at the trailing end of the distribution (Figure 4). Overall, the data presented here suggests that caseins in sodium caseinate solutions exist as a dynamic system of casein monomers, casein complexes, and aggregates.

An example of a Debye plot for a single slice of the SEC profile (shown in Figure 4) is given in Figure 5. Although the R_g value (14.9 ± 0.4 nm) was close to the lowest resolution limit for the MALLS instrument (Wyatt, 1993), a first-order Debye fit was still a good approximation of the data (as indicated by the low standard deviations for both the R_g and M_w). The ability of the MALLS instrument to report the uncertainties of its measurements is a powerful tool in evaluating the (statistical) reliability of parameters calculated from LS data.

A summary of the M_w and R_g values for commercial and laboratory-made sodium caseinates is shown in Table 2. The M_w of peak 2 for all samples ranged from ~420 to 750 kDa and those of peak 3 from 38 to 70 kDa. Laboratory made (never dried) sodium caseinate had the smallest M_w value for peak 2. The laboratory-made freeze-dried sample had a higher M_w value for both

Table 1. Effect of Ultracentrifugation[a] on the Weight-Average (M_w) and *z*-Average rms Radius (R_g) for Sodium Caseinates Using the Microbatch Mode

	M_w ($\times 10^5$ g/mol)		R_g (nm)	
sample	not centrifuged	ultracentrifuged	not centrifuged	ultracentrifuged
lab made (freeze-dried)	19.98 (0.42)	4.74 (0.05)	54.1 (1.3)	22.6 (1.4)
lab made (never dried)	14.60 (0.42)	3.35 (0.13)	62.7 (2.1)	27.4 (1.1)
commercial (standard)	12.28 (0.40)	5.49 (0.33)	59.2 (1.0)	48.7 (2.1)
experimental (extruded)	47.46 (3.88)	5.75 (0.43)	123.5 (8.4)	48.9 (3.3)

[a] Ultracentrifugation was performed at 90000*g* for 60 min at 20 °C and the subnatant used for MALLS analysis.

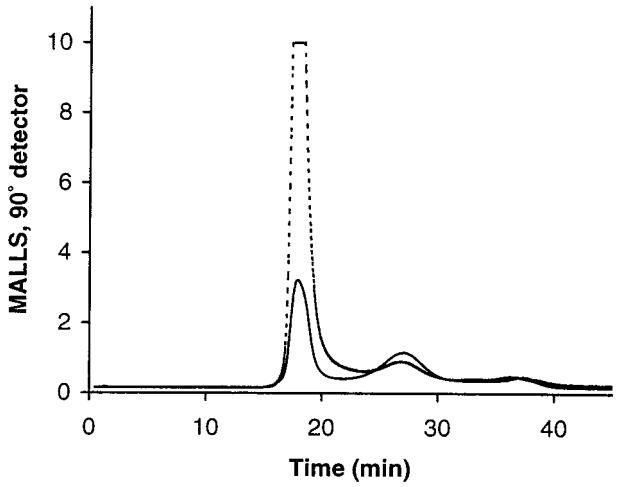

Figure 2. MALLS 90° detector responses during SEC of a 2% commercial sodium caseinate sample (···) and the subnatant after ultracentrifugation (—).

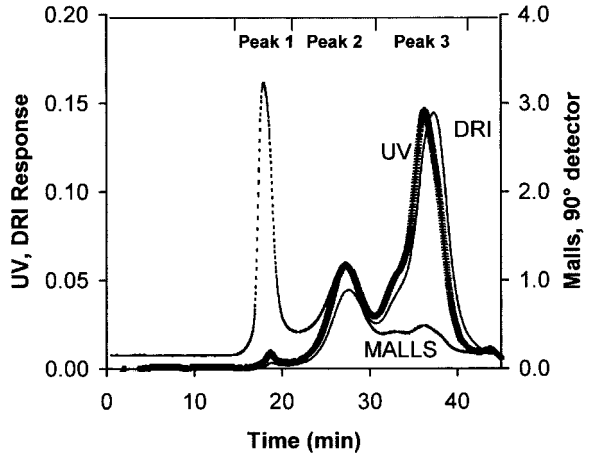

Figure 3. SEC elution profiles for a 2% commercial sodium caseinate sample after ultracentrifugation from DRI (—) and UV (+) and MALLS 90° detector (···).

