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INTRODUCTION

In general, the objectives outlined in our original proposal to Jet Propulsion Laboratory directed towards the development of bladder materials suitable for use at cryogenic temperatures have been attained. The outline presented under the heading of "Plan of Approach", has been carried out and a film has been developed having the best possible combination of properties comprising all the variables studied. During the course of the work, Mr. D. D. Lawson of the Jet Propulsion Laboratory requested that our work include consideration of the dynamic mechanical properties of gelatin at temperatures in the cryogenic region, and suggested that these might be studied using a torsional pendulum. It was particularly desired that increased flexibility in the cryogenic region be obtained, and it was hoped that these studies would provide a chemical basis for improving cryogenic flexibility. It was recognized that this could be done only by extending the span of this contract. Consequently it was extended from twelve to twenty-four months. During the twelve month extension of the contract, exhaustive studies were made of possible ways of lowering the glass transition temperature (Tg) of gelatin. For the sake of coherence we will report on the work of the original twelve months first to be followed by that of the second twelve months.

I. PROPERTIES OF GELATIN FILMS—TENSILE STRENGTH AND GAS PERMEABILITY STUDIES.

OUTLINE

The objectives to be attained in this effort for the first twelve months were:

1. Provide protein films of various thicknesses derived from:
    - a. Swift's Superclear gelatin.
    - b. Deaminated gelatin.
    - c. Carbamidated gelatin.
    - d. Cyanamidated gelatin.
    - e. Phenol-formaldehyde resin-gelatin condensate.
2. The use of plasticizers in these films at various levels:
    - a. None.
    - b. Ethylene glycol.
    - c. Polyethylene glycol.
    - d. Dimethylsulfoxide.
    - e. N-Methylpyrrolidone.
    - f. Glycerol.
3. The use of crosslinking agents at various levels:
    - a. Formaldehyde.
    - b. Glyoxal.
    - c. Glutaraldehyde.
    - d. Other.
4. The use of reenforcing fibers:
    - a. Glass fibers.
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    d. Nitrogen.
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PREPARATION OF FILMS

Swift's Superclear Gelatin

The basic product for this work was Swift's Superclear gelatin, a gelatin of 300 gram test with a natural pH of 3.9-4.1. It was extracted from acid cured pork skins for maximum jelly strength and minimum viscosity. The grind used was 6-20, a designation which signified the mesh range. Such a product was free of fines and did not hydrate excessively fast and permitted the production of almost any concentration of gelatin with minimun entrapment of air.

DEAMINATED GELATIN

Deaminated gelatin was prepared by the reaction with nitrous acid on the amino groups with the formation of hydroxy groups. Three different levels of deamination were carried out in order to select the one most suited for film preparation. For the purpose of calculations, gelatin was considered to contain 46 milliequivalents of amino groups per 100 grams of gelatin. On this basis, three lots of deaminated gelatin were prepared using the theoretical, 0.5 the theoretical and 0.1 the theoretical amount of sodium nitrite.

These samples on drying were found to be incompletely soluble and thus had to be rejected. The factors responsible for the lack of complete solubility were narrowed down to the amount of nitrous acid, temperature of deamination and insolubilization resulting from complete drying.

The preparation of a soluble lot of deaminated gelatin was accomplished as follows:

Four hundred fifty grams of gelatin were soaked in 2500 ml. of distilled water for several hours. The gel was melted on a water bath at 39-40°C. Nine grams of sodium nitrite dissolved in 100 ml. of water were added dropwise with mechanical stirring over a ten minute period. The reaction mixture was allowed to stand for twenty minutes and poured into trays and chilled. The gelatin sheets were cut into strips, dialyzed in running tap water at 15-17°C for 24 hours. The swelled strips were partially dried in the air and then cut into 0.5 inch cubes. These were held in a cooler for 48 hours to equilibrate the moisture content and then assayed for gelatin content. The gelatin content was 17.53% gelatin. The moist cubes were packaged in 250 gram lots in Mylar bags and frozen in a sharp freezer until needed.

CARBAMIDATED GELATIN

The preparation of carbamidated gelatin was carried out by reacting gelatin with potassium cyanate. Under the conditions of the experiment the cyanic acid reacted with the amino groups to yield substituted ureas. The same problem of partial insolubility was encountered if the carbamidated gelatin was dried completely, therefore, the following procedure was developed and used to produce a soluble carbamidated gelatin.

Four hundred fifty grams of Swift's Superclear gelatin were soaked in 2500 gms. of distilled water. After melting at 40°C., nine grams of potassium cyanate dissolved in 100 ml. of water were added with stirring and allowed to react for 25 minutes. The mixture was poured into trays, chilled, cut into 1/2" wide strips and dialyzed for 24 hours in 15-17°C water. The dialyzed product was partially dried in an air tunnel, cut into small pieces and packed in Mylar bags and frozen. The solids content was 20.7%.

CYANAMIDATED GELATIN

Cyanamidated gelatin was made just before the solution was cast into a film. This had to be done because of the very limited life of gelatin solutions after the addition of hydrogen cyanamide. The level of hydrogen cyanamide used was at the rate of 42 milliequivalents of hydrogen cyanamide for 100 gms of gelatin. The material was conveniently measured out with a micro pipette as a 50% aqueous solution.

PHENOL-FORMALDEHYDE CONDENSATE-GELATIN

Phenol-formaldehyde condensate solutions were prepared and added to the gelatin just before pouring onto the film plates. A number of preparations of a water soluble phenol-formaldehyde resin based on the procedure of d'Alelio, (Experimental Plastics and Synthetic Resins, John Wiley & Sons, (1960), p. 16) were prepared. None of these were found to be water soluble in all proportions. A product made according to the following directions was found to be suitable and homogeneous after having added to it 5% of ethanol.

PREPARATION OF A SOLUBLE PHENOL-FORMALDEHYDE CONDENSATE

A mixture of 376 grams of crystalline phenol and 420 grams of formalin catalyzed with 3.2 gms. of sodium hydroxide dissolved in 20 ml. of water was heated in a flask on a glycerol bath at 120 ± 5°C with stirring under reflux for 45 minutes, cooled at once in an ice bath, neutralized with 0.1 M citric acid and concentrated at 30°C in a "Rinco" evaporator under vacuum. After several days no further material was being removed and the residue was cut with water. The heterogeneous suspension was rendered homogeneous by the addition of 5% alcohol. The resin content was 22.2%.

PLASTICIZERS

The plasticizers originally considered were: glycerol, ethylene glycol, polyethylene glycol, propylene glycol, dimethyl sulfoxide, N-methyl pyrrolidone. These were made up as aqueous solutions and were colored for easy identification.

CASTING SURFACES

The casting surfaces were clean plate glass one foot by two feet. An area 55 x 15 cm. was outlined with a china-marker pencil to enclose an area of 825 cm^2. Using a density of 1.33 for gelatin, we found that 2.8 gms. of gelatin spread out over this area yielded a film one mil (.001 inch) thick.

Summarizing, the basic formula became:

2.8 grams of gelatin

28 mgs. of dried ox bile (1% basis gelatin)*

x% plasticizer basis the gelatin added as a 2.8% aqueous solution.

Add sufficient water to permit the 825 cm^2 to be covered. The total water ranged from 80 to 100 ml. depending upon the amount of dissolved solids.

*The ox bile acted as a release agent without which gelatin films could not be removed from glass surfaces.

Thicker films were made by multiplying the basic formula by the film thickness desired. The initial set of films was limited to gelatin alone, a total of 180 different films being produced. Of the 180 films prepared we have examined many to evaluate their gas permeability in a general way. The calibration and testing of our equipment using these early films indicated the following:

1. Helium consistantly diffused through the films at about twice the rate of hydrogen, thereby permitting us to speed up the screening of films using helium.

2. Our gas permeability determination when run on four replications of 4 mil gelatin films plasticized with 5% glycerol yielded the following results. Results are as cc/100 in^2/24 hrs./ atm.

2.2 cc repeating on the same sample - 2.1 cc
2.5 cc " " " " " - 2.3 cc
2.0 cc " " " " " - 2.2 cc
2.3 cc " " " " " - 2.2 cc

A statistical evaluation of the magnitude of the significant limits yielded a difference of 0.2cc/100 sq. in./ 24 hrs./atm. This is to say that differences of 0.2 cc or greater are significant in 95% of the cases. However, this is true only of these films, having this thickness, composition and formulation. Other films will no doubt have other differences.

In a preliminary way the following premeabilities to gases were measured in cc/100 in^2/24 hrs./atmos.

<u>2 mil Gelatin Film</u>

2% Glycerol Plasticizer He=5.0, A=.034, O_2=0.1
5% " " He=4,8, A=0.3, O_2=0.02

<u>5 mil Gelatin Film</u>

5% Glycerol He=1.9,2.2; O_2=0.006

5 mil Gelatin Film

3% Ethylene Glycol $He=2.9$; $O_2=0.02$

5% " " $He=2.5$; $H_2=1.8$; $O_2=0.01$

These permeabilities were re-run on the films prepared as part of a statistically designed series.

Pursuant to Mr. Lawson's request for an approximate cohesive energy density calculation we have obtained a value using the equation $\delta^2 = (\Delta Hv - RT)/_v$ as reported in the Encyclopedia of Polymer Science and Technology, Volume 3, page 834. Predicated on water being the only solvent for gelatin we have $\Delta Hv = 539.5 \times 18.02$ cal/mol; $RT = 1.387 \times 373.16$ and $V = 22.4$ liters. Therefore, $\delta^2 = 400$ approx. and $\delta = 20.$ approx.

It became evident that the number of specimens would have to be reduced because of the limitations of the contract. Also, the supply of gelatin proved to be inadequate. Therefore, a new supply of gelatin sufficient for the contract was obtained. Our Division of Statistics and Experimental Design recommended a design for the production of 165 films from 5 varieties of gelatin, with 5 plasticizers at 3 levels and 3 thicknesses of film. The design was coded as follows:

Plasticizers

1 - Ethylene glycol (green)
2 - Polyethylene glycol (red)
3 - Dimethyl sulfoxide (yellow)
4 - N-Methylpyrrolidone (purple)
5 - Glycerol (no color)

Gelatin

1 - Gelatin
2 - Deaminated gelatin
3 - Carbamidated gelatin
4 - Cyanamidated gelatin
5 - Phenol-formaldehyde condensate and gelatin.

Thickness

1 - 1 mil.
3 - 3 mil.
5 - 5 mil.

Levels

0 - 0%
1 - 2%
2 - 5%

The above variables indicated 225 trials but these were reduced of 165 because one of the levels of plasticizers was zero and one determination at this level could be used for all plasticizers. The samples were randomized so as to yield additional information about the experimental error.

