

# COMPUTER-ASSISTED METHOD TO MEASURE THE ADHESIVE PROPERTIES OF HYDROLYSIS PRODUCTS FROM COLLAGEN\*

by

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## ABSTRACT

Historically, hydrolysis products from collagen, such as technical gelatin and animal glue, were used as adhesives. With the advent of synthetic adhesives, those products from collagen became less popular. Because of the "green revolution" over environmental concerns, animal glues are again becoming appealing. Characterization of adhesives has, at best, been subjective. The Jellygram and/or the Bloom values are presently the most generally used physical tests, the higher values denoting the better quality adhesives. We will describe a method, presently being adopted by adhesive companies, that utilizes a commercially available computerized texture analyzer. The advantages of this method are that it not only measures the tackiness but also the work of adhesion, and it requires only a small amount of sample. Subtle differences in the products that cannot be measured by the prevailing technology can be determined by this technology. Furthermore, analysis of the standard deviation and coefficient of variation shows that the method has good precision when relatively simple guidelines are followed.

## INTRODUCTION

Although hide glues or lower grade gelatins once were valued for their adhesive properties,<sup>1,2</sup> synthetic adhesives

have all but replaced these products of animal origin. Only about 6,000-7,000 metric tons of hide glues are produced in the United States<sup>1</sup> at the present time and an equal amount is imported.<sup>3</sup> These numbers are down from approximately 41,000 metric tons produced in 1977.<sup>4</sup> The principal uses for hide glues today are for bookbinding, coated abrasives, creping and converting paper, metal refining and miscellaneous uses such as woodworking and the manufacture of matches and gummed-tape.<sup>1,3</sup> Among the advantages of hide glues are that they have rapid tack, they can be stored in the dry state indefinitely without loss of strength, they are soluble only in water, and they pass from a liquid to a gel state on cooling and the reverse upon heating. Dried films deposited from hide glue solutions possess great strength and resilience. A wide range of viscosities can be achieved by varying the concentration and the test grade of the hide glues. These products are thus readily available for a wide range of adhesive.<sup>3,4</sup>

Glues of animal origin are regaining their appeal because they offer an environmentally friendly alternative to the synthetics.<sup>3,5</sup> Among the disadvantages cited in the past are that hide glues have high water solubility and limited adhesive strength compared to the synthetics. It has been shown that hide glue products can be chemically modified so as to impart some of the more desirable properties of the synthetics requirements.<sup>5</sup>

In our treatment of the solid chromium-containing waste from the leather industry,<sup>6,7</sup> we have been isolating gelable and hydrolyzed protein products that, by subjective evaluation, have adhesive properties. To judge the quality of these

products we needed to find a method that could compare these products to those produced commercially. The adhesive properties of hide glue products are evaluated by measurement of the Bloom value, Jellygram value, viscosity and a variety of qualitative analyses.<sup>8</sup> If the product does not gel, these first two tests would not be applicable. This paper will describe a method, presently being adopted commercially, that measures the adhesive properties of the proteinaceous hydrolysis products from collagen. In the analysis that we will describe, the samples will be measured not only for their tackiness but also for their work of adhesion. It will also be shown that the method described can have good precision if relatively straightforward guidelines are followed. Another advantage of the method is that only a small amount of sample has to be used.

In this paper, commercial samples are used for the development of the method. We have included several samples from the treatment of chrome shavings to show that these samples compare well with the commercial products. In a following paper, we will show the effects of different alkalinity agents on the adhesive properties of the protein products that are extracted. Also, we will suggest different modifications of these products that will enable them to compete with the synthetics.

## EXPERIMENTAL

### Materials

Gelatin samples, 75 and 225 Bloom, were obtained from Sigma Chemical Company (St. Louis, MO). Gelable protein samples, extracted by processes described in previous publications,<sup>6,7</sup> were also used.

### Equipment

Determination of adhesive properties was carried out on the TA.XT2 Texture Analyzer, designed and manufactured by Stable Micro Systems (Godalming, Surrey, UK) and marketed by Texture Technologies Corp. (Scarsdale, NY). The instrument was equipped with a 5 Kg load cell, a heavy duty platform equipped with a lexan plate and a two inch acrylic probe. XT.RA Dimension software was used to set the conditions for the analysis, run the determinations and calculate the results. A Neslab constant temperature bath was used to control the temperature of the samples.