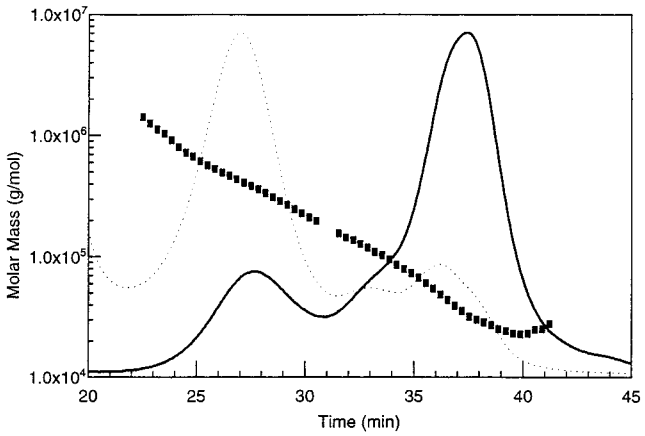

Figure 4. SEC-DRI elution profiles (—) of peaks 2 and 3 obtained for a 2% commercial sodium caseinate solution that had been ultracentrifuged. The elution profile is overlaid with the calculated molar mass (■) and MALLS at the 90° angle (···).

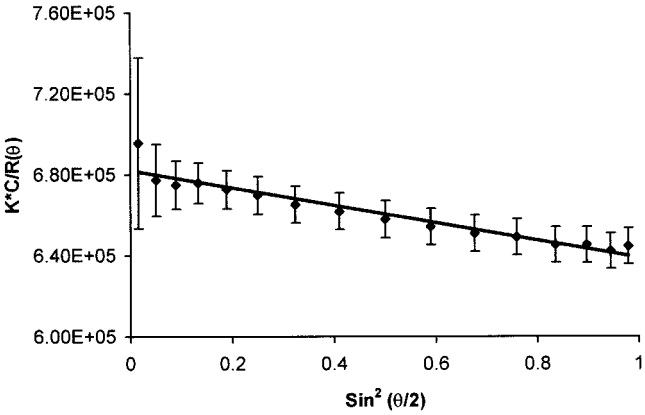

Figure 5. Debye plot showing the extrapolation of $K^*c/R\theta$ to zero angle. The data shown correspond to a slice at 24.8 min in the data for 2% commercial sodium caseinate shown in Figure 4. The extrapolated value for $K^*c/R\theta$ corresponds to a weight-average molar mass (M_w) of (6.756 ± 0.185) × 10^5 (g/mol), and the slope of the line shown yields a *z*-average root-mean-square radius (R_g) value of 14.9 ± 0.4 nm.

peaks 2 and 3, compared with laboratory-made never dried caseinate. The polydispersity ratio (M_w/M_n) of peak 3 was higher than that of peak 2. The R_g for both peaks 2 and 3 ranged from ~18 to 32 nm with no clear trends. It appeared that the R_g values of the particles in peak 3 were larger than those in peak 2, despite the much smaller M_w values for peak 3 (Table 2).

The changes in R_g during SEC-MALLS of 2% commercial sodium caseinate are shown in Figure 6. No clear trends were observed. Since R_g is proportional to the geometrical size for linear molecules, a log−log plot of R_g versus M_w permits the extraction of information on molecular conformation (Wyatt, 1993). Spherical molecules should yield a plot with a slope of 0.33; if the molecules are rods, then the slope should be unity, while for random coils in a good solvent, it should be between 0.5 and 0.6 (Wyatt, 1993). A log−log plot of R_g versus M_w for a 2% sodium caseinate sample is shown in Figure 7a. Peak 1 was not included as it was considered that this peak was produced by residual lipid material. It should be noted that some of the R_g data were close to the lower limit of resolution for the MALLS instrument so the R_g data should be used with caution. No clear trend was obvious, and there appeared to be a number of different behaviors present at different parts of the M_w distribution spectrum. For the high molar mass (400−970 kDa) part of the curve a straight line with a slope of 0.91 was observed (Figure 7b); since this is only a relatively small molar mass range no definitive conclusions should be made about the conformation of

