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# Fractionation of Sodium Caseinate by Ultrafiltration

J. M. Murphy & P. F. Fox

Department of Food Chemistry, University College,
Cork, Republic of Ireland

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*ABSTRACT*

*A method for the fractionation of sodium caseinate into a β-casein-rich permeate and an $\alpha_s$/κ-casein-rich retentate has been developed using ultrafiltration through 300 000 dalton cut-off membranes at 4°C. The β-casein fraction was 80% homogeneous, γ-caseins being the principal contaminants; most of the β-casein could be recovered in the permeate by diafiltration or repeated ultrafiltration. For the successful recovery of β-casein, it was essential to dissolve the sodium caseinate in deionized or distilled water but pH in the range 7–9 was not significant. A caseinate concentration of 1% was optimal in terms of yield and ease of operation.*

## INTRODUCTION

Caseins and caseinates are widely used as functional food proteins; ~200 000 tonnes per annum are currently used in cheese analogues, coffee creamers, whipped toppings, meat products, dietary formulations, etc. Bovine casein is a mixture of four proteins, $\alpha_{s1}$-, $\alpha_{s2}$-, β- and κ-casein, the functional properties of which differ significantly (see Mulvihill and Fox (1989)). β-Casein is the most surface active of the caseins, owing to its random structure, hydrophobicity and non-uniform distribution of hydrophobic and hydrophilic residues. Therefore, fractionation of sodium caseinate may yield products with improved functionality.

The protein system in bovine milk differs markedly from that in human milk in several respects (see Blanc (1981); Lonnerdal (1985)). *Inter alia*, the



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principal casein in human milk is β-casein, which is highly homologous to bovine β-casein (Greenberg *et al.*, 1984); α-like caseins, the principal caseins in bovine milk, are present at low concentration, or absent, in human milk. Although the casein:whey protein ratio in 'humanized' infant formulae is adjusted to approximate that in human milk, neither the caseins nor whey proteins in these formulae are modified to simulate the protein pattern in human milk. The availability of bovine β-casein on an industrial scale would offer the possibility of developing improved infant formulae.

To date, β-casein is normally prepared from sodium caseinate by differential solubility of the individual caseins in urea solutions at pH 4·5–5·0 (Hipp *et al.*, 1952; Fox & Guiney, 1972) or by ion-exchange chromatography, with or without urea (Davies & Law, 1977). These methods are cumbersome and are not amenable to scale-up. Recently, methods for fractionating casein on a potentially industrial scale have been published. These are based on the association characteristics of the caseins which are dependent on ionic strength and/or temperature. At low temperatures, β-casein exists in solution as monomers (Payens & van Markwijk, 1963), a characteristic exploited by Allen *et al.* (1985) to prepare β-casein by renneting calcium caseinate at 4°C; under these conditions, β-casein remains soluble while $\alpha_s$- and *para*-κ-caseins coagulate. In our experience, the β-casein prepared by this method is extensively hydrolysed by chymosin to β-casein-I. A method for the isolation of β-casein by microfiltration of calcium caseinate at 5°C was reported by Terre *et al.* (1986). The same technique was optimized by Famelart *et al.* (1989) who purified β-casein from whole casein at 4°C and pH 4·2–4·6.

This paper reports the development of a method for the preparation of β-casein from sodium caseinate by ultrafiltration.

## MATERIALS AND METHODS

### Sample preparation and ultrafiltration

Sodium caseinate was prepared from unpasteurized skim milk (University herd) or from commercially-produced acid casein (Mitchelstown Co-op, Cork, Republic of Ireland). Solutions of the caseinate were made up in pre-cooled distilled/deionized water, the pH adjusted to 7·0 and allowed to equilibrate for a minimum of 3 h at 0·4°C. Fractionation was achieved by ultrafiltration (UF) through a tangential flow filtration system using polysulfone membranes with a nominal molecular weight cut-off (NMWCO) of 100 000 or 300 000 daltons or by microfiltration using microporous polyvinylidene difluoride membranes, average pore size,