### Procedure

A 6.67% wt/wt concentration (1.44 g/20 ml water) of the sample to be analyzed was prepared on the day of the analysis. Three aliquots of the sample solution were prepared

and these were stored at 4°C until ready to use. The samples were placed in a constant temperature bath at either 33, 35 or 39°C for 15 minutes to equilibrate prior to beginning the analysis. The appropriate amount of sample, 0.3-0.4 g (enough to be completely and evenly dispersed under the acrylic probe), was weighed onto the lexan plate. The plate was then placed on the platform and was screwed tightly in place. The appropriate parameters were programmed into the instrument and the test was begun. The acrylic probe approached the sample at a speed of 1 mm/sec. When it sensed the sample, the speed increased to 2 mm/sec. A force of 200 grams was applied for 3 seconds and the probe was pulled away from the sample at a speed of 10 mm/sec for a distance of 5 mm. The determination, from weighing of the sample to completion of the test, took no longer than 75 seconds. Keeping within this time frame prevented excessive heat loss from the sample and thus insured the precision of the analysis.<sup>9</sup> The XT.RA Dimension software calculated the tackiness (peak, #1) in grams and the work of adhesion (area) in gram seconds (g's) as shown in Figure 1. The ratio of tackiness (g) to the work of adhesion (g's) was calculated upon completion of the determinations. This value can be reported as sec<sup>-1</sup> (smaller values would indicate higher grade adhesives).

## RESULTS AND DISCUSSION

By subjective evaluation, the gel and hydrolyzed protein products that we have isolated from the treatment of chromium-containing waste have adhesive properties. The usual methods for determining the strength of the adhesive are by Bloom value, Jellygram value or viscosity.<sup>1</sup>

ASTM has many methods for measuring the adhesion of one product to another but there are very few methods that will measure the adhesion of adhesives. The methods that are described are qualitative methods, not quantitative.<sup>8</sup>

To evaluate the adhesiveness of protein products that we are isolating we needed a method that would measure the different adhesive properties (tackiness and work of adhesion) and would give statistically meaningful data. We had been using the TA.XT2 Texture Analyzer for determination of the Bloom and Jellygram values. The literature accompanying the instrument suggested that it could be used for adhesive testing.<sup>9</sup> The instrument manufacturer informed us that this method was being adopted commercially and he assisted us in determining the appropriate accessories and the basic instrument conditions to run this test. A lexan plate and acrylic probe were used to test the samples and the instrument was controlled and data collected and calculated with XT.RA Dimension software.