Table 2. Number- (M_n) and Weight-Average (M_w) Molar Masses and *z*-Average rms Radius (R_g) for Sodium Caseinate Samples Analyzed Using SEC/MALLS[a,b]

	peak 2				peak 3			
sample	M_n (g/mol) $\times 10^5$	M_w (g/mol) $\times 10^5$	M_w/M_n	R_g (nm)	M_n (g/mol) $\times 10^4$	M_w (g/mol) $\times 10^4$	M_w/M_n	R_g (nm)
lab made (freeze-dried)	6.58 (0.40)	7.46 (0.50)	1.13	19.1 (0.8)	3.96 (0.08)	4.99 (0.13)	1.26	21.9 (2.0)
lab made (never dried)	5.50 (0.08)	5.99 (0.08)	1.09	18.4 (0.9)	3.41 (0.03)	3.88 (0.04)	1.14	20.4 (1.3)
commercial (standard)	5.09 (0.04)	6.04 (0.07)	1.19	28.0 (0.7)	5.35 (0.11)	6.92 (0.14)	1.29	32.4 (1.8)
experimental (extruded)	3.69 (0.06)	4.20 (0.07)	1.14	22.8 (1.0)	4.05 (0.09)	5.28 (0.11)	1.30	28.7 (2.0)

[a] Peak 1, which eluted at ~16−18 min, was omitted due to excessive light scattering and a very low protein concentration. [b] Samples were ultracentrifuged at 90000*g* for 60 min at 20 °C and the subnatant used for SEC-MALLS analysis.

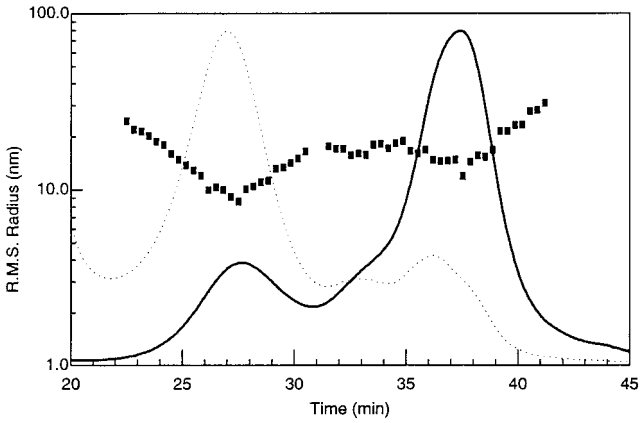

Figure 6. Plot of log *z*-average root-mean-square radius (R_g) as a function of time (■) obtained for a 2% commercial sodium caseinate solution that had been ultracentrifuged. The elution profile is overlaid with the DRI signal (—) and MALLS at the 90° angle (···).

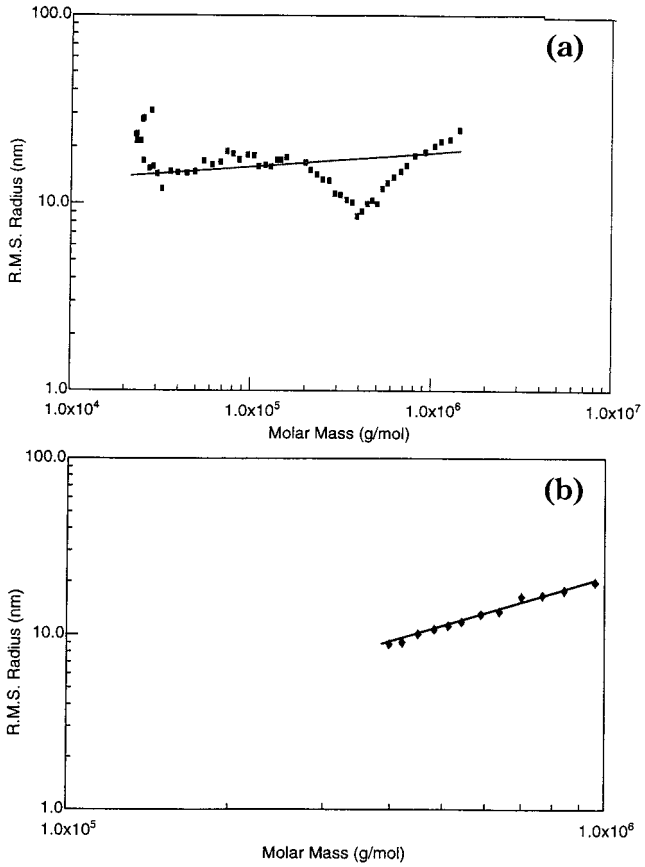

Figure 7. Plot of log *z*-average root-mean-square radius (R_g) as a function of log molar mass obtained for a 2% commercial sodium caseinate solution that had been ultracentrifuged (a) the complete SEC-DRI spectrum and (b) the molar mass fraction between 400 and 970 kDa. The slopes of the regression lines for (a) and (b) were 0.07 (±0.01) and 0.91 (±0.01), respectively.