0·1 μm (Millipore (UK) Ltd, Middlesex, UK). The concentrated retentate was freeze-dried immediately. Initially, permeate protein was recovered by precipitation at pH 4·9 and 20°C and centrifugation at 5000 × *g* for 10 min. The pellet was resuspended in water and the pH adjusted to pH 7·0 prior to freeze-drying. In later experiments, the β-casein-enriched permeate was concentrated to the required degree using ultrafiltration with 100 000 NMWCO polysulfone membranes at 50°C and freeze-dried prior to analysis. The yield of permeable protein was calculated from the weight of freeze-dried protein recovered.

**Analysis**

The nitrogen content of all protein preparations was determined in duplicate by the standard macro-Kjeldahl method (AOAC, 1975) and converted to protein by multiplying by 6·38. Protein composition of the preparations was determined by polyacrylamide gel electrophoresis with urea-containing buffers (Andrews, 1983) and 200 μg protein load. Gels were stained with Coomassie Brilliant Blue G250 for 8–12 h and destained in distilled water (Blakesley & Boezi, 1977). The electrophoretograms were scanned densitometrically at 600 nm using a densitometer attachment for an SP8-400 Pye Unicam spectrophotometer (Pye Unicam Ltd, Cambridge, UK). *N*-Acetylneuraminic acid was determined by the colorimetric method of Warren (1959).

## RESULTS AND DISCUSSION

The ability of available UF/MF membranes to fractionate sodium caseinate was investigated initially. Sodium caseinate (0·1% w/v) at pH 7·0 was equilibrated at 4°C for 12–16 h before filtration at 4°C using 100 000 or 300 000 NMWCO polysulfone membranes or 0·1 μm NMWCO microporous membranes.

Although β-casein was concentrated in the permeate (Fig. 1), approximately 97% of the total protein was retained by the 100 000 NMWCO membrane. The yield of permeable protein was greater for the other membranes used: ~18% and ~20% for the 300 000 and 0·1 μm microporous membranes, respectively. However, α-casein was not selectively retained by the 0·1 μm membranes (Fig. 1, Sample 9) and subsequent optimization of the method was performed using the 300 000 NMWCO membrane.