FILM PREPARATION

The preferred method of casting a film was as follows: The required amount of gelatin or frozen gel in the case of deaminated or carbamidated gelatin was combined with 1% dry ox bile, the required amount of aqueous plasticizer and enough water to total 80cc of water for 1 mil films, 90 ml of water for 3 mil films and 100 ml of water for 5 mil films. The mixture was melted in 130-140°F water bath and held long enough to be free of bubbles before pouring. The cyanamidated and phenol-formaldehyde resin treated samples had the required amount of additive added at this point and were poured on glass. Samples which could be expected to release poorly were poured on Mylar film cemented to glass plates. Generally, these were the cyanamidated and phenol-formaldehyde resin-gelatin preparations.

Generally, the best films were obtained when the drying took place at a leisurely rate. In the case of relative humidity extremes, difficulties were encountered. Drying and release were poor in the case of high relative humidity. At low humidity, static attraction was so great that the films on snapping off the support would be strained and often would shatter because they were attracted so strongly that they could not adjust to the changing forces in the film. The films were stored flat in envelopes until required for testing. Samples of film were conditioned at 73°F and 50% relative humidity for at least 18 hours before being tested for helium transmission and tensile strength.

Gas transmissions were determined in accordance with ASTM Method D 1434-58 using a Dow Gas Transmission Cell manufactured by Custom Scientific Instruments, Inc.

Gas transmission rate in the method is determined from measurements of the pressure and volume change of the gas transmitted through the specimen from a chamber of gas at a specified temperature and at a static and essentially constant pressure and into an initially evacuated, calibrated manometer. The gas transmission rate is the steady state volume of test gas, converted to standard temperature and pressure which passes through a known area of speciman per unit of time. Steady State is defined as that state of test conditions at which the volume of test gas transmitted becomes linear with time.

The Gas Transmission Cell was operated using the adapter designated by manufacturer for the determination of slow or low range transmission rates. The gas used was Grade A Helium manufactured by the Dept. of Interior, Bureau of Mines. The average pressure difference between opposite sides of the cell was 750 mm mercury and the manometer deflection was observed through a 50 mm mercury change. The test area of the specimen was 10./sq. cm. The tests were conducted at 73°F and 50% RH.

The rates were reported in cubic centimeters per square inch per 24 hours corrected to standard temperature and pressure.

The Tensile Breaking Strengths were determined in accordance with ASTM Method D 828-60 using a Schaffer Tensile Apparatus manufactured by Testing Machine Inc., of New York.

The test specimens were cut to 1 inch width and the test conducted with a 2" initial gap between the jaws at a speed of 1 foot per minute. The tests being conducted at 73°F and 50% RH.

A portion of the evaluation was complicated by a change in the character of the films themselves arising from a change in the environment. All of these films were prepared by casting onto a glass surface and permitting the film to dry at ambient temperature and humidity. As the Fall season advanced, the relative humidity in the laboratory underwent a continuous change to lower values. The rate of film drying was speeded up correspondingly resulting in uneven drying. This in turn produced strains in the film at the point where the dry film joined the wet film resulting in the formation of ridges or waves on the film surface. These ridges then made the formation of a gas tight seal in the gas transmission cell quite impossible. About thirty-one specimens were repeated and dried in the test room at 50% relative humidity. The majority of these replicates formed satisfactory films under these conditions and those that did not were repeated and dried in a cooler at 35°F. All but two yielded usable films.

The complete set of results is given below using symbols to prevent cluttering of the table. The code used for the different variables is given below:

Plasticizers

1 - Ethylene glycol.
2 - Polyethylene glycol.
3 - Dimethyl sulfoxide.
4 - N-Methylpyrrolidone.
5 - Glycerol.

Gelatins

1 - Swift's Superclear gelatin.
2 - Deaminated gelatin.
3 - Carbamidated gelatin.
4 - Cyanamidated gelatin.
5 - Phenol-formaldehyde condensate and gelatin.

Thicknesses

1 - 1 mil.
3 - 3 mil.
5 - 5 mil.

Levels

0 - 0% basis gelatin.
1 - 2% " "
2 - 5% " "

GELATIN	PLASTICIZER	LEVEL	THICKNESS	HELIUM TRANSMISSION cc/100 in.^{2}/ 24 hrs./atm.	TENSILE STRENGTH POUNDS PER SQ. INCH.
1	1	0	1	13.06	7845
2	1	0	1	27.87	3410
3	1	0	1	16.1	7490
4	1	0	1	13.4	6630
5	1	0	1	8.8	7920
1	1	1	1	13.81	6185
2	1	1	1	17.2	6870
3	1	1	1	15.9	6960
4	1	1	1	15.9	6660
5	1	1	1	11.7	6720
1	2	1	1	20.92	7040
2	2	1	1	26.09	7960
3	2	1	1	8.5	10410
4	2	1	1	22.3	6150
5	2	1	1	15.42	5400
1	3	1	1	15.1	4560
2	3	1	1	16.9	5870

GELATIN	PLASTICIZER	LEVEL	THICKNESS	HELIUM TRANSMISSION cc/100 in.^{2}/ 24 hrs./atm.	TENSILE STRENGTH POUNDS PER SQ. INCH.
3	3	1	1	16.75	6750
4	3	1	1	12.6	5370
5	3	1	1	1.98	7220
1	4	1	1	12.6	4340
2	4	1	1	15.57	6490
3	4	1	1	26.17	2975
4	4	1	1	11.96	6500
5	4	1	1	15.0	7080
1	5	1	1	14.88	6270
2	5	1	1	15.04	6390
3	5	1	1	16.31	9940
4	5	1	1	10.9	5490
5	5	1	1	12.7	5400
1	1	2	1	21.18	7000
2	1	2	1	31.3	6000
3	1	2	1	16.38	5780
4	1	2	1	11.7	6640
5	1	2	1	13.4	6330
1	2	2	1	not available	
2	2	2	1	18.58	7220
3	2	2	1	18.6	6950
4	2	2	1	13.4	6370
5	2	2	1	19.82	6530
1	3	2	1	9.3	6700
2	3	2	1	24	5650
3	3	2	1	34.4	5260

GELATIN	PLASTICIZER	LEVEL	THICKNESS	HELIUM TRANSMISSION cc/100 in.^{2}/ 24 hrs./atm.	TENSILE STRENGTH POUNDS PER SQ. INCH.
4	3	2	1	14.2	6780
5	3	2	1	13	8020
1	4	2	1	13.2	8200
2	4	2	1	25.75	6820
3	4	2	1	20.94	7190
4	4	2	1	15.42	6580
5	4	2	1	13.3	6630
1	5	2	1	10.1	4880
2	5	2	1	20.9	6140
3	5	2	1	15.1	4130
4	5	2	1	9.9	8330
5	5	2	1	6.7	3540
1	1	0	3	4.6	13130
2	1	0	3	13.2	6850
3	1	0	3	3.14	12330
4	1	0	3	5.51	11490
5	1	0	3	5.2	11900
1	1	1	3	4.2	7800
2	1	1	3	8.1	13890
3	1	1	3	7.4	9080
4	1	1	3	3.64	11850
5	1	1	3	12.1	10340
1	2	1	3	4.41	11670
2	2	1	3	6.08	13030
3	2	1	3	4.71	14830
4	2	1	3	10.46	12380
5	2	1	3	5.13	11430

GELATIN	PLASTICIZER	LEVEL	THICKNESS	HELIUM TRANSMISSION cc/100 in.^{2}/ 24 hrs./atm.	TENSILE STRENGTH POUNDS PER SQ. INCH.	
1	3	1	3	15.1	6040	
2	3	1	3	11.41	6450	
3	3	1	3	5.39	12370	
4	3	1	3	36.6	14450	Pinholes suspected
5	3	1	3	3.11	11590	
1	4	1	3	9.9	8330	
2	4	1	3	5.7	12140	
3	4	1	3	6.32	12100	
4	4	1	3	2.4	11550	
5	4	1	3	3.3	9340	
1	5	1	3	2.68	11930	
2	5	1	3	7.5	9800	
3	5	1	3	3.5	12340	
4	5	1	3	3.94	12000	
5	5	1	3	2.64	17420	
1	1	2	3	3.3	9821	
2	1	2	3	6.6	9700	
3	1	2	3	6.65	10460	
4	1	2	3	4.96	11680	
5	1	2	3	9.4	10290	
1	2	2	3	7.69	11450	
2	2	2	3	5.7	11370	
3	2	2	3	8.8	12950	
4	2	2	3	4.92	12890	
5	2	2	3	5.02	13490	
1	3	2	3	4.5	6470	

GELATIN	PLASTICIZER	LEVEL	THICKNESS	HELIUM TRANSMISSION cc/100 in.^{2}/ 24 hrs./atm.	TENSILE STRENGTH POUNDS PER SQ. INCH.	
2	3	2	3	7.85	7110	
3	3	2	3	4.3	12630	
4	3	2	3	5.23	8370	
5	3	2	3	not available		
1	4	2	3	3.3	12100	
2	4	2	3	10.1	7230	
3	4	2	3	73.7	12600	Flyer-N.G.
4	4	2	3	4.3	11580	
5	4	2	3	4.71	11460	
1	5	2	3	3.94	11740	
2	5	2	3	2.34	6410	
3	5	2	3	4.54	11870	
4	5	2	3	3.05	7680	
5	5	2	3	2.54	10880	
1	1	0	5	2.73	16610	
2	1	0	5	4.23	12930	
3	1	0	5	4.28	14640	
4	1	0	5	3.8	8780	
5	1	0	5	2.44	15600	
1	1	1	5	2.58	12800	
2	1	1	5	5.4	12620	
3	1	1	5	7.91	7590	
4	1	1	5	2.3	13950	
5	1	1	5	2.1	10200	
1	2	1	5	2.26	14710	
2	2	1	5	4.78	13030	
3	2	1	5	not available		

GELATIN	PLASTICIZER	LEVEL	THICKNESS	HELIUM TRANSMISSION cc/100 in.^{2}/ 24 hrs./atm.	TENSILE STRENGTH POUNDS PER SQ. INCH.
4	2	1	5	8.17	16380
5	2	1	5	7.1	11770
1	3	1	5	2.59	14960
2	3	1	5	3.38	12050
3	3	1	5	3.7	13320
4	3	1	5	3.3	11700
5	3	1	5	3.14	8230
1	4	1	5	2.9	12030
2	4	1	5	2.86	14090
3	4	1	5	3.85	15070
4	4	1	5	2.51	11410
5	4	1	5	2.64	16550
1	5	1	5	3.16	9730
2	5	1	5	3.8	10720
3	5	1	5	3.81	8390
4	5	1	5	2.23	11530
5	5	1	5	1.82	15420
1	1	2	5	1.8	11450
2	1	2	5	2.79	15420
3	1	2	5	4.28	12620
4	1	2	5	1.97	16180
5	1	2	5	2.46	8500
1	2	2	5	3.38	11610
2	2	2	5	3.1	14460
3	2	2	5	2.8	9350
4	2	2	5	3.8	10890
5	2	2	5	3.1	12590

GELATIN	PLASTICIZER	LEVEL	THICKNESS	HELIUM TRANSMISSION cc/100 in.^{2}/ 24 hrs./atm.	TENSILE STRENGTH POUNDS PER SQ. INCH.
1	3	2	5	4.59	9170
2	3	2	5	3.68	11530
3	3	2	5	3.22	12110
4	3	2	5	2.7	9600
5	3	2	5	3.17	9990
1	4	2	5	3.3	12670
2	4	2	5	9.8	14700
3	4	2	5	2.8	15000
4	4	2	5	4.2	5670
5	4	2	5	2.5	11020
1	5	2	5	1.6	14260
2	5	2	5	5.81	8980
3	5	2	5	3.3	9400
4	5	2	5	3.73	6110
5	5	2	5	2.7	11250

The analysis of the data supplied by our Experimental Statistics Division is made part of this report and is attached below:

One hundred fifty points were taken from the data available to get estimations of effects of gelatins, plasticizers, levels of plasticizers and film thicknesses on gas transmission. The points analyzed did not take into account the zero level of plasticizers.