the particles. This slope is larger than that expected for a random coil but slightly less than that was expected for a rod (Wyatt, 1993). Higher molecular weights were not selected, as it was considered that there was relatively poor separation of peak 1 and peak 2, which might influence these calculations. For a large part of the spectrum (400−30 kDa) the overall slope of the line was <0.1 and at even smaller M_w there was a departure from linear behavior (Figure 7a). These results suggest that in caseinate there could be a number of different types of aggregates and complexes, which might have very different conformations depending on their M_w. It is also possible that better chromatographic separation of peaks 2 and 3 (e.g., by using additional SEC columns) would have assisted in the interpretation of the log−log plot of R_g versus M_w data.

Lynch et al. (1997) reported that SEC of sodium caseinates showed that caseinates contained different levels of high molecular weight proteins, which were present in low levels in the laboratory prepared caseinate samples. SEC results revealed modifications to the structure and aggregation state of caseinates during manufacture, which may influence their functional properties (Lynch et al., 1997).

The effect of caseinate concentration (0.5−5.0%) on the SEC-DRI profiles for a commercial sodium caseinate sample is shown in Figure 8. As the protein concentration increased the size of both peaks increased. Peak 1 could be clearly observed at protein concentrations >2% despite the previous ultracentrifugation of these samples. The retention time for peak 3 decreased at high protein concentrations while the retention time for peak 2 was relatively unchanged. The M_w values for peaks 2 and 3 ranged from ~412−495 and ~49−63 kDa, respectively (Table 3). R_g values for both peaks appeared to decrease slightly with increasing protein concentration. Concentration of casein had a major effect on the M_w of casein aggregates and complexes. The increase in M_w values of caseins in peak 3 was consistent with the results of Pepper and Farrell (1982) who reported, using gel permeation chromatography, that, for whole casein in solution, changes in protein concentration resulted in a variation in the association behavior.

Estimation of the Shape of Caseinate Molecules. The R_g value for sodium caseinate was very large if the shape of the molecule was similar to that of most globular proteins that had a comparable similar M_w value. Some initial calculations were performed to estimate if the conformation of sodium caseinate was spherical. The value for R_g can be used to roughly estimate the shape of a molecule by comparing the measured value (R_{gm}) with that expected for a sphere

Table 3. Effect of Protein Concentration on the Number- (M_n) and Weight-Average (M_w) Molar Masses and *z*-Average rms Radius (R_g) for a Commercial Sodium Caseinate Sample Analyzed Using SEC/MALLS[a,b]

	peak 2				peak 3			
protein concentration (%)	M_n (g/mol) $\times 10^5$	M_w (g/mol) $\times 10^5$	M_w/M_n	R_g (nm)	M_n (g/mol) $\times 10^4$	M_w (g/mol) $\times 10^4$	M_w/M_n	R_g (nm)
0.5	4.07 (0.19)	4.95 (0.39)	1.22	20.1 (0.7)	4.29 (0.09)	5.81 (0.14)	1.35	19.2 (1.5)
1.0	3.57 (0.03)	4.18 (0.05)	1.17	14.4 (0.5)	3.56 (0.03)	4.87 (0.03)	1.37	14.7 (1.1)
2.0	3.54 (0.05)	4.16 (0.09)	1.17	13.2 (0.6)	3.91 (0.04)	5.15 (0.05)	1.31	16.6 (1.1)
3.0	3.62 (0.03)	4.43 (0.09)	1.23	11.9 (0.8)	4.32 (0.04)	5.76 (0.04)	1.33	13.2 (1.1)
4.0	3.39 (0.02)	4.12 (0.02)	1.21	10.9 (1.3)	4.37 (0.05)	5.63 (0.04)	1.29	13.9 (1.6)
5.0	3.88 (0.02)	4.58 (0.02)	1.18	11.7 (0.9)	4.72 (0.03)	6.29 (0.03)	1.33	12.2 (1.1)

[a] Peak 1, which eluted at ~16−18 min, was omitted due to excessive light scattering and a very low protein concentration. [b] Samples were ultracentrifuged at 90000*g* for 60 min at 20 °C and the subnatant used for SEC-MALLS analysis.