The effect of sodium caseinate concentration in the range 0·25 to 3·0%

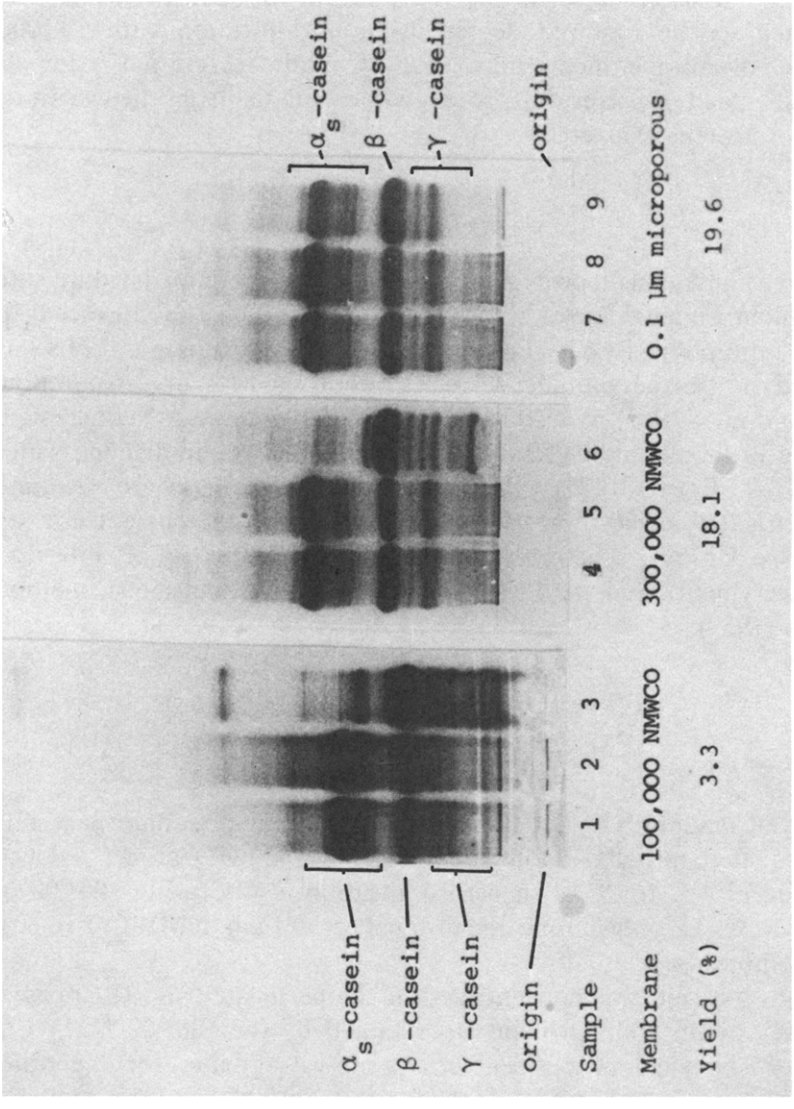


**Fig. 1.** Polyacrylamide gel electrophoretograms of the fractions of sodium caseinate (0·1%, pH 7·0) obtained by ultrafiltration at 4°C. Sodium caseinate was concentrated 10-fold using the different membranes; protein not retained by the membrane was recovered from the permeate by isoelectric precipitation and centrifugation. Yield, i.e. % of original protein recovered in the permeate, was determined by measuring protein content of the weighed dried precipitate. 1, 4 and 7, Sodium caseinate; 2, 5 and 8, retentates; 3, 6 and 9, permeates.