Analysis of variance showed most main effects as significant and no interactions (only 2 way interactions were examined) as significant.

The mean gas transmission values are given for the main effects which were significant.

I. Film Thickness

Thickness	Average
1 mil.	16.07 cc.
3 mil.	5.89 cc.
5 mil.	3.70 cc.

The mean values all differ significantly from each other. Taking 1 mil and 16.07 cc's as a base value, and assuming transmission is inversely related to thickness, the expected values for 3 mil would be 5.36 (as opposed to 5.89) and for 5 mil 3.21 (as opposed to 3.70). The data thus substantiates the inverse relationship fairly well.

II. Plasticizers

Type	Average
1. Ethylene glycol	8.95 cc
2. Polyethylene glycol	9.46 cc
3. Dimethyl sulfoxide	8.74 cc
4. N-Methyl pyrrolidone	8.84 cc
5. Glycerol	6.79 cc
6. Control (Zero Level)*	8.56 cc

*15 observation for mean rather than 45 as with 1, 2, 3, 4, 5.

The basic difference is that plasticized glycerol results in significantly lower gas transmission. All other plasticizers did not significantly differ from one another.

III. Gelatin

1.	Gelatin	7.64
2.	Deaminated gelatin	11.02
3.	Carbamidated gelatin	9.74
4.	Cyanamidated gelatin	7.43
5.	Phenol formaldehyde condensate and gelatin	6.95

At 95% confidence, the following differences are significant. #2 and 3 are significantly higher than #1, 4 and 5. There are no differences between 1, 4 or 5.

IV.

The level of plasticity did not significantly affect gas transmission. The mean for 2% was 8.491, for 5%, 8.621 with plasticizer at 0 level, the mean is 8.560.

In summary, the lower gas transmissions are associated with thick films, glycerol and either gelatin alone, cyanamidated gelatin or phenol-formaldehyde condensate and gelatin. The differences stated as significant are based on an error term estimated by the higher interaction. There were also four missing values which were estimated by a row x column mean value.'

The significantly lower gas transmission value for films containing glycerol when compared with the control without glycerol suggested that the presence of glycerol improves the resistance of the film to helium transmission.

The fact that the 5% level of glycerol was better than the 2% level indicated only that the higher level was better but provided no information as to the optimum level. The determination of this optimum level of glycerol will be taken up later. The similarity of the values found for the other four plasticizers when compared with zero level suggested that they are inert as far as influencing the gas transmission. This was further evidence that glycerol had a special place as a gelatin plasticizer.

On the basis of the information contained in the statistical analysis, the following selections were made:

A. In the area of the type of gelatin:
  1. Swift's Superclear Gelatin.
  2. Cyanamidated gelatin.
  3. Phenol-formaldehyde condensate and gelatin.

B. In the area of plasticizers:
  1. Glycerol - 5%
  2. None

C. In the area of film thickness:
  1. Nominally one mil.

Summarized this means that we had six variations to be evaluated with the selected crosslinking agents.

CROSSLINKING AGENTS

The need for a suitable method for introducing known amounts of crosslinking agents into a film restricted us to the addition of known amounts of the crosslinking agent in liquid or solid form. For example, treatment of films with gaseous formaldehyde would have been too inexact because there would have been no adequate control over the actual amount of crosslinking agent picked up by the film.

The initial object of this search for a way to crosslink our films was to determine the amounts of crosslinking agent that could be added to a formulated gelatin solution without causing it to gel and at the same time form a dry film that would be hot water insoluble. It was assumed that the minimum amount of crosslinking required to do this would also bring about a significant change in the gas transmission of the film.

In order to prevent premature gelation, all crosslinking agents were added as 1.0% solutions. The basic gelatin formulation used was:

14 gms Swift's Superclear Gelatin
140 mgs. ox bile.
25 ml. of 2.8% Glycerol.
75 ml. water

The chosen amount of formaldehyde was added to each melted sample, mixed and cast onto a glass plate. After drying the films were judged by their behavior in hot water. The results so obtained are summarized in the table below:

No.	ml. of 1.0% Formaldehyde	% Formaldehyde basis Gelatin	Behavior in Hot Water
1.	1.4 ml	0.1	dissolved
2.	4.2 ml	0.3	soluble but less readily
3.	5.6 ml	0.4	insoluble
4.	7.0 ml	0.5	insoluble
5.	10.0 ml	0.75	insoluble

The above results were obtained with concentrations of gelatin used to produce films nominally five mils thick. Formulation number four was chosen as being most applicable to all film thicknesses.

GLYOXAL AS A CROSSLINKING AGENT

Glyoxal levels and concentrations were chosen in a similar manner as shown in the table below:

No.	ml. of 1% Glyoxal	% Glyoxal basis Gelatin	Solubility in hot water	Film at 72°C for 1 hr.- Sol. in hot water
1.	1.4	0.1	soluble	
2.	5.0	0.36	soluble	
3.	10.0	0.72	soluble	insoluble
4.	15.0	1.07	soluble	insoluble

The less reactive nature of the glyoxal could be explained by its pronounced tendency to hydrate and to form acetals with the hydrated forms. Since this reaction is reversed by heat, the crosslinking after one hour at 72°C brought about the required water insolubility. The level of glyoxal selected was the 0.72% level basis gelatin.

GLUTARALDEHYDE

The optimum level of glutaraldehyde was chosen from the following series:

No.	ml. of 1% Glutaraldehyde	% Glutaraldehyde basis Gelatin	Solubility in hot water
1	1.4	0.1	Soluble
2	5.0	0.36	Partially soluble
3	7.5	0.54	Insoluble
4	10.0	0.71	Insoluble
5	15.0	1.07	Insoluble

Allowing for some margin of safety we elected to use the 0.7% level basis gelatin.

OTHER CROSSLINKING AGENTS

The use of hexamethylene tetramine was tried because of the slow rate with which it reacts with proteins. However, none of its reaction products with gelatin were insoluble and because it offered no advantages, no further effort was made to use it.

The initial set of eighteen crosslinked unsupported gelatin films had to be repeated because the films could not be removed from the plate glass without destruction. A second set of films was cast onto Mylar films laminated to plate glass using a pressure sensitive adhesive. The material used was obtained from the Universal Coating Company, 2320 E. 26th Street, Minneapolis 6, Minn.

The range of conditions of these eighteen films included three types of gelatin, namely, Swift's Superclear gelatin, carbamidated gelatin and phenol formaldehyde condensate-gelatin. The glycerol plasticizer was held at two levels, namely, 0% and 5% basis gelatin. Finally, all samples were crosslinked with one of three crosslinking agents; formaldehyde, glyoxal and glutaraldehyde.

The experimental conditions for preparing these films were so similar that the procedure for a gelatin film plasticized with 5% glycerol and crosslinked with formaldehyde could serve as a model for all of them.

2.8 Grams of Swift's Superclear gelatin, 28 mg. of powdered ox bile, 80 ml. of distilled water and 5 ml. of 2.8% aqueous glycerol were mixed and allowed to swell for one hour. The swelled gelatin was melted at 50°C in a water bath while covered with a watch glass. After melting, complete mixing by careful stirring was apparent by the absence of schlieren. Immediately prior to casting the film, 1.4 ml of 1% aqueous formaldehyde was added and gently stirred in. The resulting clear, bubble free solution was poured onto the casting surface and allowed to dry spontaneously without any artificial air movement. Twenty four hours later the film was peeled off, conditioned at least eighteen hours at 73°F and 50% relative humidity and evaluated for helium transmission and tensile strength.

The following table summarizes the compositions and the test results:

NO.	GELATIN GRAMS	OX BILE mg.	WATER	ml. of 2.8% GLYCEROL	ml. of 50% HYDROGEN CYANAMIDE	ml. of 32.8% PHENOL FORMALDEHYDE CONDENSATE	ml. of 1% FORMALDEHYDE	ml. of 1% GLYOXAL	ml. of 1% GLUTARALDEHYDE	HELIUM TRANSMISSION cc/100in^2/24 hrs/atm.	TENSILE STRENGTH lbs/in^2
1	2.8	.28	80	5			1.4			10.07	5100
2	2.8	.28	85	-			1.4			21.54	4560
3	2.8	.28	80	5	0.2		1.4			11.08	4570
4	2.8	.28	85	-	0.2		1.4			Holes	5695
5	2.8	.28	80	5		.06	1.4			11.3	4360
6	2.8	.28	85	-		.06	1.4			12.97	5530
7	2.8	.28	80	5				2		9.46	4930
8	2.8	.28	85	-				2		12.51	4540
9	2.8	.28	80	5	0.2			2		10.89	4300
10	2.8	.28	85	-	0.2			2		35.2	5200
11	2.8	.28	80	5		.06		2		9.5	4095
12	2.8	.28	85	-		.06		2		Holes	6010
13	2.8	28	80	5					2	10.7	3710
14	2.8	28	85	-					2	24.9	2296
15	2.8	28	80	5	0.2				2	13.8	3670
16	2.8	28	85	-	0.2				2	19.17	5180
17	2.8	28	80	5		.06			2	13.82	3630
18	2.8	28	80	-		.06			2	22.16	5820

The analysis of data revealed that crosslinking had no significant effect on the helium transmission. However, the level of glycerol had a very strong positive effect on the impermeability of the film to helium. The tensile strength was much less effected by the various changes in the film formulations.