## Stable Micro Systems - XT.RA Dimension V3.7G

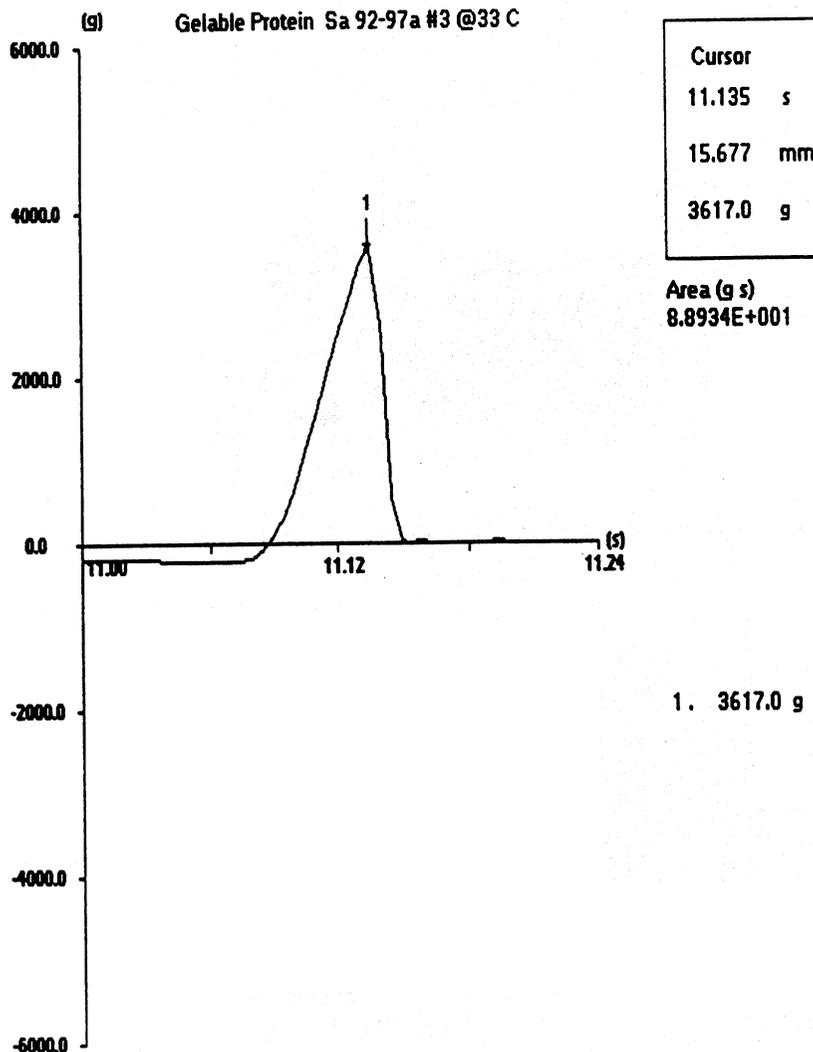


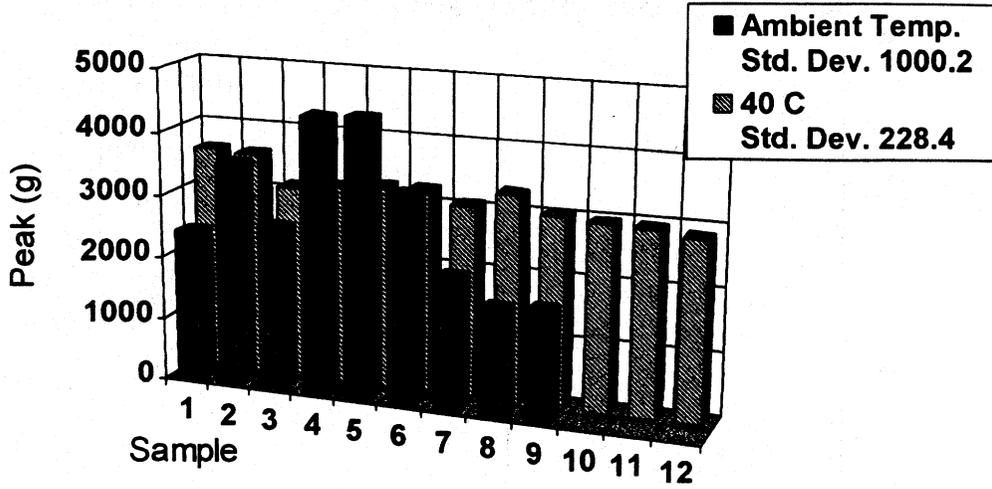
FIGURE 1. — Graph showing the tackiness (peak) in grams and work of adhesion (area) in gram seconds as calculated by XT.RA Dimension software.

In the initial tests, many samples with multiple determinations were run. The precision of the analysis, however, was poor. We attempted to vary the force, speed and time of the probe. This, however, did not improve the results. Figure 2 shows the tackiness (peak) in grams of one of the samples that was examined (gel extracted from chrome shavings by 6% aqueous magnesium oxide). The data show that the standard deviation was quite high when nine replicates were run. Also shown in this figure is the work of adhesion (area) in g s and ratio of peak to area, respectively. These standard deviations are also quite poor. The samples that were being examined existed as a gel at room temperature and, before the determination could begin, it had to be heated until liquid. It took almost one hour to run the set of replicates and during that time the viscosity of the sample had increased as the temperature of the sample returned to room temperature.