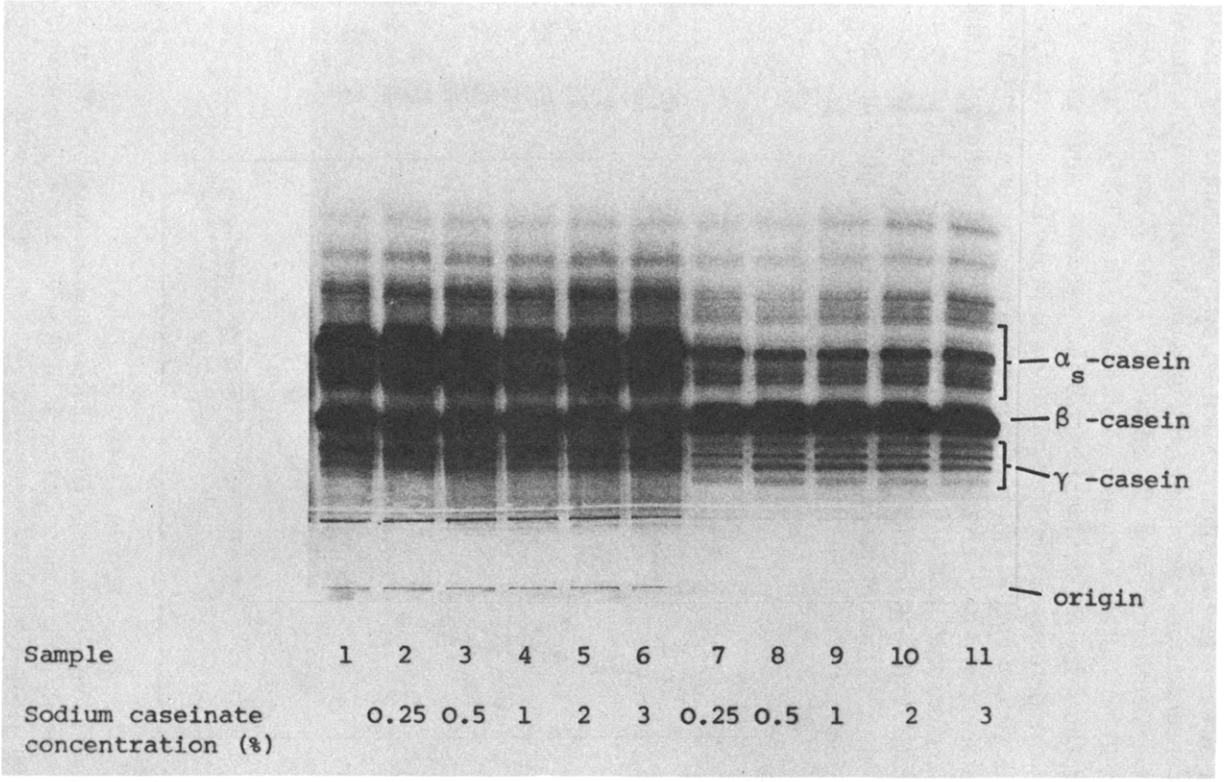


**Fig. 2.** Polyacrylamide gel electrophoretograms of retentates and permeates obtained by ultrafiltration of sodium caseinate of increasing concentration (0·25–3·0%) at 4°C. Two successive ultrafiltration steps using 300 000 NMWCO membranes were carried out with 70% volume reduction at each step except for the 3% sodium caseinate sample where 60% volume reduction was used due to fouling. Protein was recovered from permeate as described in Fig. 1. 1, Sodium caseinate; 2–6, retentates; 7–11 permeates.

(w/v) on the efficacy of fractionation was investigated. The proportion of β-casein retained by the membrane was least at low casein concentrations and increased with increasing casein concentration (Fig. 2, Samples 2–6). β-Casein was enriched in all permeate fractions but, with increasing sodium caseinate concentration, increasing amounts of minor proteins passed through the membrane (Fig. 2, Samples 7–11). Typically, β-casein represented 70–80% of the total permeable protein from 300 000 NMWCO membranes, the principal contaminants being γ-caseins which were also enriched in the permeate. The amount of total protein recovered in the permeate, i.e. yield, was inversely related to sodium caseinate concentration, as was the amount of β-casein fractionated (Fig. 3). Ultrafiltration of a 1·0% sodium caseinate solution at 4°C using 300 000 NMWCO membranes was found to be optimal with respect to amount of purified β-casein recovered (Fig. 3). With 3% sodium caseinate, processing proved difficult due to membrane fouling.