OPTIMUM GLYCEROL LEVEL

The indisputably lower helium transmission of the 5% glycerol samples suggested that there might be a still more effective level of glycerol. In order to find out if this was indeed so, the following screening test was performed:

NO.	GELATIN GMS.	OX BILE mg.	% GLYCEROL BASIS GELATIN	WATER cc	He TRANS-MISSION	TENSILE STRENGTH
1	2.8	28	5	90	15.84	7620
2	2.8	28	8	87	17.06	5720
3	2.8	28	11	84	13.3	4090
4	2.8	28	14	81	9.84	5560
5	2.8	28	17	78	8.05	6230
6	2.8	28	20	75	28.8*	4990
7	2.8	28	23	72	11.4	5950
8	2.8	28	26	69	9.8	5510
9	2.8	28	29	66	10.07	4780

* - Suspected outlier

The results of the screening test suggested that the most useful glycerol content probably lay between 8 and 18% glycerol basis the gelatin. The following series was set up in order to determine this value for each type of gelatin.

SWIFT'S SUPERCLEAR GELATIN

% GLYCEROL	GELATIN GMS.	OX BILE mg.	2.8% GLYCEROL cc	WATER ml.	HELIUM TRANS-MISSION cc/100in.^{2}/ 24 hrs/atm.	TENSILE STRENGTH lbs/in.^{2}
8	2.8	28	8	90	17.73	4340
10	2.8	28	10	90	15.53	5950
12	2.8	28	12	90	14.25	4330
14	2.8	28	14	90	13.85	4400
16	2.8	28	16	90	11.46	6780
18	2.8	28	18	90	12.12	4020

CYANAMIDATED GELATIN

8	2.8	28	8	90	15.28	6020
10	2.8	28	10	90	13.12	5020
12	2.8	28	12	90	17.73	5560
14	2.8	28	14	90	12.32	4660
16	2.8	28	16	90	16.15	4860
18	2.8	28	18	90	21.54	5520

PHENOL FORMALDEHYDE CONDENSATE - GELATIN

8	2.8	28	8	90	brittle	
10	2.8	28	10	90	"	
12	2.8	28	12	90	11.75	5550
14	2.8	28	14	90	19.50	5800
16	2.8	28	16	90	10.80	4280
18	2.8	28	18	90	15.4	4750

Inspection of the data indicated that there were no substantial differences between gelatins and that unmodified gelatin yielded the most consistent results. In the handling of these films, it was noticed that the films made from modified gelatins tended to break more easily and tended to invite damage more rapidly. For these reasons we have decided to restrict further experimentation to Swift's Superclear gelatin alone with a plasticizer content of 16 percent.

REENFORCING FIBERS

a. Glass Fibers.

The use of chopped glass fibers as reenforcing agents for gelatin films was abandoned because it was found impossible to mat the individual fibers into the film. The probable explanation is that the solvent had no effect on the fibers and consequently these were too stiff to form a mat.

b. Cattle Hair.

The use of cattle hair appeared to be impractical because films made with it behaved as if they were full of pin holes. Presumably, the gas is able to travel along or through the hair shaft and thus pass through the film. In addition, the relatively large size of the fibers when compared to the film thickness made it unlikely that a satisfactory combination could be found.

c. Collagen.

The use of reconstituted collagen from dry collagen fibers failed to yield lump free films. In connection with other work we have made films using ratios of gelatin to collagen comprising 2 to 1, 1 to 1 and 1 to 2 using fresh collagen fibers.

The method of preparing films from this form of collagen was as follows: The gelatin was made up as a 2 1/2% aqueous solution after swelling and melting at 45°C. After cooling to 30-35°C, the pH was adjusted to 2.6 with lactic acid. The gelatin solution was then blended with the required amount hydrated collagen dispersed in the necessary amount of water. The blending was done in a Hobart dough mixer and forced through a filter screen. Deaeration was effected in a vacuum chamber without gelation difficulties. The mass was cast into non-oriented films on Mylar coated plates and allowed to dry in a stream of unheated air. Films of this type exhibited tensile strengths of 12000 ± 2000 psi which was comparable to gelatin alone, however nothing was known of their behavior regarding gas transmission or elastic properties at cryogenic temperatures. In order to develop such information we proposed to make films by the above described method limiting ourselves to a ratio of two parts of gelatin to one part of collagen. The excessively high viscosity of the mixture caused us to revise the proportions of collagen to gelatin to a one to one ratio.

Gelatin-Collagen Ratio, 1 to 1

150 gms of frozen collagen and 850 of distilled water were slurried in a Hobart mixer at 21°C. 41.5 gms of gelatin (containing 37.5 gm of anhydrous gelatin) were swelled and dissolved in 1500 gms of distilled water. After melting and cooling to 35°C, the pH was adjusted to 3 with 85% lactic acid (app. 7 gms. required). An additional 12.4 gms was added and then diluted to a net of 2000 gms. for a final pH of 2.6. After cooling to 30°C the gelatin solution was added to the collagen slurry with stirring over a fifteen minute period, mixed twenty minutes longer and filtered through a stainless steel screen to remove any lumps. The filtrate was deaerated in a vacuum chamber at 30 inches of vacuum. The final slurry was then used to cast films of varying thickness on Mylar surfaces.

COLLAGEN - GELATIN FILMS 1 : 1

FILM KNIFE SETTING	FILM THICKNESS Mils.	He TRANSMISSION cc/100in.^{2}/24 hrs./atm.	TENSILE STRENGTH lbs./sq. in.
0.025"		porous	-
.032"	-	porous	-
.040"	1.65	25.84	2710
.050"	2.4	26.24	2550
.060"	2.3	88.6	3512
0.075"	2.95	17.8	3142
0.100"	3.55	14.2	5120
0.150"	4.48	8.09	6060
0.200"	4.78	6.05	6690

A second set of films was prepared using an improved process for the preparation of the collagen in which we used 65% isopropyl alcohol to dewater the fibers. The mixing of the gelatin and collagen was performed more slowly resulting in better collagen dispersion in the mix. The amount of collagen was decreased to a collagen-gelatin ratio of 1 to 1.25.

100 gms of dewatered collagen fibers (containing 30% solids) and 900 gms of distilled water were slurried and cooled to 20°C. 41 gms of gelatin were swelled in 1500 gms of water, melted, cooled to 35°C and 7 gms of 85% lactic acid were added. 12.4 gms more of 85% lactic acid were added and the mixture diluted to 2000 gms. This gelatin solution was added dropwise to the collagen slurry using a dropping funnel over an hour's period of time. The addition was particularly slow at the beginning so that the paddle had a maximum opportunity to work the doughy collagen. This working resulted in a more effective dispersion of the collagen as the addition proceeded. The final mix was filtered through a stainless steel screen, deaerated in a vacuum chamber and cast into films using a #361 Gardner knife.

COLLAGEN - GELATIN FILMS 1 TO 1.25

KNIFE SETTING	FILM THICKNESS IN MILS.	He TRANSMISSION cc/100in.^{2}/24 hrs./atm.	TENSILE STRENGTH lbs./sq. in.
0.050"	1.57	39.89	1735
0.060"		Porous	
0.070"		Porous	
0.080"	2.47	21.1	2724
0.090"	2.64	15.1	3937
0.100"	2.98	17.18	3380

Inspection of the data showed that the use of collagen appeared to impart no beneficial characteristics to a gelatin film. While the values are somewhat erratic, their average indicated no superior qualities either in helium impermeability or in tensile strength. Consequently, we have eliminated collagen fiber reinforced films from the program.

d. Cellulose Fibers.

The use of cellulose fibers appeared to offer the best chance of success. Dispersion of cellulose fibers in a gelatin solution prior to casting has usually resulted in an uneven distribution of the fibers in the film. A more workable approach has been the use of a preformed layer of fibers and applying this to the gelatin solution to yield a film containing cellulose reenforcing fibers. The material best suited for this application has been lens paper. It is very thin, has long fibers and is free of fillers. This paper has a definite fiber direction, but this appeared to have little effect upon the film properties. It was easier to keep flat if the paper direction was maintained perpendicular to the long direction of the film. Also, a slight tension on the paper while in the gelatin solution yielded better specimens. A pull of not more than 33 grams per centimeter of paper edge seemed to be adequate. Thus our films of 15 cm. width were subjected to a 500 gm. pull uniformly distributed along the edge. It was discovered early that if dry portions of the cellulose sheet projected beyond the gelatin solution, there would be wrinkles due to the stretching of the wet sheet. The best results were obtained when the cellulose sheet was cut smaller than the gelatin film. However, in order to permit handling, the sheet was made larger than the film in the long direction. Applied to an actual example this meant that for a gelatin film 55 x 15 cm the cellulose sheet was 65 cm x 14 cm and was laid into the fluid gelatin. The cellulose sheets were cut with, across and on a bias with the direction of the cellulose fibers. The least amount of wrinkling occurred when the fibers were perpendicular to the long dimension of the film. Almost as good results were obtained with the fibers on a bias.

Evaluation of the films disclosed that only one of the three films had the necessary impermeability, namely, the film in which the cellulose fiber direction was perpendicular to the film length. Since the permeability is not likely to be directly related to the fiber direction, we must conclude that the incidence of successful film preparation with cellulose reenforcing fibers is low. The film itself was good having a helium transmission of 6.56 cc/100in.^{2}/24 hrs/atmosphere and the tensile strength was 8370 lbs/in.^{2}. These values compare favorably with the best values obtained using gelatin alone but because of the difficulties encountered during the preparations we elected to eliminate the cellulose fiber reenforced films from further consideration until a definite need for them is indicated.

ORIENTATION OF FILMS

Attempts were made at orientating the molecules in a gelatin film by heating three mil films containing five percent glycerol under tension. The specimens, 40 x 15 cm were stretched between two 500 gram loads and heated by infra-red lamps to 167-170°C. The films shrank rather than stretched, the amount of shrink being about 0.5% or 2 to 3 mm for the specimens being used. The lack of stretching probably meant that no appreciable amount of orientation had taken place.

FINAL SELECTION OF FILM FORMULATION

The film composition chosen as possessing the optimum properties had the following composition:

Gelatin	100 parts
Glycerol	16 parts
Ox Bile	1 part

A stock pile of twenty-four films, 55 x 30 cm was prepared using the above formula with the necessary amount of water.