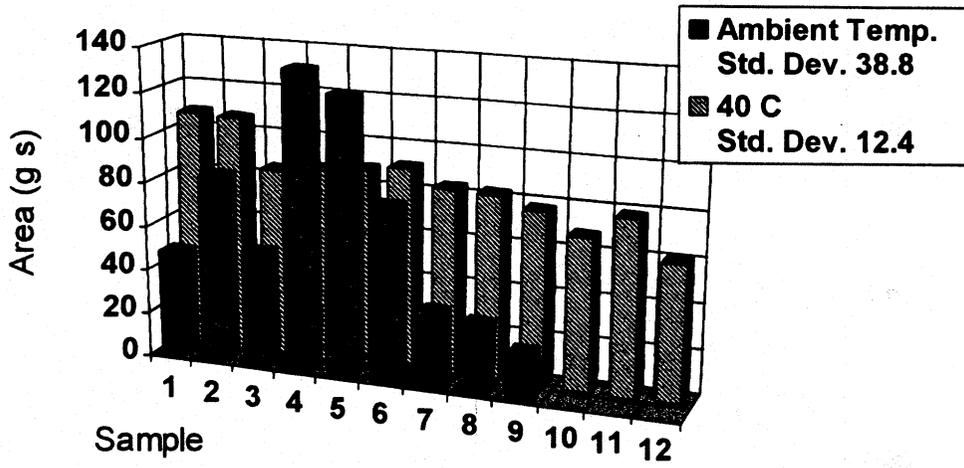
We designed an experiment to determine if, by preparing the sample at a controlled temperature, the precision of the determination could be improved. For the first set of experiments, a controlled temperature of 40°C was tried. The sample was equilibrated at this temperature for 15 minutes. The sample was quickly weighed onto the lexan plate and the determination was run. The results of this analysis are also shown in Figure 2. The standard deviations for this sample improved significantly over the determinations that were run at an ambient temperature.

In the next set of experiments, we examined whether the temperature at which we prepared the sample would affect the properties and the precision of the method. We obtained commercial samples of 225 and 75 Bloom gelatin. The appropriate concentration, 6.67% wt/wt, was prepared and an aliquot of each of the samples was equilibrated at 33, 35

### Measurement of Tackiness (Peak)



### Measurement of Work of Adhesion (Area)



### Ratio of Peak to Area

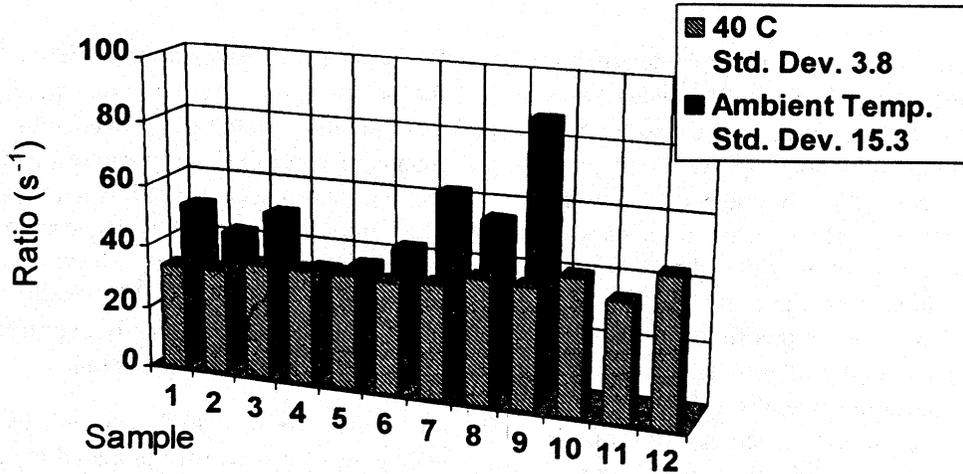


FIGURE 2. — Effect of ambient and controlled temperature on the reproducibility of TA.XT2 Adhesive Test.

and 39°C. Figure 3 shows the results from the tests run on the 225 Bloom gelatin. The sample that had been equilibrated at 33°C had a tackiness value of almost 5000 g, a value approaching the limit of the instrument. The samples equilibrated at the higher temperatures (35 and 39°C) had lower values with good standard deviation (Table III). With respect to this high Bloom gelatin, it appears that temperature affects the adhesive properties.

A 75 Bloom gelatin sample was also equilibrated at 33, 35 and 39°C (Figure 3). The standard deviation of these samples was quite good (Table III) and, upon examining the tackiness and work of adhesion values, it appears that the temperature did not have a significant effect on the adhesive properties of this lower Bloom gelatin.

As a baseline for the sample studies, we examined the adhesive properties of the solvent used in these tests, water. Figure 4 shows the results, with different ordinate scales, of the adhesive tests on water at 33, 35 and 39°C. These results show that water does indeed have adhesive properties as measured by this test, but when one compares these values to those from the 225 and 75 Bloom gelatin, as shown in Table I, one can see that the tackiness and work of adhesion values are quite low, whereas the ratio is extremely high, indicating a rather poor adhesive sample. This figure also shows that there is a significant difference in the properties of the water sample measured at 39°C.