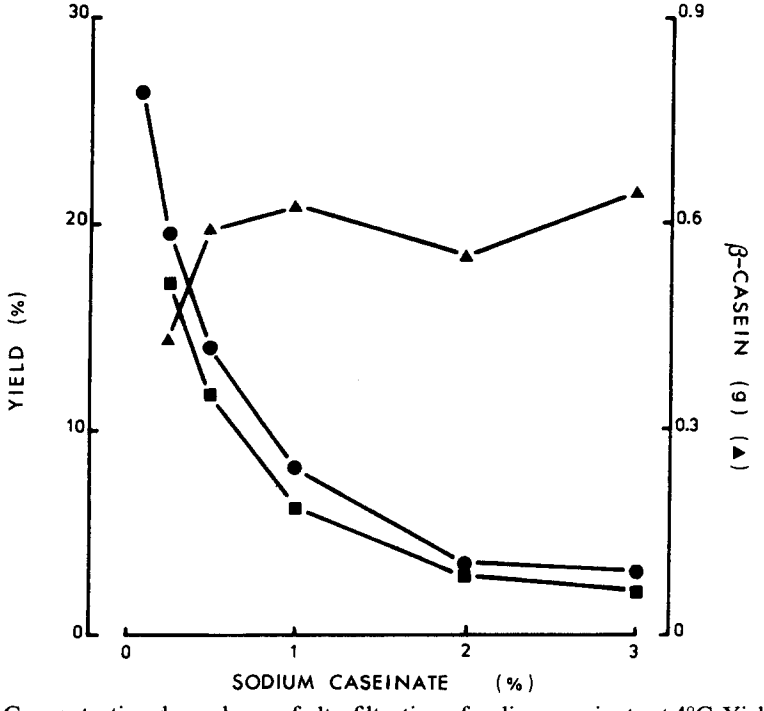


**Fig. 3.** Concentration dependence of ultrafiltration of sodium caseinate at 4°C. Yields were calculated, as described in Fig. 1 using experimental data from Fig. 2, on the basis of two 70% volume reduction cycles except for 3% protein solution where two 60% reduction cycles were used since fouling occurred on more extensive concentration. ●, % total protein recovered from permeates; ■, % β-casein recovered from the permeates, calculated from densitometric analysis of permeate gel patterns; ▲, calculated amount of pure β-casein isolated from ultrafiltration of 1 litre sample at 4°C.

**TABLE 1**
Effect of pH and Water Quality on the Fractionation of Sodium Caseinate by Ultrafiltration using 300 000 NMWCO Membranes at 4°C. (Experimental conditions were as described in Fig. 1.)

| *pH* | *Yield* (%) | *Water quality* (*pH 7*) | *Yield* (%) |
|---|---|---|---|
| 7·0 | 17·5 | Distilled | 18·8 |
| 8·0 | 18·5 | Deionized | 20·0 |
| 9·0 | 16·6 | Tap | 5·2 |

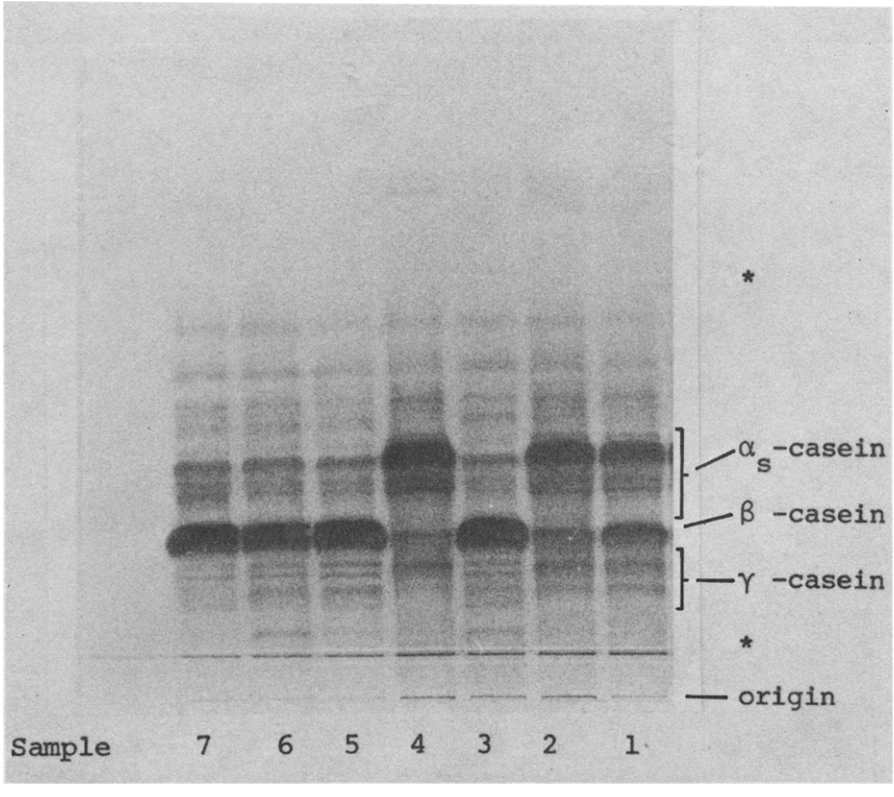


**Fig. 4.** Polyacrylamide gel electrophoretogram of the fractions of sodium caseinate (0·1%, pH 7·0) obtained by ultrafiltration through 300 000 NMWCO membranes at 4°C. 1, Sodium caseinate; 2 and 3, retentate and permeate, respectively, after diafiltration with three volumes; 4, retentate after three successive 90% volume reduction steps with equilibration for 8 h at 100% volume between each step; 5, 6 and 7, permeate collected at each step. Permeate protein was recovered by isoelectric precipitation.