Samples of these films after the usual conditioning treatment at 73°F and 50% relative humidity for a minimum of twenty-four hours, were evaluated for gas transmission and tensile strength with the following results:

UNTREATED CONTROL

GAS TRANSMISSION

Hydrogen	12.15	cc/100sq.in./24 hrs./atm.
Helium	23.08	" " " "
Oxygen	0.302	" " " "
Nitrogen	0.0258	" " " "
Carbon Dioxide	2.6	" " " "

TENSILE STRENGTH

5040 pounds/sq. in.

HEAT TREATED

Four samples of the film described above were heat treated in a forced draft oven at 101 ± 1°C for six days.

On evaluation for gas transmission and tensile strength the following results were obtained:

GAS TRANSMISSION

Hydrogen	4.66	cc/100sq.in./24 hrs./atm.
Helium	9.97	" " " "
Oxygen	0.0958	" " " "
Nitrogen	0.053	" " " "
Carbon Dioxide	0.616	" " " "

TENSILE STRENGTH

4705 pounds/sq. in.

ULTRA-VIOLET IRRADIATION

Two 55 x 30 cm films were exposed to two 100 watt mercury vapor lamps for a total time of thirteen days. After the irradiation, the films were conditioned at 50% relative humidity at 73°F. and evaluated for gas transmission and tensile strength.

GAS TRANSMISSION

Hydrogen	6.15	cc/100 sq.in/24 hrs./atm.
Helium	16.62	" " "
Oxygen	0.172	" " "
Nitrogen	0.032	" " "
Carbon Dioxide	1.57	" " "

TENSILE STRENGTH

3,790 lbs./sq. in.

The twelve samples of gelatin film were subjected to a -195.6°C. treatment with liquid nitrogen for two to three hours. After conditioning at 50% relative humidity and 73°F. for twenty four hours, the samples were evaluated with the following results:

GAS TRANSMISSION

Hydrogen	9.77	cc/100	sq.in./24	hrs./atm.
Helium	21.54	"	"	"
Oxygen	0.466	"	"	"
Nitrogen	0.051	"	"	"
Carbon Dioxide	5.52	"	"	"

TENSILE STRENGTH

3,620 lbs./sq. in.

II. MECHANICAL PROPERTIES OF GELATIN FILMS.

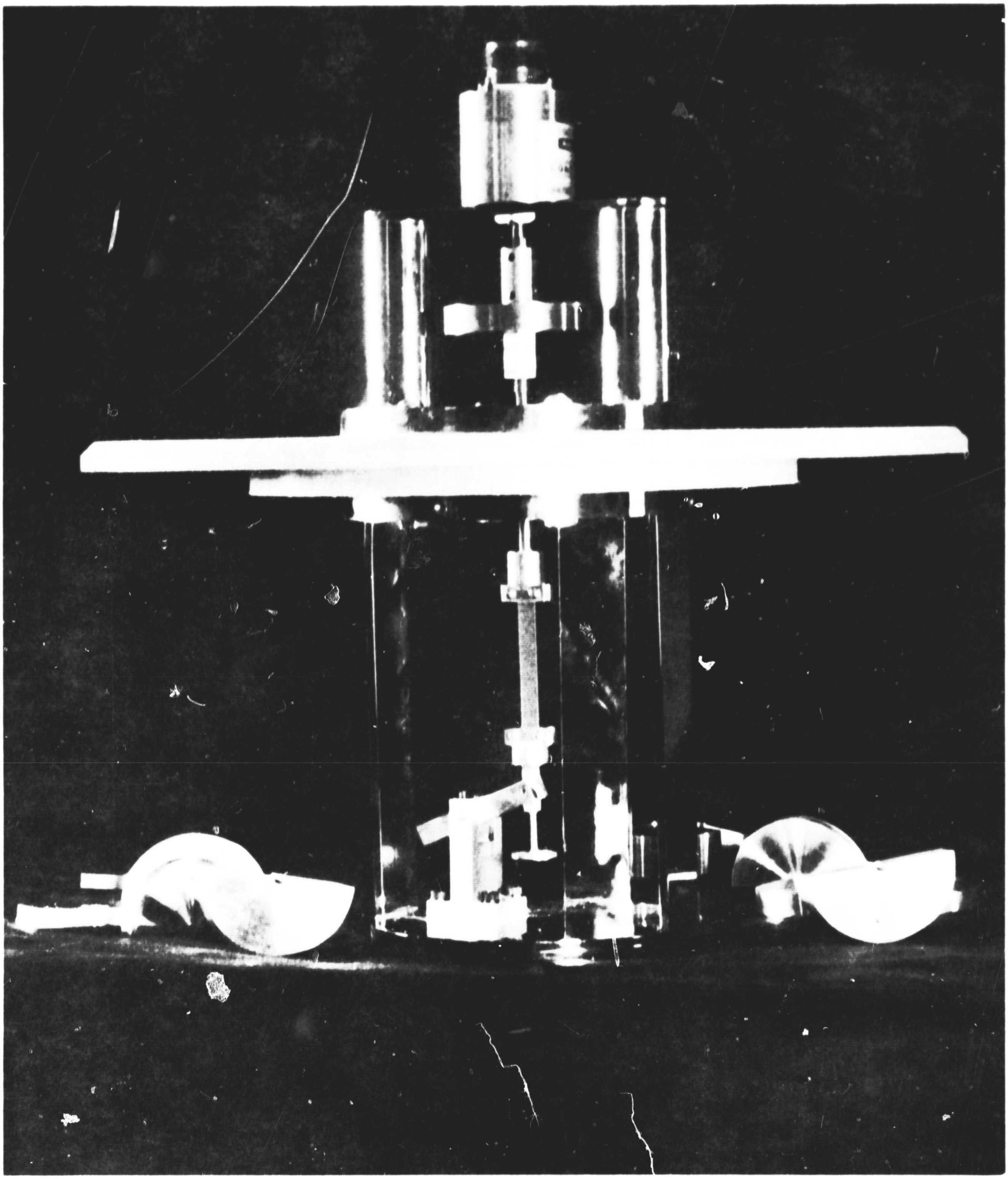
Because of the amazingly low permeability of gelatin films, it was deemed appropriate to investigate the mechanical properties of these films. Impetus for this work resulted in part from the observation that, on exposure to liquid helium, a previously flexible gelatin film passed into an apparently irreversible glassy state by virtue of which it shattered. Further preliminary investigations, at the Jet Propulsion Laboratory, indicated that gelatin films maintained energy absorbing properties at quite low temperatures. It was therefore proposed that a study be undertaken in an attempt to lower the temperature range in which sensible energy absorption was manifested in gelatin films, and to relate this to chemical changes in the gelatin molecules. It was suggested by the project monitor, Mr. D. D. Lawson, that this energy absorption might best be followed by measuring dynamic mechanical properties with a torsion pendulum.

It was originally intended to construct a torsion pendulum such as described by Koppelman (Kolloid Z., 144, 22 (1955)) for these studies. However, investigation of about 3 commercially available assemblies (Askania, Plas-Tech, and Metavac-Lenscote), plus consultation with E. F. Cuddihy, of the Jet Propulstion Laboratory, resulted in the purchase of an assembly consisting of the following components:

1 - Direct Recording Torsional Pendulum, Item #100,628 Master Test Unit, including Test Stand, Transducer, Inertial Discs, and Environmental Housing, 345,000, from Plas-Tech Equipment Corp., Natick, Mass. This apparatus is pictured in Fig. 2.

2 - #500-603-1866-6-002-001-145-146 Speedomax Type N Recorder using chart paper #491,866 (-200 to + 150°C) from Leeds and Northrup Co., Park Ridge, Ill.

FIG. 2: TEST FRAME FOR DIRECT RECORDING TORSIONAL PENDULUM



Acquisition and reduction of the data required the following government property received from the Jet Propulsion Laboratory on a loan basis:

1.	Oscilloscope, Tektronix 532	J-270-F-47436
2.	Type H. Plug-in	LA-61817
3.	Hewlitt-Packard Oscillator	J-270F-2883
4.	Voltmeter H H.P. 400D	J-270F-8243
5.	Camera, C-12	J-270F-14369

This equipment was assembled and checked out with the aid of Mr. D. D. Lawson. It was then applied to the study of the dynamic mechanical properties of gelatin films.

Throughout the work to be described here, it was intended that the variation with temperature, of the elastic and inelastic components of the complex shear modulus be determined with the torsion pendulum. This was done using the procedures of ASTM Tentative Method D 2236-64T. Here the film sample is used as the energy absorbing element of the torsion pendulum. In the evaluation of each sample, the largest appropriate inertial disc was used, in order that the pendulum assembly might have as long a period as possible. For most of the specimens investigated in this work, this was the most massive inertial disc provided with the pendulum, and had a moment of inertia of 882 g-cm^2.

With the Plas Tech pendulum, the magnitude of the torsional oscillations is followed by the output of a rotary variable differential transformer. This output is displayed as the vertical component on an oscilloscope, with time being the horizontal component. The pattern formed by a series of oscillations of the pendulum assembly on the oscilloscope is photographed. From such photographs, the amplitudes of several successive oscillations are measured as are the periods. The data so accumulated are processed electronically, using the appropriate parameters of sample specimen and inertial disc, to give the elastic and inelastic components of the complex shear modulus. These components of shear modulus are then plotted versus temperature. A plot of the inelastic component is used to estimate transition temperatures from the positions of peaks. An example of these plots is given in Fig. 3.

All the samples to be reported here have shown maxima in the temperature region obtainable with liquid nitrogen. We have taken the temperature at which the maxima occur as the glass transition temperature, Tg, and are so reporting throughout this report. It may in fact, however, not be the glass transition temperature, since that is reported by Yannas and Tobolsky (J. Macromol. Chem., 1, 723 (1966), to be about 190°C for anhydrous, non-crosslinked gelatin, a material which cannot be prepared experimentally, due to the cross-linking of gelatin which occurs with a high degree of dehydration. This has recently been observed by Yannas and Tobolsky (Nature, 215, 509 (1967), in an apparent re-confirmation of the insolubilization, which occurs on heating gelatin at 130°C, believed by Hofmeister (Z. Physiol. Chim., 2, 299 (1878), to represent the reconversion of gelatin to collagen. The maximum observed by us seems to be the same mechanical loss peak observed by Koleske and Faucher (J. Phys. Chem., 69, 4040 (1965). In spite of this, it will be cited throughout this report as a glass transition temperature, T_g.

Our initial efforts toward the goal of finding chemical methods for lowering this glass transition temperature consisted of the preparation of film samples in which as many chemical parameters as possible were varied for the purpose of detecting directions to pursue in order to obtain beneficial results.

With this objective in mind the following films were prepared and tested.