Equilibrating the water at this temperature gave properties that were inferior to those water samples equilibrated at the lower temperatures.

It should be noted that the average amount of time to run the analysis, from removal of the sample from the bath to completion of the test, took less than 75 seconds. The test could be run using an aluminum probe and plate, but there would be rapid heat transfer to the plate from the sample, thus negating the effect of temperature equilibrium. Using the lexan plate, in which heat transfer would be slower, would enable one to determine the effect, if any, of the temperature on the properties.

We calculated the coefficient of variation (CV) of the samples that had been examined.<sup>10</sup> This value is calculated by dividing the standard deviation by the mean and multiplying by 100. The CV is often used to describe the amount of variation in the population. It is often a preferred measure because it is unitless. A value of five or below indicates good precision where the actual analytical value is large. The following tables give the mean, the standard deviation and the CV of the samples that we had tested. In Table II, one can see that as the samples were subjected to controlled temperatures, the CV improved dramatically.

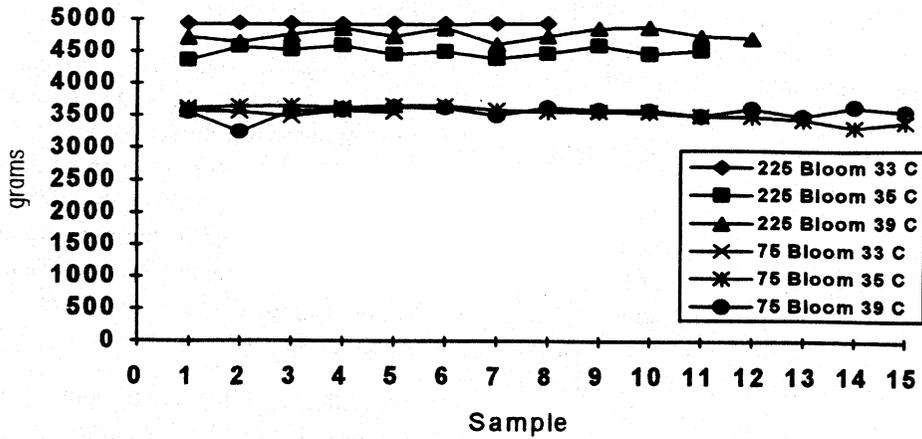
In Table III, the CV of 225 Bloom samples at 33, 35 and 39°C are given. These values, for the most part are under

**TABLE I**  
**Adhesive Properties (Averages) of Water and Gelable Proteins**

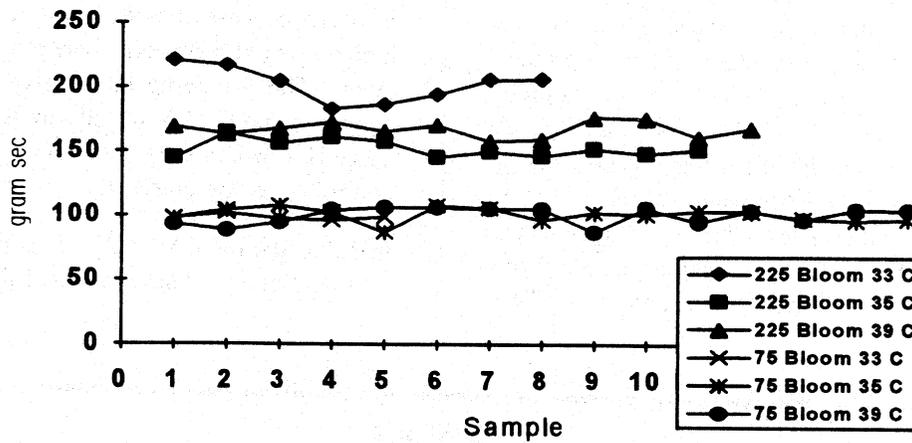
Substrate	N	Peak (g)	Area (g s)	Ratio (1/s)
<b>Water</b>				
33 C	15	914.4	9.2	88.5
35 C	15	864.4	9.9	87.3
39 C	15	795.9	5.8	137.3
<b>Bloom 75<sup>a</sup></b>				
33 C	4	3546.4	98.5	36.0
35 C	15	3546.6	100.8	35.3
39 C	15	3551.6	100.2	35.6
<b>Bloom 225<sup>a</sup></b>				
33 C	10	4926.8	202.0	24.5
35 C	11	4492.7	152.5	29.5
39 C	12	4760.8	167.1	28.5

