Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Semiquantitative Gel Electrophoresis

Charles W. Gehrke[a]; Don A. Craven[a]; Joseph Absheer[a]; K. K. Tripathi[a]
[a] DEPARTMENT OF AGRICULTURAL CHEMISTRY, UNIVERSITY OF MISSOURI, COLUMBIA, MISSOURI

To cite this Article Gehrke, Charles W. , Craven, Don A. , Absheer, Joseph and Tripathi, K. K.(1967) 'Semiquantitative Gel Electrophoresis', Separation Science and Technology, 2: 2, 199 — 210
To link to this Article: DOI: 10.1080/01496396708049927
URL: http://dx.doi.org/10.1080/01496396708049927

SEPARATION SCIENCE, 2(2), 199–210 (1967)

Semiquantitative Gel Electrophoresis*

CHARLES W. GEHRKE, DON A. CRAVEN,
JOSEPH ABSHEER, and K. K. TRIPATHI
DEPARTMENT OF AGRICULTURAL CHEMISTRY,
UNIVERSITY OF MISSOURI,
COLUMBIA, MISSOURI

Summary

Known weights of α_{s_1}-, β_s-, and K-caseins were studied by polyacrylamide gel and starch gel electrophoresis under conditions of high concentrations (4.5 and 6.0 *M*) of urea to determine the minimum detectible amounts of these caseins, the sample loading volume and weight giving the optimum resolution, and other quantitative factors pertaining to gel electrophoresis.

In examining purified preparations of α_{s_1}- and β_s-caseins it was shown that 2 to 4 μg of either could be detected by polyacrylamide gel electrophoresis. Starch gels showed stained α_{s_1}- and β_s-casein zones at sample loading levels of 6 to 10 μg. However, the starch gels were sliced prior to staining, thereby decreasing the "effective" sample loading weight by a factor of one-half. Larger quantities (25 μg) of reduced K-casein were required for detection in both types of gels, owing to the splitting of the K-casein molecule and the resultant appearance of several zones.

Seemingly "homogeneous" preparations of α_{s_1}- and β_s- caseins showed multiple bands at low loading sample weights. This increased resolution probably demonstrates genetic variants in both caseins, since the starting material for these preparations was from pooled milk.

PGUE patterns of 200-μg mixtures of α_{s_1}- and β_s-caseins showed no alteration of the detectibility of either casein. Both gave stained zones at levels of 2 to 4 μg (i.e., 1 to 2% of total protein).

Comparison of the lower detectible limits of α_{s_1}- and β_s-caseins from the individual samples with those of the mixtures definitely showed that there was no interaction between the two strong enough to deplete either casein zone.

In evaluating the relative merits of polyacrylamide gel and starch gel

---

* Contribution from the Missouri Agricultural Experiment Station, Journal Series 5054. Approved by the Director.

electrophoresis the former is decidedly the preferred method. Polyacrylamide gels are clear and resilient, and the electrophoretic process requires roughly 6 hr from start to finish, whereas starch gel is extremely difficult to work with and 2 days is required to complete an electrophoretic analysis.

In general, the resolution obtained from both types of gels is comparable. K-casein exhibits itself better on starch gel in both its native and reduced states and certain other minor casein components show a stronger appearance on starch gel. It is convenient to use PGUE for preliminary investigation of casein fractions; however, a complete evaluation should be supported by SGUE.

The importance of gel electrophoresis for characterization and analysis of proteins cannot be overemphasized. Smithies (*1*) improved starch gel electrophoresis for detecting abnormalities in human blood sera. His techniques were later applied to analysis of caseins by Wake and Baldwin (2), Neelin et al. (3), and others. High concentrations of urea showed a profound increase in electrophoretic separation of the caseins. More recently, synthetic polymers have been introduced as supporting electrophoretic media to replace starch gel. The application of polyacrylamide gel as an electrophoretic medium and its advantages have been reported by Raymond and Nakamichi (*4*). Peterson (5) demonstrated the high resolution of caseins on polyacrylamide gel, and, at present, there is conflicting opinion as to the comparative merits of polyacrylamide and starch gels in casein analysis.