1. Gelatin
    - a. Gelatin unplasticized.
        - (1) Gelatin
        - (2) Gelatin crosslinked
    - b. Gelatin plasticized
        - (1) Gelatin and glycerol.
        - (2) Gelatin and ethylene glycol.
    - c. Gelatin plasticized and crosslinked.
        - (1) Gelatin, glycerol with formaldehyde.
        - (2) Gelatin, ethylene glycol with formaldehyde.

2. Deaminated gelatin.
    - a. Deaminated gelatin, unplasticized.
        - (1) Deaminated gelatin.
        - (2) Deaminated gelatin, crosslinked.
    - b. Deaminated gelatin, plasticized
        - (1) Deaminated gelatin and glycerol.
        - (2) Deaminated gelatin and ethylene glycol.
    - c. Deaminated gelatin, plasticized and crosslinked.
        - (1) Deaminated gelatin with glycerol, crosslinked.
        - (2) Deaminated gelatin with ethylene glycol crosslinked.

3. Cyanamidated gelatin.
    a. Cyanamidated gelatin, unplasticized
        (1) Cyanamidated gelatin.
        (2) Cyanamidated gelatin, crosslinked
    b. Cyanamidated gelatin, plasticized
        (1) Cyanamidated gelatin and glycerol
        (2) Cyanamidated gelatin and ethylene glycol
    c. Cyanamidated gelatin, plasticized and crosslinked
        (1) Cyanamidated gelatin and glycerol, crosslinked.
        (2) Cyanamidated gelatin and ethylene glycol, crosslinked.

THE PREPARATION OF FILMS

Before any samples of gelatin films for testing on a torsion pendulum could be prepared a method of producing such films had to be developed. The casting of such films using our standard formulation at higher concentration yielded films which tended to distort as they dried. Pouring these films so as to yield disks rather than rectangular sheets brought about an improvement by minimizing the internal strains as the films dried. The films, if removed from the glass plate too soon, tended to curl up as they finished drying. The shaping of test specimens from such films by various means was investigated. The dry films could not be cut either by knife or scissors. Sawing with circular saws, band saws, scroll saws, hand saws or coping saws was not successful. The extreme brittleness of the films resulted in too much chipping. A workable but impractical method was found based on the power sanding of the specimen to the specified shape. The shaping of specimens from oversized pieces by means of machine tools was considered and rejected as being impractical for the number of samples we needed to prepare.

After a review of all the results, it was decided that the method most likely to be successful was the casting procedure followed by shaping the specimen from the dry film. Gelatin films were cast on glass and removed before complete drying. Attempts at keeping these films flat by restraining them between filter paper backed up with wire gauzes failed because the surfaces of the films became marked from the pressure exerted to keep them flat. Attempts at drying the films on Mylar surfaces failed because the film released too soon and curled badly. The one workable method found was as follows: The films were cast from a 20% solids solution onto plate glass and allowed to dry until dry but not brittle. This stage was reached in twenty-four hours. The films were conditioned to pliability by holding them at 100% relative humidity in a sealed pressure cooker for 24 hours at room temperature. At this point the films, even those lacking any organic plasticizers, could be cut readily with scissors. The one cm. wide strips in duplicate, 15 cm. long were stretched between two paper clips using a rubber band for tension and dried over phosphorus pentoxide in a desiccator. The phosphorus pentoxide was renewed daily until it failed to form phosphoric acid. Usually a week was required. These specimens then were equilibrated in the desiccator until measured on the torsion pendulum.

GELATIN FILMS

In the preparation of the gelatin films the solutions were poured onto glass plates until the solution filled 20 cm. circles outlined with a wax pencil. These solutions had the following compositions:

SAMPLE NO.	GELATIN	WATER	OX BILE	GLYCEROL	ETHYLENE GLYCOL	.37% FORMALDEHYDE
4815-33A	100g	400g	1g	16g	-	-
4815-33B	100g	400g	1g	-	-	-
4815-33C	100g	300g	1g	-	-	100g
4815-34D	100g	300g	1g	16g	-	100g
4815-34E	100g	400g	1g	-	16g	-
4815-34F	100g	300g	1g	-	16g	100g

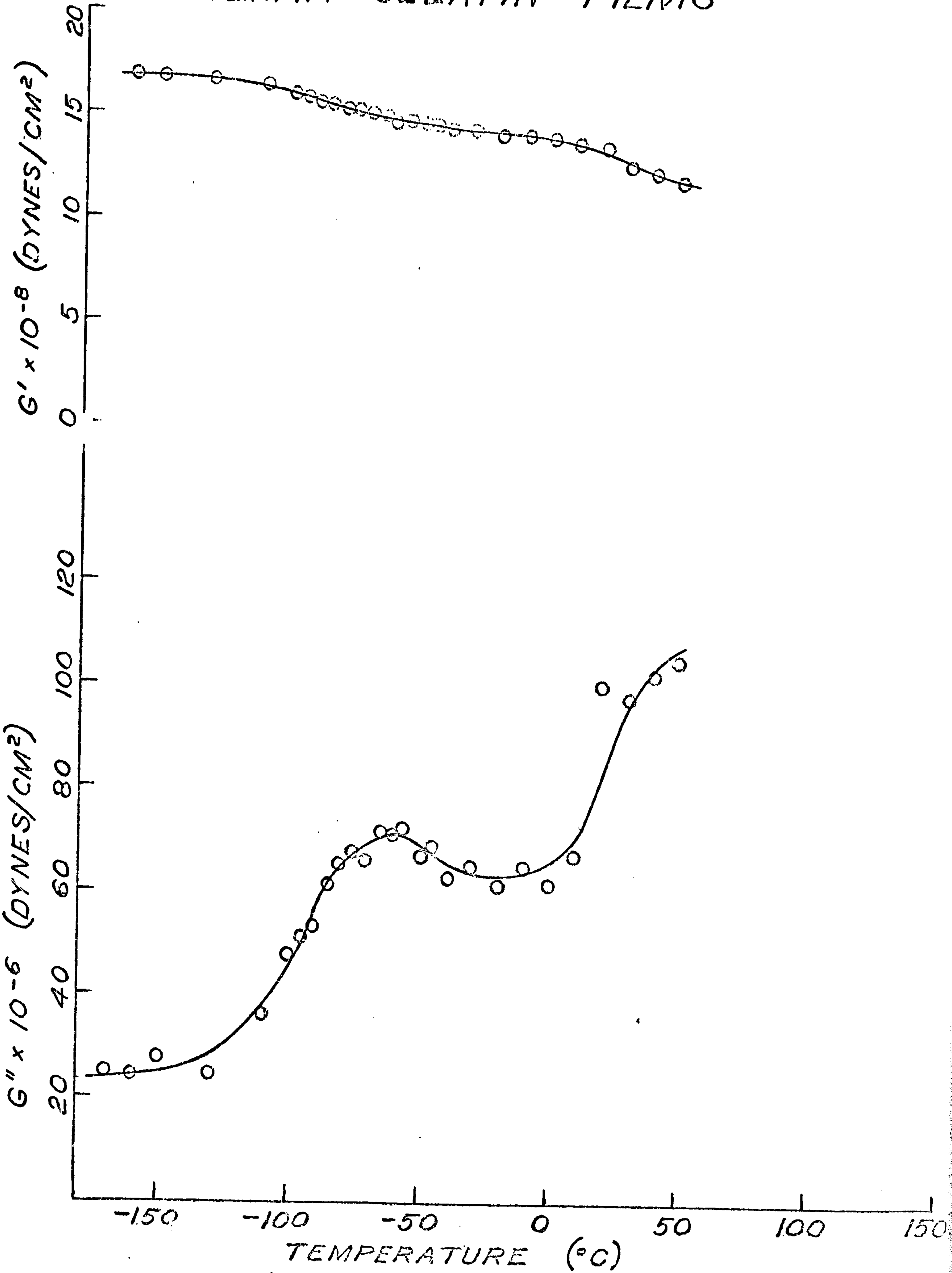
After conditioning at 100% relative humidity these films were cut to a width of 1.0 cm., and a length of approximately 15 cm. The final dry samples were evaluated on the torsion pendulum as described above.

Figure 3 gives a picture of the behavior of the elastic and elastic components of the complex shear modulus typical of the speciments. The elastic component G' is calculated using the equation:

$$G' = \frac{631.3\ LI}{CD^3\ \mu P^2}$$

as given by Nielsen (Mechanical Properties of Polymers, p. 141, Reinhold Publishing Corp., New York, 1962). Here

FIGURE 3
INELASTIC COMPONENTS OF THE COMPLEX SHEAR MODULUS OF SUPERCLEAR GELATIN FILMS



L = length of specimen between clamp in cm.

I = moment of inertia of assembly in gm-cm.^{2}

C = width of specimen in cm.

D = thickness of specimen in cm.

P = period in sec.

μ = shape factor.

The inelastic component G'' is computed from the elastic component G' using the equation:

$$G'' = \frac{\Delta}{\pi} G'$$

(Neilsen, op. cit., p. 150). Here:

Δ = logarithmic decrement, the logarithm of the ratio of the amplitudes of two successive deflections of the torsion pendulum.

All of the samples listed above showed similar performance in the torsion pendulum, and gave results typified by those presented in Fig. 1, that is, they showed a small region of damping near -70°C and a region of great damping above +100°C. In terms of Fig. 3, our objective is to decrease the temperature at which the small peak appears, and to obtain a plateau region between the two peaks. Because all of the above samples showed results not different from each other, this series of experiments failed to show any guidance toward obtaining this objective.

DEAMINATED GELATIN

Preliminary work with deaminated gelatin indicated that more dilute solutions such as 10% solids yielded more satisfactory films. These could be deaired more readily and spread with fewer striations. The following procedure was used successfully to prepare six different variations of deaminated gelatin.