<sup>a</sup> 6.67% wt/wt concentration

### Measurement of Tackiness (Peak)



### Measurement of Work of Adhesion (Area)



### Ratio of Peak to Area

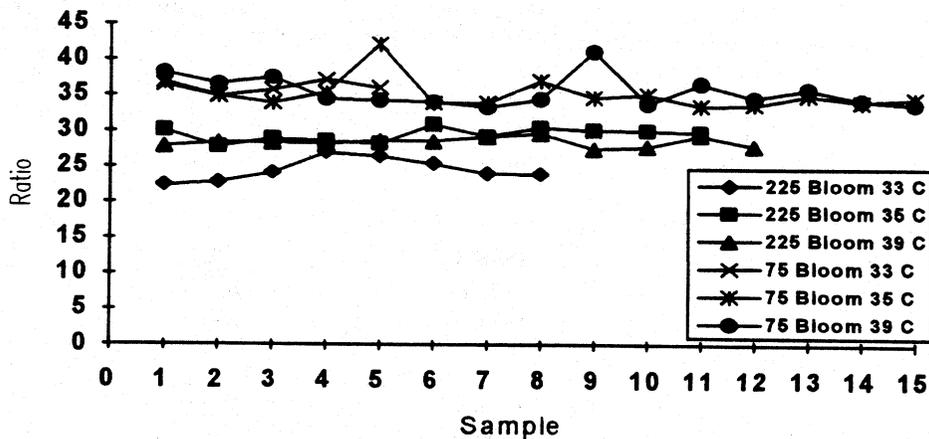
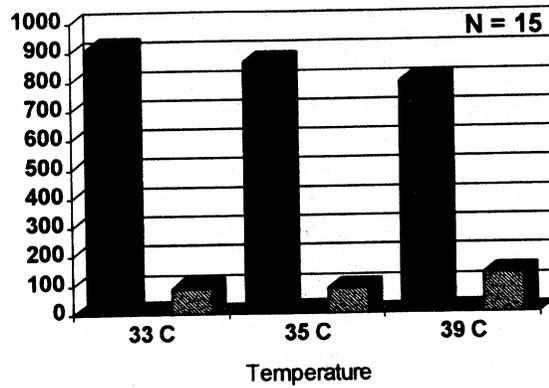
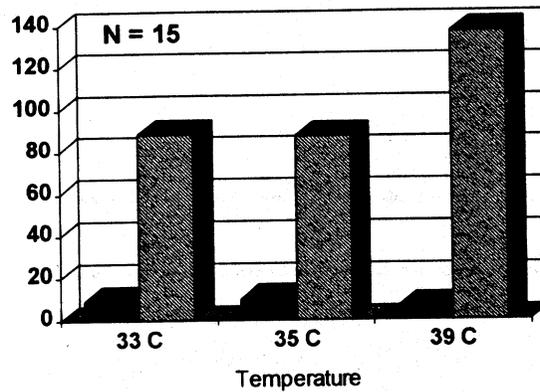


FIGURE 3. — Effect of temperature on the adhesive properties of 225 and 75 Bloom gelatin.



■ Peak (g) ■ Area (g sec) ■ Ratio (peak/area)



■ Area (g sec) ■ Ratio (peak/area)

FIGURE 4. — Adhesive properties of water as measured by the TA.XT2 test.

**TABLE II**  
**Effect of Controlled Temperature on Precision of Adhesive Test Method<sup>a</sup>**

Property	Temp	Mean	Std	CV
Peak (g)	Ambient <sup>b</sup>	2921.1	1000.2	34.2
Area (g s)		67.5	38.8	57.5
Ratio (l/s)		50.7	15.3	30.3
Peak (g)	40 <sup>c</sup>	3168.6	228.4	7.2
Area (g s)		84.6	12.4	14.7
Ratio (l/s)		38.0	3.8	9.9

<sup>a</sup> MgO extracted gel

<sup>b</sup> N = 9

<sup>c</sup> N = 12

**TABLE III**  
**Statistical Analysis of Adhesive Properties of 225 Bloom Gelatin**

Property	Temp	Mean	Std	CV
Peak (g)	33 C <sup>a</sup>	4926.8	2.0	0.0
Area (g s)		202.0	12.8	6.3
Ratio (l/s)		24.5	1.6	6.4
Peak (g)	35 C <sup>b</sup>	4492.7	74.1	1.6
Area (g s)		152.5	6.3	4.1
Ratio (l/s)		29.5	1.0	3.3
Peak (g)	39 C <sup>c</sup>	4760.8	84.6	1.8
Area (g s)		167.2	5.7	3.4
Ratio (l/s)		28.5	0.6	2.3