This research was aimed at obtaining more quantitative data from gel electrophoretic patterns. Prior to this work no determination had been made on the minimum detectible limits of the major casein components (α_{s_1}-, β_s-, and K-caseins) by starch and polyacrylamide gel electrophoresis. In addition, no investigation has been made of the optimum sample loading volumes and weights of the various caseins which provide the desired electrophoretic representation and resolution. This paper answers these questions and a number of others that arise through continued use of gel electrophoresis for examination of the caseins. The α_{s_1}-, β_s-, and K-caseins were chosen because they are the major components of whole casein for which isolation and purification procedures have been developed that yield sufficiently homogeneous products (*6*–*8*).

Full utilization of the data provided by this research will significantly increase the extent of information one can obtain by observing the gel electrophoretic patterns of the major casein components.

EXPERIMENTAL

Polyacrylamide Gel-Urea Electrophoretic (PGUE) Conditions

The E-C vertical gel apparatus (E-C Apparatus Company, Philadelphia, Pa.) was used for the PGUE analyses. Gels and buffer were prepared by the method of Thompson et al. (9), using 7% Cyanogum 41, 4.5 *M* urea, and a continuous buffer system of tris-EDTA-borate, pH 9.2 (121 g THAM, 15.6 g Na_2-EDTA, and 9.2 g H_3BO_3 in 12 liters of distilled water). Sample solutions containing 4.5 *M* urea and 8% sucrose were prepared in the same buffer. For disulfide reduction of K-casein all buffers were made to contain 0.2% of 2-mercaptoethanol (2-ME). The slot former used in all cases gave a wedge-shaped slot 10 mm long and 3 mm deep. The electrophoretic analyses were carried out under an applied potential of 200 V for 5 to 6 hr.

Starch Gel-Urea Electrophoretic (SGUE) Conditions

The starch gel experiments were performed on a Buchler Vertical gel apparatus (Buchler Instruments, Inc., Fort Lee, N.J.). "Starch Hydrolyzed," obtained from the Connaught Medical Research Laboratories (Toronto, Canada) was used to prepare the gels. A discontinuous buffer system was used consisting of 0.076 *M* tris-citrate (gel buffer) and 0.3 *M* Na-borate (bridge buffer), both at pH 8.6, and the gels contained 6 *M* urea. The sample solution was prepared with tris-citrate buffer containing 6 *M* urea and as with PGUE all buffers contained 0.2% 2-ME for the reduction of K-casein.

The gel was prepared by heating a suspension of 56 g of starch in 350 ml of tris-citrate buffer, and quickly adding 126 g of urea after the gel point was exceeded. The gel solution was subjected to a partial vacuum, via a suction flask, to remove the air bubbles, then poured into the gel mold while at a temperature of 40 to 50°C. The gel was covered with a glass plate and allowed to set for 15 to 18 hr. The sample slots, having dimensions 8 × 6 × 1 mm, were filled with 0.05 ml of sample solution and sealed with petrolatum. The complete apparatus was placed in a cold room (5°C) and a potential of 200 V was applied immediately. After 20 hr the gels were removed, sliced, and stained.

Both polyacrylamide gels and starch gels were stained for 3 to 5 min with a saturated solution of Amido Black 10 B in 5:5:1, v/v/v, methanol, water, and glacial acetic acid; and destained with 12:1:1, v/v/v, water, methanol, and glacial acetic acid.

RESULTS AND DISCUSSION

The PGUE patterns of α_{s_1}-casein shown in Fig. 1 illustrate the effect of loading volume when the weight of protein is constant. Resolution is shown to decrease with sample volumes above 40 μl. Thus, loading volumes of 20 to 30 μl were chosen for the different sample weight studies with PGUE.

In SGUE experiments the sample loading volume in all cases was 50 μl, since this is the volume required to completely fill the gel slot.

The PGUE patterns for various sample loading weights of α_{s_1}-casein are given in Fig. 2. Overloading tends to obscure the true electrophoretic representation of α_{s_1}-casein, and only at sample weights of less that 150 μg does the α_{s_1}-casein show the three characteristic zones. The only value in overloading with sample is to test for any minor contamination present in the preparation. Figure 2 also shows that 6.3 μg of α_{s_1}-casein can be detected by PGUE.