Fifty grams of gelatin were swelled in 300 ml. of distilled water. After melting at 45°C and cooling to 40°C., one gram of sodium nitrite dissolved in 25 ml. of water was added. The nitrous acid was allowed to react for one hour before being neutralized by the addition of five ml. of concentrated ammonium hydroxide. After standing overnight, the solution was melted, skimmed and decolorized by the addition of a minimal amount of sodium hydrosulfite (Lycopon). The clear solution was cast onto a glass plate using an iron ring having an internal diameter of 19.4 cm. and an external diameter of 25 cm. and a thickness of 0.9 cm. as a retaining wall. The ring was ground flat on one side to make even contact with the plate glass surface and seal the joint. In use the ring was chilled in an ice bath, dried and placed onto the casting surface. The solution was poured into the space enclosed by the ring and after gelling the ring was removed. The disk of gelatin was dialyzed in a 35°F cooler in a mixture of ice and water for five days. Several plates were prepared in this fashion all of which were stacked horizontally with 4 cm. separators between the plates. Dialysis was carried out in a plastic vessel containing the ice and distilled water. After five days of dialysis, the disks of gelatin in which each disk of gelatin represented 50 gms. of gelatin solids were then used to make up the individual formulations. The calculations for these films are given below:

Inside diameter of ring = 19.4 cm.
radius = 9.7 cm.
Area ($r^2 \pi$) = 295 cm.^{2}
For a 1 mil film, .001 x 2.54 = .00254 cm.
Vol. of film (A x h)
$2.54 \times 10^{-2} \times 2.95 \times 10^{2} = 7.5 \times 10^{-1}$ cc
Density of gelatin = 1.33
$7.5 \times 10^{-1} \times 1.33$ = 1 gm. gelatin for 1 mil film.
and for 25 mil film = 25 gm. gelatin or 1/2 circle from dialysis operation.

COMPOSITION OF DEAMINATED GELATIN FILMS

LAB. NO.	GMS. OF DEAMINATED GELATIN AS A DIALYZED GEL	GLYCEROL	ETHYLENE GLYCOL	1% FORMALDEHYDE	OX BILE
4815-56-1	25 gms	-	-	-	250 mg.
4815-56-2	25 gms	4 gms	-	12.5 ml	250 mg.
4815-56-3	25 gms	-	-	12.5 ml	250 mg.
4815-56-4	25 gms	4 gms	-	-	250 mg.
4815-56-5	25 gms	-	4 gms	-	250 mg.
4815-56-6	25 gms	-	4 gms	12.5 ml	250 mg.

These mixtures were melted, poured onto glass using the cooled metal ring to create a wall of gel at the edges of the pool to retain the mix until gelled by natural cooling. After drying in air for twenty-four hours, the dried films were replasticized in the pressure cooker over water at a relative humidity of 100% for twenty-four hours. Two specimens 1.0 cm. wide and about 15 cm. long were cut from each sample, dried under slight tension in a desiccator over phosphorus pentoxide. The finished, dry films were stored in a desiccator until needed for torsion pendulum evaluation.

The torsion pendulum results were actually fairly parallel with the results on gelatin films, that is, the transition temperatures were, by and large, unaffected by the treatments. The most noticeable change was in an ethyleneglycol plasticized sample treated with formaldehyde. It should be pointed out, however, that if amino groups were the primary site of formaldehyde attack, then the deaminated gelatin should not be different after treatment with formaldehyde. If this were true, samples 4815-56-5 and 4815-56-6 should have given quite similar results, while in fact they differed most from each other of any pair of these samples. This raises questions of the chemistry of the deamination process as well as of the formaldehyde reaction, both of which were explored later in the program.

A workable method of preparing films from deaminated gelatin and glycidol was worked out. A sample of deaminated gelatin prepared earlier, dialyzed and concentrated to 12% solids was used as the starting material. This was preserved with chlorinated phenols and in addition was stored at 35°F in a refrigerator. Because of the strong and destructive adhesive properties of the films, the following special procedure was employed. a Teflon-nylon laminate manufactured by the Cadillac Plastics and Manufacturing Co., was laminated to a 1 x 2 ft., piece of plate glass using a two component epoxy resin cement. A second piece of glass was used to flatten the film and the addition of 250 lbs., of weights assured the squeezing out of excess cement and air bubbles resulting in a smooth flat Teflon surface having only negligible calendering marks. No release problems were encountered when this surface was used. Films containing 0, 10, 20 and 30% glycidol were prepared by pouring onto the Teflon and allowing the film to dry. The dry films were heated at 55°C for four hours to complete the reaction. The excess glycidol was removed by dialysis in ice water in the refrigerator. Finally the wet films were laid onto the Teflon casting surface, air dried and reconditioned at 100% relative humidity prior to cutting into strips 7 mm x 100 mm and dried under slight tension using phosphorus pentoxide as the desiccant. Evaluation of these specimens was difficult because they were too thin, they however, were evaluated using a smaller inertial mass, and no significant change in Tg was observed. A second set of films was prepared from an assayed solution of deaminated gelatin so as to get a 20 mil thickness. Of these only the 0 and 10% glycidol specimens could be processed; the 20 and 30% samples simply disintegrated during the dialysis step intended to remove excess glycidol. These two samples were evaluated as 4974-24-A and 4974-24-B. After drying over phosphorus pentoxide, these films were tested on the torsion pendulum with no promising results.

CYANAMIDATED GELATIN FILMS

The procedure used for producing gela in films was also used to prepare cyanamidated gelatin films. The required amount of hydrogen cyanamide was added as a 50% aqueous solution just before casting the film. The following table gives the amounts of each component for the various films.

LAB. NO.	GMS. OF GELATIN	ml. OF 50% HYDROGEN CYANAMIDE	mg. OX BILE	WATER	GMS. GLYCEROL	GMS. ETHYLENE GLYCOL	ml. OF 1% FORMALDEHYDE
4815-57A	25	0.8	250	100	-	-	-
4815-57B	25	0.8	250	100	4	-	-
4815-57C	25	0.8	250	100	-	-	12.5
4815-57D	25	0.8	250	100	4	-	12.5
4815-57E	25	0.8	250	100	-	4	-
4815-57F	25	0.8	250	100	-	4	12.5

These quantities when cast onto glass plates using the metal ring yielded films of the required thickness. After drying, conditioning at 100% relative humidity and cutting to size followed by complete drying over phosphorus pentoxide, the films were stored in a desiccator until tested on the torsion pendulum.

The results suggested that cyanamidation followed by plasticization might well be showing the beginnings of the desired effect, that is, increased damping capacity at lower temperatures. Once again, however, the formaldehyde reaction effects seemed puzzling.

DEAMINATION STUDIES

As was pointed out earlier the samples of deaminated films on treating with formaldehyde should have been similar to untreated samples which in fact they were not. A fundamental study of the deamination reaction of gelatin was undertaken to clear up this anamoly.

It had been assumed that molecules containing primary amino groups would readily deaminate to yield the corresponding hydroxy compounds when treated with nitrous acid at room temperature. Evolution of nitrogen has been considered as a measure of the amount of amino functions available (Van Slyke Determination).

As the first step to a greater understanding of what happens during the treatment of gelatin with nitrous acid, we have determined the iso-electric points and some lysine contents of gelatins deaminated by various means.

ISOELECTRIC POINTS OF DEAMINATED GELATINS

SAMPLE NO.	TREATMENT	% NITRITE BASIS GELATIN	NITR. ANHY-DROUS BASIS	ISO-ELECTRIC pT	LYSINE % AT TOTAL A.A.
4815-73-1	100g gelatin-40°C-several hrs.	1	17.06	-	
4815-73-1A	100g gelatin-40°C-several hrs.	0	17.96		
4815-73-2	100g gelatin-pH 4-40°C-sev. hrs.	3	17.24	6.0	2.9
4815-73-3	100g gelatin-pH 4-40°C-sev. hrs.	5	18.03	6.0	2.82
4815-74-4	100g gelatin -pH 2.5-40°C-sev. hrs.	1	18.02	7.6	-
4815-74-4A	100g gelatin-pH 2.5-40°C-sev. hrs.	0	17.46	7.6	-
4815-74-5	100g gelatin-pH 2.5-40°C-sev. hrs.	3	18.02	6.6	3.2

SAMPLE NO.	TREATMENT	% NITRITE BASIS GELATIN	NITR. ANHY-DROUS BASIS	ISO-ELECTRIC pT	LYSINE % AT TOTAL A.A.
4815-74-6	100g gelatin-pH 2.5-40°C-sev. hrs.	5	17.9	6.1	2.75
4815-75-7	100g gelatin-pH 4-60°C-sev. hrs.	1	17.7	7.5	
4815-75-8	100g gelatin-pH 4-60°C thin pH 7	1	18.07	7.7	
4815-75-9	100g gelatin-pH 4-60°C-sev. hrs.	3	16.88	6.6	
4815-75-10	100g gelatin-pH 4-60°C-sev. hrs. pH 7 and cast	3	17.16	-	
4934-1-11	100g gelatin, pH 4-60°C-sev. hrs.	5	17.88	5.8	
4934-1-12	100g gelatin, pH 4-60°C-sev. hrs. then pH 7, cast	5	17.98	5.7	2.7
4934-1-13	100g gelatin-no nitrite	0	-	7.5	-
	Same as #11				
4934-1-14	100g gelatin-Same as #12	0	17.65	7.4	
4934-2-	50g gelatin, slow addition, 40-50°C-4 hrs.-pH 4	10	17.5	6.0	
4934-3-	50g gelatin-same as #2 pH 3	10	18.07	6.0	3.31
4934-4-	50g gelatin-30°C-400% HAc	100	16.56	5.0	0.13

Failure to achieve a significant reduction in the lysine content of gelatin indicated that the desired deamination was not taking place. Examination of the data shows that the use of a large excess of nitrous acid seems to be required. The use of a weak acid like acetic is also indicated in that it does not liberate the unstable nitrous acid in excessive amounts at any one time.

Thus the nitrous acid is present continuously in a concentration high enough to be effective throughout the entire reaction period.

Using the method of Hitchcock (J. Gen. Physiol. 6, 96 (1923)) as a basis the following manner of operation has yielded good results:

Fifty grams of Swift's Superclear gelatin were swelled in 950 ml. of water. The gel was melted at 40-45°C and cooled to 30°C. Fifty grams of sodium nitrite dissolved in 50 ml. of water were added with stirring. Two hundred grams of glacial acetic were added next and stirring was continued with no further heating until the gas evolution ceased. The solution tended to foam but was controlled by the addition of a few cc of ethyl ether. After several hours the solution was cast into a gel on a tray and chilled in a refrigerator. Because of the high level of electrolytes and the low concentration of gelatin, the gel tended to be weak and have a low melting point. The dialysis was carried out using running tap water at about 5°C for a period of forty-eight hours. The color changed to a brown olive drab. The gel swelled and gained in strength. No attempt was made to dry the dialyzed strips because we knew from prior experience that drying would insolubilize the modified gelatin. Films poured using this dialyzed material were found to be excessively long in drying resulting in some mold growth on the surface. A satisfactory solution to these problems was worked out by melting the strips at 30°C and concentrating the liquor to 13% solids at 30°C using reduced pressure. The addition of 0.5% of a proprietory mixture of chlorinated phenols based on the weight of the gelatin served as a preservative. This solution was then used for casting films for torsion pendulum evaluation.