<sup>a</sup> N = 10

<sup>b</sup> N = 11

<sup>c</sup> N = 12

**TABLE IV**  
**Statistical Analysis of Adhesive Properties of 75 Bloom Gelatin**

Property	Temp	Mean	Std	CV
Peak (g)	33 C <sup>a</sup>	3546.4	35.4	1.0
Area (g s)		98.5	2.2	2.2
Ratio (l/s)		36.0	0.9	2.4
Peak (g)	35 C <sup>b</sup>	3546.6	95.4	2.7
Area (g s)		100.8	5.1	2.1
Ratio (l/s)		35.3	2.1	5.9
Peak (g)	39 C <sup>c</sup>	3551.6	94.0	2.6
Area (g s)		100.2	6.6	6.5
Ratio (l/s)		35.6	2.0	5.7

<sup>a</sup> N = 4

<sup>b</sup> N = 15

<sup>c</sup> N = 15

**TABLE V**  
**Statistical Analysis of Adhesive Properties of MgO-Na<sub>2</sub>CO<sub>3</sub> Extracted Gel**

Property	Temp	Mean	Std	CV
Peak (g)	33 C <sup>a</sup>	3260.6	85.6	2.6
Area (g s)		84.0	5.7	6.8
Ratio (l/s)		39.0	2.7	6.9
Peak (g)	35 C <sup>a</sup>	3331.5	140.2	4.2
Area (g s)		87.0	9.0	10.4
Ratio (l/s)		38.6	2.5	6.4
Peak (g)	39 C <sup>a</sup>	2857	92.5	3.2
Area (g s)		65.8	5.4	8.1
Ratio (l/s)		43.6	2.4	5.4

<sup>a</sup> N = 15

five. The values for the 33°C testing are a little higher, and that may be due to the sample's approaching the limits of the load cell.

In Table IV, the adhesive properties of 75 Bloom gelatin are given. This table is not only showing the precision of the individual temperatures but is also showing that temperature did not affect the physical properties of this sample. The values for the tackiness, area and ratio are quite similar at each temperature.

We examined the adhesive properties and the precision of the measurement of a gel that had been extracted with 5% magnesium oxide and 1% sodium carbonate.<sup>6,7</sup> As shown in Table V, the values for the 33 and 35°C samples were quite similar but those for the 39°C sample were lower. This sample still had ash present and we cannot compare these samples to the low ash commercial gelatins. This sample had a Bloom value of 108 g but the adhesive properties were a little lower than the 75 Bloom commercial sample.

Generally, it appears that the higher Bloom values will give better adhesive properties as measured by the TA.XT2 analysis, which is similar to the results that were observed previously. It appears that temperature affects the higher Bloom samples, but, as shown in Table II, it does not affect the 75 Bloom sample. Temperature also appeared to have an effect on the water samples run at the high temperature. Many more samples will have to be run to see if change in temperature will significantly affect the properties. We showed that if the gelatin samples are not run at a controlled

temperature, the values will have a high standard deviation and a high CV, thus making the results statistically meaningless.

In our next study we will be examining the products that we isolated after a variety of alkali extraction treatments. These samples will be analyzed before the ash is removed and then after deionization. At that time we will be able to make a statement on the effect of pretreatment and the presence of salts on adhesive properties and the subsequent comparison of these samples with the commercial products. We will also be looking at the adhesive properties of the hydrolysates, products that do not have Bloom or Jellygram values but do, by qualitative observations, have adhesive properties. We will also look at the effect that modification of these protein products, e.g., to improve the water resistance, will have on the adhesive properties.

### CONCLUSION

In conclusion, a computer-assisted method, presently being adopted commercially, is available to measure the adhesive properties, tackiness and work of adhesion, of hydrolysis products from collagen. Because of the nature of gelatin to be in the gel form at room temperature, it is necessary to equilibrate the samples at a controlled temperature (33, 35 or 39°C) for a short time before each of the replicates of the samples is run. The results from these tests show that a precise and statistically meaningful method is available that will give values that one could use to compare the adhesive properties of hydrolysis products from collagen.

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