PGUE patterns for β_s-casein of sample weights of 6.3 to 600 μg are shown in Fig. 3. Again, resolution is shown to improve with decreasing amounts of applied sample. The lower detectible limit for β_s-casein is on the same order of magnitude as for α_{s_1}-casein. An explanation for the appearance of more than a single zone for both α_{s_1}- and β_s-caseins is the presence of genetic variants, since these preparations were isolated from pooled milk.

It was of interest to study synthetic mixtures consisting of different weight ratios of α_{s_1}- and β_s-caseins. The PGUE patterns of these purified caseins are shown in Figs. 4 and 5. The first important observation is that the presence of 1% of either casein was detectible in 200-μg mixtures. In addition, the lower detectible limit for either casein was not changed, and the electrophoretic separation is complete (i.e., no interaction effects).

Also, the effect of sample loading weight on the electrophoretic appearance of reduced K-casein was investigated and the results are shown in Fig. 6. Twenty-five micrograms of reduced K-casein was required for detection by PGUE because of the large number of zones present. Extremely large amounts of reduced K-casein

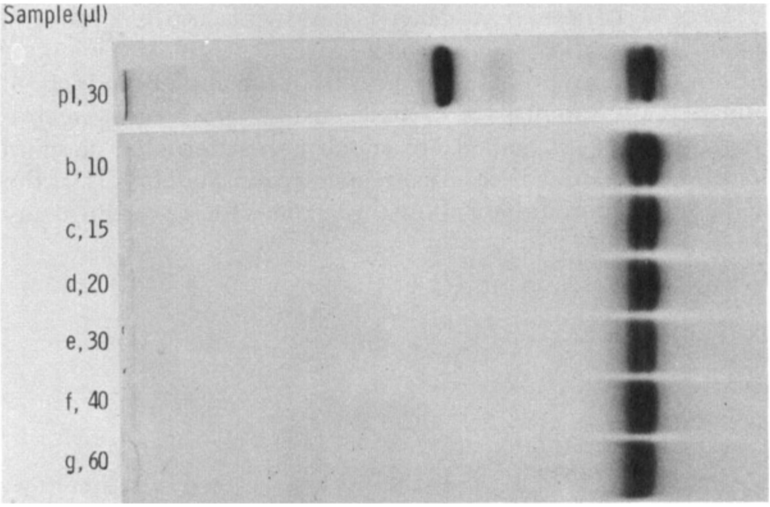

FIG. 1. Effect of sample loading volume on PGUE pattern of α_{s_1}-casein. Sample weight constant, pI 300 μg; b to g, 200 μg.

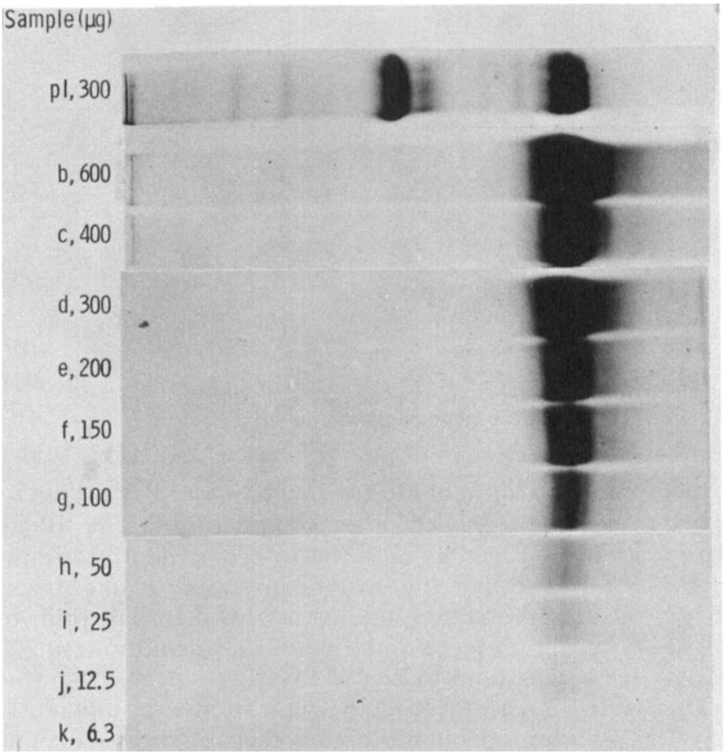

FIG. 2. Effect of sample loading weight on PGUE pattern of α_{s_1}-casein. Sample volume constant at 30 μl.

gave a diffuse pattern probably as a result of incomplete reduction.