The effect of pH and water quality on the yield of permeable protein, i.e. β-rich fraction, was examined. The pH in the range 7·0–9·0 did not significantly affect yield (Table 1). However, the use of tap water, with higher ionic strength than either deionized or distilled water, significantly reduced the yield with only 5% of total protein recovered in the permeate (Table 1).

Virtually all β-casein may be removed from the retentate by diafiltration, i.e. constant volume washing (Fig. 4, Sample 2) or by repeated ultrafiltration steps (Fig. 4, Sample 4). Gel electrophoretograms of the retentate obtained after three successive, 90% volume reduction, filtration cycles were scanned using a densitometer at 600 nm. The pattern obtained was compared to that of sodium caseinate and the first permeate (Fig. 5). Enrichment of $\alpha_s$-casein in the retentate was evident as was a reduction of the staining intensity of the

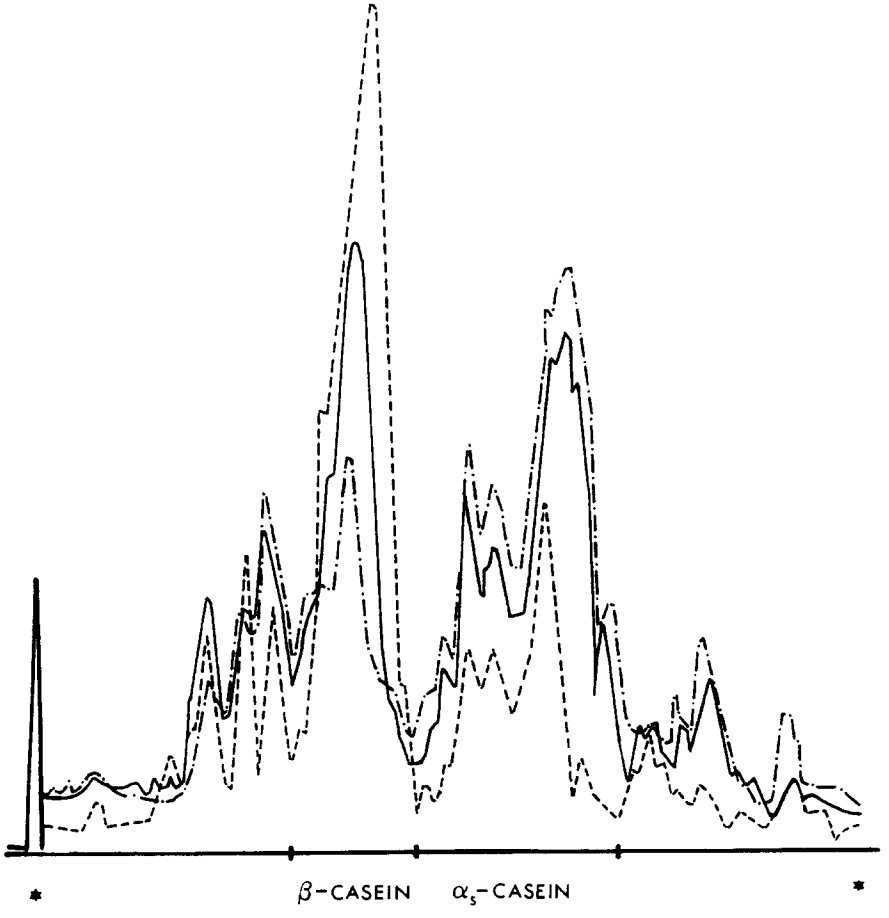


**Fig. 5.** Densitometric profile of the fractions of sodium caseinate (0·1%, pH 7·0) obtained by ultrafiltration. The protein resolved by PAGE (Fig. 4) was scanned at 600 nm from * to *. ——, Sodium caseinate (sample 1); – · –, retentate (sample 4); – – –, permeate (sample 5).