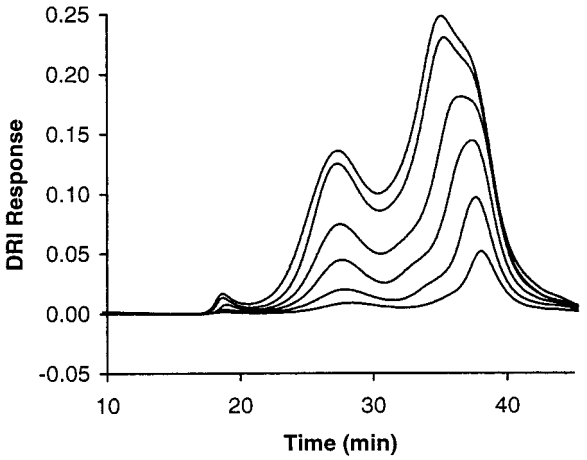

Figure 8. SEC-DRI elution profiles for a commercial sodium caseinate sample as a function of protein concentration: (a) 0.5, (b) 1.0, (c) 2.0, (d) 3, (e) 4, and (f) 5.0%.

(R_{gs}) (Freifelder, 1982). The R_{gs} of a sphere (with uniform density) is given by

$$R_{gs} = \sqrt{3/5}r \quad (1)$$

where r is the radius of a sphere.

The volume of a sphere is $4\pi r^3/3$ or Mv/N_a (Freifelder, 1982) where M is the molecular weight, v is the partial specific volume, and N_a is Avogadro's number. Thus

$$r = \left(\frac{3Mv}{4\pi N_a}\right)^{1/3} \quad (2)$$

Assuming that v is 0.75×10^{-6} m^3/g (McKenzie and Wake, 1959), which is also a typical value for many proteins such as myoglobin (Freifelder, 1982), and using data from ultracentrifuged commercial caseinate in Table 1 for $M_w = 549$ kDa we get $R_{gs} = 5$ nm. Comparing this R_{gs} with R_{gm} (48.7 nm) we find that our measured value is ~10× higher than our estimated value for a sphere, which suggests that our caseinate sample had a very nonspherical conformation. Using smaller values for v had little effect on this calculation. If we assume that caseinate formed thin rods we can calculate that the length L (nm) of the rod is (Freifelder, 1982)

$$L = R_g \times \sqrt{12} \quad (3)$$

Using the previous R_{gm} data, we get an L of 169 nm. The volume of a rod (V_r) is $Lr^2\pi$ or Mv/N_a, where r is the radius of the rod. Thus, this rod would have an r of 1 nm.

Additional support for the suggestion that caseinate molecules could have nonspherical conformation comes from the results of Fang and Dalgleish (1995), who suggested that the shape of sodium caseinate particles, studied by cryo-fracture electron microscopy, appeared to be elongated and nonspherical. Recently, Farrer and Lips (1999) reported that sodium caseinate particles appeared to be weakly branched rodlike aggregates when studied using transmission electron microscopy (and osmotic pressure measurements). Further work is necessary to confirm our initial findings on (unpurified) sodium caseinate using techniques, such as other light-scattering approaches and electron microscopy.

Conclusions. We have shown that size-exclusion chromatography in combination with MALLS is a very useful technique for studying the M_w of casein aggregates and complexes that are present in sodium caseinate solutions. The overall M_w of unfractionated sodium caseinate was also be determined using MALLS in microbatch mode. Compared with conventional SEC, where the column is calibrated with proteins with a known molar mass, the SEC-MALLS system has several advantages. In particular calibration of SEC columns is dependent on the conformation of the protein (Billingham, 1977) and as was evident in this study caseinate molecules may not be spherical but highly elongated in contrast to most protein standards used for calibration of SEC columns. A small amount of residual lipid material in caseinates was responsible for a large light scattering peak, which could be greatly reduced by ultracentrifugation. An attempt was made to estimate the conformation of caseinate molecules, and this also suggested that they were probably not spherical.

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