Duplicate experiments were made using starch gel electrophoresis. Figures 7, 8, and 9 are starch gel patterns of standard weights of α_{s_1}-, β_s-, and K-caseins, respectively. Starch gels of the specified composition did not show three zones for α_{s_1}-casein at any loading concentration. This is not to say that α_{s_1}-casein could not be resolved better in starch gels of other compositions. The 10 μg sample of α_{s_1}-casein in Fig. 7 is near the lower perceptible limit. It should be noted here that the weight of stained protein was roughly 5 μg because the starch gels were sliced in half prior to staining. Therefore, the detectability, if not the resolution, of α_{s_1}-casein by SGUE is equivalent to that of PGUE.

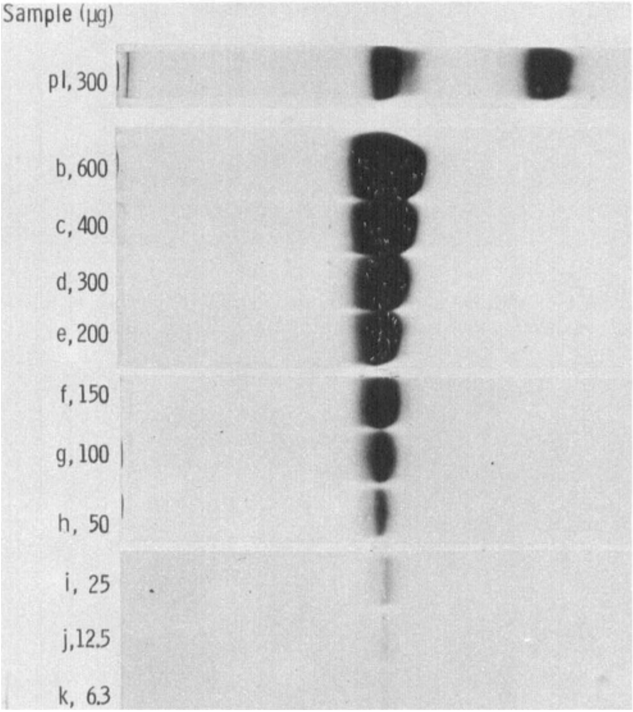

FIG. 3. Effect of sample loading weight on PGUE pattern of β_s-casein. Sample volume constant at 30 μl.

Unlike α_{s_1}-casein, β_s-casein (Fig. 8) was resolved sufficiently well by SGUE, as verified by the appearance of the two zones

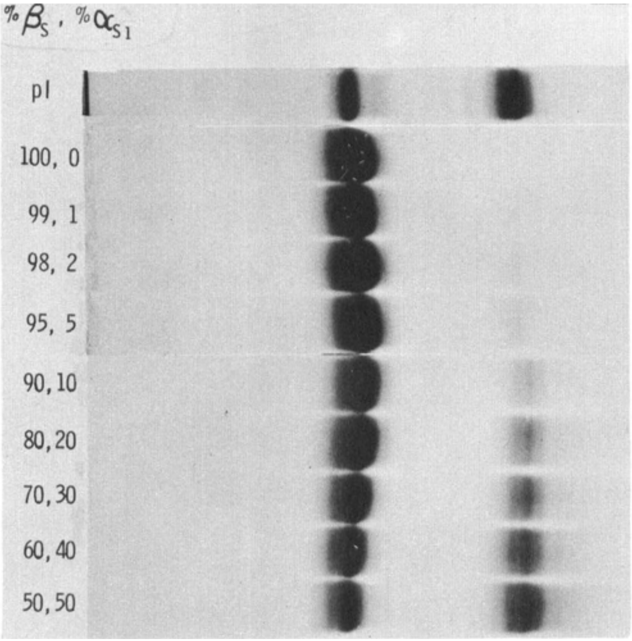

FIG. 4. PGUE patterns of different w/w% mixtures of β_s- and α_{s_1}-caseins. Sample volume constant at 20 μl and total weight at 200 μg.