β-casein band. Further elucidation was necessary to establish whether staining of κ-casein contributed to protein staining in the β-casein region. *N*-Acetylneuraminic acid content was measured as an indicator of the distribution of κ-casein. From 0·06 to 0·07 g *N*-acetylneuraminic acid/100 g permeate was detected compared to approximately 0·6 g *N*-acetylneuraminic acid/100 g retentate. It is possible that κ-casein contributed to staining in the β-casein region of the retentate sample but in the case of the permeate, the contribution of κ-casein may be ignored, thus highlighting the enrichment of β-casein achieved by ultrafiltration at 4°C (Fig. 5).

A combination of quantitative PAGE and *N*-acetylneuraminic acid content was used to calculate the relative distribution of individual caseins achieved by ultrafiltration of 0·1% sodium caseinate, pH 7·0, using 300 000 NMWCO membranes at 4°C. Table 2 shows that 50% of β-casein may be isolated from sodium caseinate. This is probably an underestimate since the contribution of κ-casein to staining in the β-casein region in the case of the retentate could not be calculated. However, the fractionation results in an $\alpha_s$-/κ-casein-enriched retentate and a β-casein-enriched permeate.

Isoelectric precipitation of β-casein from the permeate and recovery by centrifugation was not suitable on a large scale and concentration of the fraction by ultrafiltration was therefore investigated. Polymerization of β-casein occurs at temperatures above 8°C and the ability of 10 000, 100 000 or 300 000 NMWCO ultrafiltration membranes to retain β-casein at temperatures above 20°C was investigated. Results are presented in Fig. 6. Concentration using 10 000 NMWCO membranes achieved 100% recovery

**TABLE 2**
Distribution of Caseins achieved by Ultrafiltration of Sodium Caseinate at 4°C using 300 000 NMWCO Membranes

| *Fraction* | *Relative distribution of casein*[a] (%) | | | *Recovery*[c] (%) |
|---|---|---|---|---|
| | $\alpha_s$- | β- | κ-[b] | |
| Retentate | 77 | 44 | 95 | 65 |
| Permeate | 19 | 56 | 5 | 32 |

[a] Relative distribution of $\alpha_s$- and β- caseins was calculated from densitometric scans of slots 1, 4, 5, 6 and 7 (Fig. 3) and total protein content of the various fractions.
[b] κ-casein was estimated using *N*-acetylneuraminic acid content.
[c] Recovery, defined as % of total protein found in the two fractions.

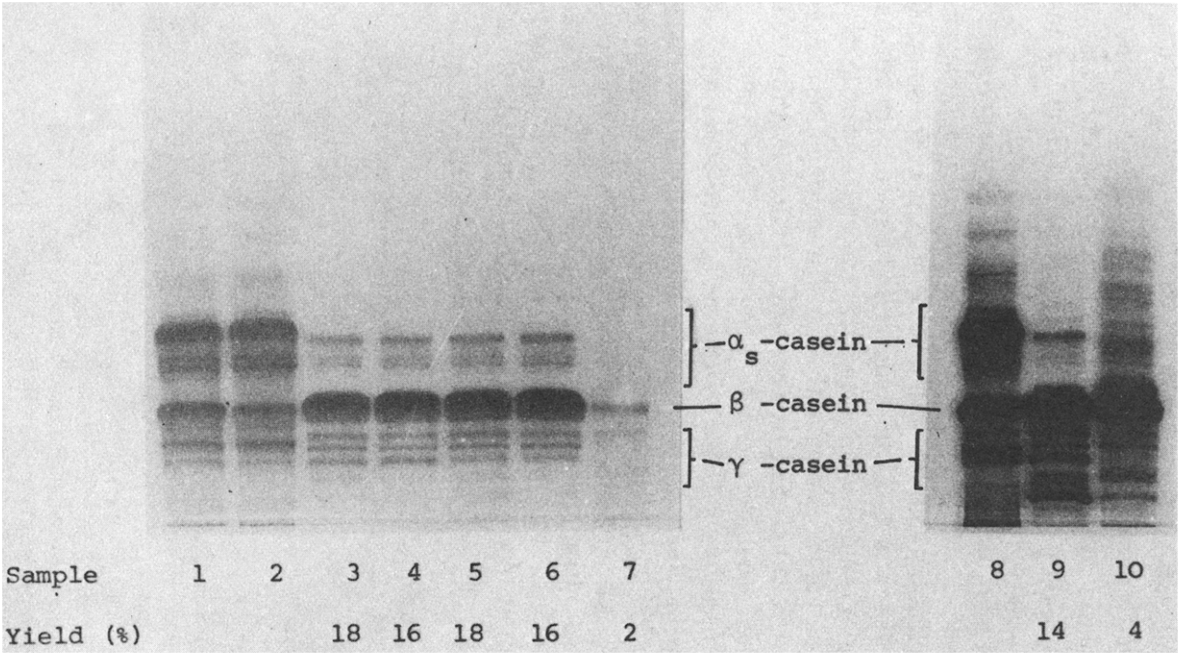


**Fig. 6.** Polyacrylamide gel electrophoretograms of recovered protein from β-enriched, dilute permeate. Permeate was obtained after ultrafiltration of 0·1% sodium caseinate pH 7·0 at 4°C using 300 000 NMWCO membranes. 1 and 8, Sodium caseinate; 2, retentate; 3, freeze-dried permeate; 4, isoelectrically precipitated protein (pH 4·9); 5, permeate concentrated by ultrafiltration at 25°C using 10 000 NMWCO membranes; 6, as in 5 but using 100 000 NMWCO membranes; 7, protein not retained by 100 000 NMWCO membrane; 9, permeate concentrated by ultrafiltration at 40°C using 300 000 NMWCO membranes; 10, protein not retained by 300 000 NMWCO membranes.

of permeable proteins (Sample 5). However, processing was considerably more efficient, i.e. higher flux; using 100 000 NMWCO membranes (Sample 6), the yield compared well to that found for isoelectric precipitation (Sample 4) and very little protein was lost at the concentration step (Sample 7). On the other hand, a significant amount of $\beta$-casein was lost during concentration using 300 000 NMWCO ultrafiltration membranes at 40°C (Sample 10). The yield achieved by this method, 14% (Sample 9), was lower than that by other methods and the loss of protein was too high for commercial viability.

Thus, a method for the preparation of a $\beta$-casein-enriched fraction of sodium caseinate has been developed and optimized. Sodium caseinate (0·1–3·0% w/v) equilibrated at 4°C and pH 7·0 may be fractionated using ultrafiltration through 300 000 NMWCO polysulfone membranes at 0–4°C. The resultant retentate was enriched in $\alpha_s/\kappa$-casein and the permeate enriched in $\beta$- and $\gamma$-caseins. Concentration of the dilute permeate was achieved using ultrafiltration through 100 000 NMWCO polysulfone membranes at 40°C. Presumably, temperatures $>40$°C would also be suitable for concentration of permeate but 50°C is recommended as the upper limit of the temperature range for the membranes used. Thus, all the steps involved in producing the $\beta$-casein-enriched caseinate are amenable to scale-up to a commercial level. The $\beta$-casein-enriched caseinate was found to be 70–80% $\beta$-casein with $\gamma$-caseins as the principal contaminants: virtually all $\beta$-casein may be fractionated using repeated ultrafiltration steps or diafiltration at 4°C and 1% sodium caseinate (w/v) was found to be optimal in terms of yield and ease of ultrafiltration.

The economic viability of the fractionation would depend on the potential uses of both the $\beta$-casein-enriched permeate and the $\alpha_s/\kappa$-casein-enriched retentate as functional food proteins and the potential use of the former caseinate as a base for 'humanized' infant formula. The functional properties of the fractions were compared to those of sodium caseinate and the results of this study will be presented separately.

## ACKNOWLEDGEMENT

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