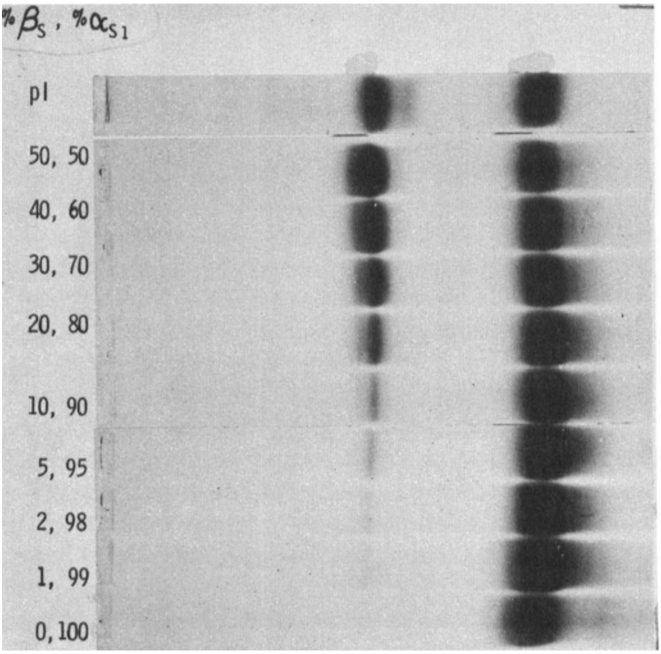

FIG. 5. PGUE patterns of different w/w% mixtures of β_s- and α_{s_1}-caseins. Sample volume constant at 20 μl and total weight at 200 μg.

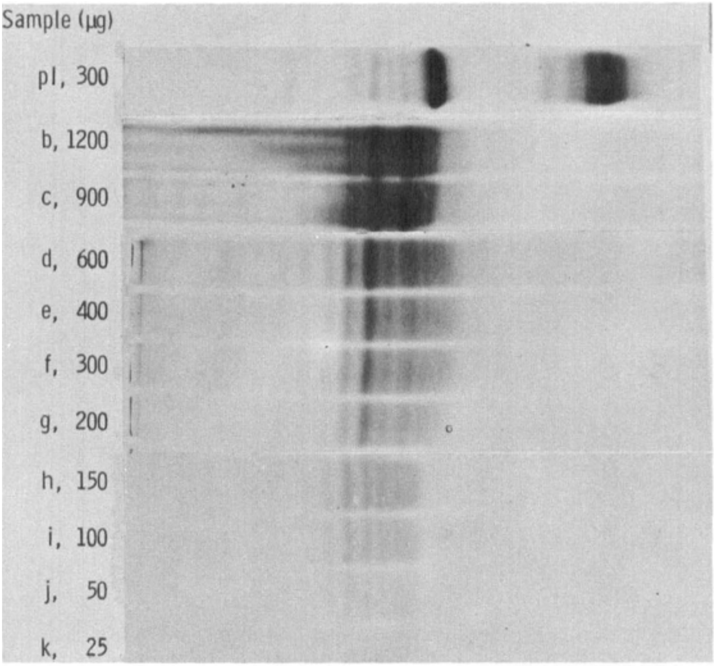

FIG. 6. Effect of sample loading weight on PGUE pattern of reduced K-casein. Sample volume constant at 30 µl.

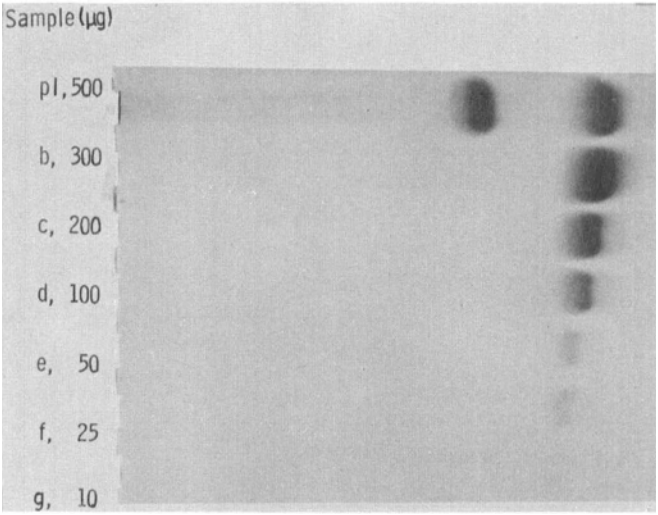

FIG. 7. Effect of sample loading weight on SGUE pattern of α_{s_1}-casein. Sample volume constant at 50 µl. Gels sliced to one-half original thickness.

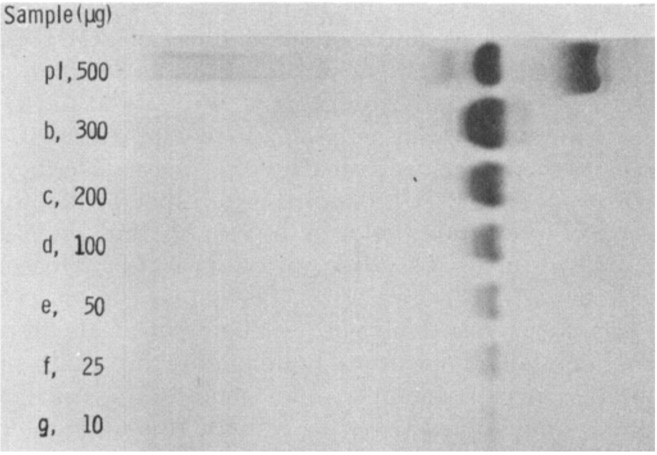

FIG. 8. Effect of sample loading weight on SGUE pattern of β_s-casein. Sample volume constant at 50 µl. Gels sliced to one-half original thickness.

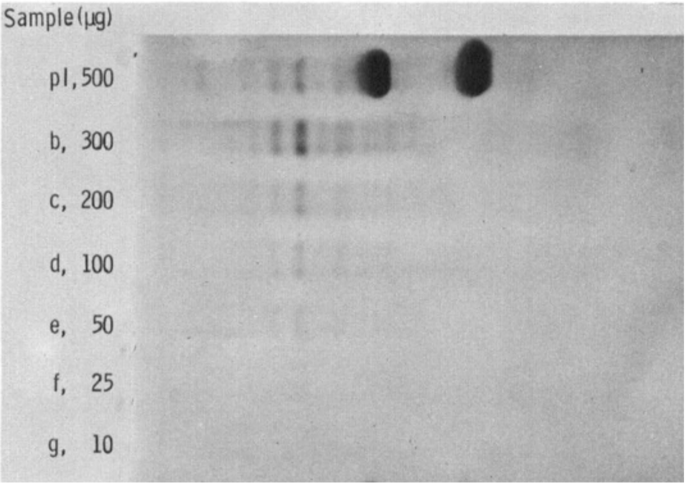

FIG. 9. Effect of sample loading weight on SGUE pattern of reduced K-casein. Sample volume constant at 50 µl. Gels sliced to one-half original thickness.

shown earlier with PGUE, but in no instances were the casein zones of SGUE as sharp and distinct as those of PGUE. The one primary advantage of starch gel was its ability to separate reduced K-casein zones to a greater extent (Fig. 9). Figures 10 and 11 are the starch gel patterns for different sample weight ratios of α_{s_1}- and β_s-caseins and the results were analagous to those described earlier for the corresponding PGUE patterns. More specifically, there was no evidence of interaction between the caseins, and 2% concentrations (in 300 μg of total loading) of either α_{s_1}- or β_s-casein gave perceptible zones.

Since this study was done with a single set of electrophoretic conditions, i.e., gel composition, buffers, etc., the results are similarly limited. The experiment was designed for a general gel electrophoretic examination of casein fractions. It is undoubtedly true that there are optimum conditions for each casein component and these should be worked out separately. Nevertheless, it is believed that in routine casein analysis the electrophoretic techniques and conditions described here are not too unlike those used in most laboratories. Therefore the major objectives of determining the

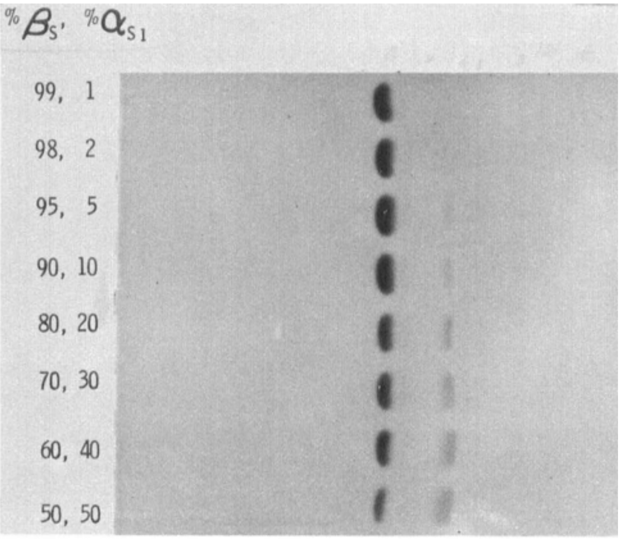

FIG. 10. SGUE patterns of different w/w% mixtures of β_s- and α_{s_1}-caseins. Sample volume constant at 50 μl and total weight at 300 μg.

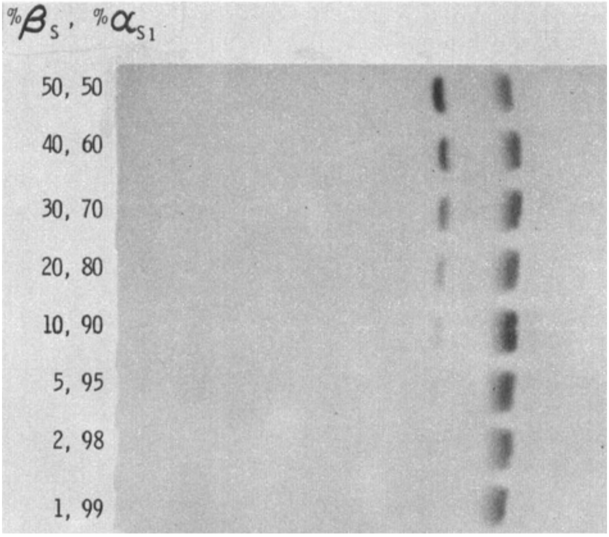

FIG. 11. SGUE patterns of different w/w% mixtures of β_s- and α_{s_1}-caseins. Sample volume constant at 50 μl and total weight at 300 μg.

lower detectible limits for the major casein components and semiquantitatively evaluating the amount of each component present in a preparation by observing the gel pattern have been accomplished. The sensitivity of gel electrophoresis is appropriately reemphasized at this point. It was shown that 1 to 2% concentrations (2 to 4 μg) of α_{s_1}- and β_s-caseins in loading weights of 200 μg of total protein could be detected by PGUE; thus, it is likely that 2% zonal concentrations of any casein component could be detected in casein preparations. An assay of this type cannot be equaled by such techniques as analytical ultracentrifuge patterns, amino acid analyses, phosphorus content, etc.

REFERENCES

1. O. Smithies, *Biochem. J.*, **71**, 585 (1959).
2. R. G. Wake and R. L. Baldwin, *Biochim. Biophys. Acta*, **47**, 225 (1961).
3. J. M. Neelin, D. Rose, and H. Tessier, *J. Dairy Sci.*, **45**, 153 (1962).
4. S. Raymond and M. Nakamichi, *Anal. Biochem.*, **3**, 23 (1962).
5. R. F. Peterson, *J. Dairy Sci.*, **46**, 1136 (1963).
6. C. W. Gehrke, C. W. Freeark, Y. H. Oh., and P. W. Chun, *Anal. Biochem.* **9**, 423 (1964).

7. C. W. Gehrke, P. W. Chun, and Y. H. Oh, *Separation Sci.*, 1(4), 431 (1966).
8. C. W. Gehrke, P. W. Chun, and K. K. Tripathi, unpublished ms.
9. M. P. Thompson, C. A. Kiddy, J. O. Johnson, and R. M. Weinberg, *J. Dairy Sci.*, **47**, 378 (1964).

Received by editor January 19, 1967
Submitted for publication January 31, 1967