THE EFFECT OF ADDITIVES ON DEAMINATED GELATIN

A series of massive films prepared from deaminated gelatin using various plasticizers and additives was prepared according to the following schedule:

SAMPLE NO.	GMS. OF DEAMINATED GELATIN AS 13% SOLN.	MG. OF OX BILE	GMS. OF GLYCEROL	GMS. OF ETHYLENE GLYCOL	ML. OF 1% FORMALDEHYDE
4934-23-A	25	250	0	0	0
4934-23-B	25	250	4	0	0
4934-23-C	25	250	0	4	0
4934-23-D	25	250	0	0	12.5

These films after drying were conditioned in a 100% relative humidity chamber for 48 hours, cut into duplicate 1 cm x 15 cm strips and evaluated on the torsion pendulum. The performance curves of the samples all had the same shape as those in Fig. 1. The values for T_g are as given below:

SAMPLE NO	T_g
4934-23-A	-60°C
4934-23-B	-40°C
4934-23-C	-50°C
4934-23-D	-50°C

These values suggested that all additives used raised rather than lowered the glass transition temperature being used as a criterion for following this work.

THE EFFECT OF INTERNAL PLASTICIZERS ON DEAMINATED GELATIN

Another lot of deaminated gelatin was prepared by the method described earlier yielding a 13.5% solution of finished deaminated gelatin. This solution was used to formulate four films as follows:

SAMPLE NO.	GMS. OF 13.5% SOLUTION OF DEAMINATED GELATIN	GMS. OF GLYCIDOL
4934-32-A	185	-
4934-32-B	185	2.5
4934-33-C	185	5.0
4934-33-D	185	7.5

These films were cast onto glass using a cold cast iron ring to support and retain film depth. It was intended that these films while still on the glass would be heated at 40-45°C for 4 hours, followed by dialysis to remove any unreacted glycidol and then redried on the glass to yield a film suitable for treating on the torsion pendulum. Actually the films pulled a layer of glass from the plate glass surface as they curled while drying spoiling the test. Samples of the film with adhering glass were forwarded to Mr. David Lawson as a "conversation piece" since such adhesive strength sufficient to peel the surface from plate glass is not a common occurrence.

INTERNAL PLASTICIZERS FOR GELATIN FILMS

Concurrently with our work on deaminated gelatin we have investigated the feasibility of plasticizing gelatin internally. Based on the known plasticizing effects of glycerol, we considered the introduction of glyceryl and of hydroxyethyl groups into the gelatin molecule as offering a good chance of imparting softening characteristics to the protein. Accordingly, we have reacted gelatin, Superclear as well as neutralized, dialyzed gelatin, with reagents such as ethylene chlorohydrin, glycidol and propylene oxide at different pH's. The table below summarizes the results of this survey:

PLASTICIZER GRAFTS ON GELATIN

SAMPLE NO.	TREATMENT	NITROGEN	ISO ELECTRIC PT.	% LYSINE BASIS TOTAL A.A.
4815-67-2	9.9g propylene oxide on 50g gelatin 120°F, pH 9	17.43%	7.1	-
4815-67-3	17.8g propylene oxide on 50 gelatin 120°F, 2 hrs.	-	-	2.25
4815-67-4	17.8g propylene oxide on 50g gelatin 120°F, pH 9, 2 hrs.	18.05	-	-
4815-68-0	Gelatin dialyzed	18.4		
4815-68-A	10cc ethylene chlorohydrin, 50g gelatin, pH 11.7 to 9, T = 46°C, 1 hr.	18.23	6.8	
4815-68-C	20cc propylene oxide, 50g gelatin, pH 11.2, T = 34°C, 1 hr.	18.03	7.4	
4815-69-A	20cc propylene oxide, 50g gelatin, pH 9, T = 46°C, 1 1/2 hrs.	18.03	7.4	
4815-69-B	20cc ethylene chlorohydrin, 50g gelatin, pH 9, 50°C, 1 hr.	18.01	7.2	
4815-69-C	5g glycidol, 50g neutral gelatin	17.06	7.3	0.80
4815-69-D	5g glycidol, 50g Superclear gelatin	15.98		3.96

Examination of the table shows that only glycidol and gelatin appear to react to yield a product having either less nitrogen or less lysine. The value of the isoelectric point was inconclusive.

In order to exploit this observation a series of tests was set up using regular Swift's Superclear gelatin as well as gelatin that had been neutralized to a pH of 7, then dialyzed to remove the electrolytes and dried and ground. All gelatin test samples were soaked and melted at 40°C before being subjected to the specifid treatment intended for that specimen.

GLYCIDOL-GELATIN REACTIONS

						EXTRA TREATMENT				
SAMPLE NO.	KIND OF GELATIN	AMT. OF GELATIN	AMT. OF WATER	GLY-CIDOL	STAND-ARD TREAT-MENT	HEAT TO 40°C FOR 4 HRS.	HEAT TO 55°C FOR 7 1/2 HRS.	DIALYZED	DRIED	GROUND
4934-25A	Neutralized	100	400	10	dried	-	-	-	-	-
4934-25A-1	"	"	"	10	dried	-	-	yes	yes	yes
4934-25A-2	"	"	"	10	dried	yes	-	yes	yes	yes
4934-25A-3	"	"	"	10	dried	-	yes	yes	yes	yes
4934-27-A-1	Neutralized	100	400	20	dried	-	-	yes	yes	yes
4934-27-A-2	"	"	"	"	dried	yes	-	yes	yes	yes
4934-27-A-3	"	"	"	"	"	-	yes	yes	yes	yes
4934-28-A-1	Neutralized	100	400	30	dried	-	-	yes	yes	yes
4934-28-A-2	"	"	"	"	"	yes	-	yes	yes	yes
4934-28-A-3	"	"	"	"	"	-	yes	yes	yes	yes
4934-29-A	Superclear	100	400	10	not dried	-	-	yes	yes	yes
4934-29-B	"	"	"	"	dried	-	-	yes	yes	yes
4934-29-C	"	"	"	"	"	yes	-	yes	yes	yes
4934-29-D	"	"	"	"	"	-	yes	yes	yes	yes

GLYCIDOL-GELATIN REACTIONS (CONT'D)

SAMPLE NO.	KIND OF GELATIN	AMT. OF GELATIN	AMT. OF WATER	GLY-CIDOL	STAND-ARD TREAT-MENT	EXTRA TREATMENT				
						HEAT TO 40°C FOR 4 HRS.	HEAT TO 55°C FOR 7 1/2 HRS.	DIALYZED	DRIED	GROUND
4934-30-A	Superclear	100	400	20	not dried	yes	yes	yes	yes	yes
4934-30-B	"	"	"	"	dried	-	-	yes	yes	yes
4934-30-C	"	"	"	"	"	yes	-	yes	yes	yes
4934-30-D	"	"	"	"	"	-	yes	yes	yes	yes
4934-31-A	Superclear	100	400	30	not dried	-	-	yes	yes	yes
4934-31-B	"	"	"	"	dried	-	-	yes	yes	yes
4934-31-C	"	"	"	"	"	yes	-	yes	yes	yes
4934-31-D	"	"	"	"	"	-	yes	yes	yes	yes

These samples on being evaluated by nitrogen determination and torsion pendulum yielded the following results. A number of samples were found to be insoluble and thus had to be eliminated. Those that were soluble were cast to yield a film of about 15-20 mil thickness. The table below summarizes the results:

GELATIN - GLYCIDOL REACTION PRODUCTS

SAMPLE NO.	PERCENT NITROGEN ANHYDROUS BASIS	SOLUBILITY IN WATER	CASTABILITY
4934-25-A			
4934-25-A-1	17.33%	Insol.	
4934-25-A-2	17.02%	Insol.	
4934-25-A-3	17.3%	Insol.	
4934-27-A-1	17.68%		
4934-27-A-1-1	16.94%	Insol.	
4934-27-A-2	16.74%	Insol.	
4934-27-A-3	16.56%	Insol.	
4934-28-A-1-1	16.64%	Insol.	
4934-28-A-2	16.64%	Insol.	
4934-28-A-3	16.64%	Insol.	
4934-29-A	17.65%	Sol.	Cast
4934-29-B	17.6%	Sol.	"
4934-29-C	17.55%	Sol.	"
4934-29-D	17.22%	Sol.	"
4934-30-A	18.32%	Sol.	"
4934-30-B	17.03%	Sol.	"
4934-30-C	16.4%	Sol.	"
4934-30-D	16.35%	Insol.	
4934-31-A	18.06%	Sol.	Cast
4934-31-B	16.61%	Insol.	
4934-31-C	15.32%	Insol.	
4934-31-D	15.6%	Insol.	

The use of neutralized dialyzed gelatin resulted in insolubilization, probably due to self-condensation of the gelatin catalyzed by the added glycidol. We expected that if the glycidol grafted onto the gelatin, its water solubility would not have been changed. Examination of the nitrogen values appears to show some reaction of the gelatin with glycidol as evidenced by a lowered nitrogen content. However, any solubilizing effect of the glycerol residue seems to have been ineffective in overcoming the insolubilizing effect of the self-condensation.

Not all of these samples were evaluated on the torsion pendulum. Instead, a series of most different samples were selected with the following results.

SAMPLE NO.	Tg
4934-27-A-1	-60°C
4934-28-A-1	-50°C
4934-29-A	-60°C
4934-29-D	-60°C
4934-30-A	-50°C
4934-30-C	-55°C
4934-31-A	-55°C

These results suggested no significant or desireable changes to have resulted from these tests. As a further check of these conclusions, the following test was carried out.

To 100 gms of neutralized, dialyzed gelatin and 400 gms of water and 20 gms of glycidol were added and reacted for 30 minutes at 40°C. Four film samples were poured and treated as follows:

4974-15-A; not dried, but dialyzed then dried.

4974-15-B; dried, dialyzed, dried.

4974-15-C; dried, 40° for 4 hrs., dialyzed, dried.

4974-15-D; dried, 55°C for 7 hrs., dialyzed, dried.

Sample 4974-15-D failed to survive the complete treatment by splitting into two layers during the dialysis step. Accordingly, 4974-15-C was heated at 55°C for one hour to represent conditions between samples 4974-15-C and 4974-15-D.

A, B and C were evaluated on the torsion pendulum, and the glass transition temperature were found to be about -60°C which is indicative of no change in Tg, and therefore eliminated our previous postulation of possible beneficial effects of glycidol in gelatin.

DEHYDRATION OF TEST SPECIMENS FOR THE TORSION PENDULUM

A group of specimens prepared as previously described for evaluation of dynamic mechanical properties on the torsion pendulum was examined for moisture loss over phosphorus pentoxide. The following chart indicates that 12 days over the dessicant is probably adequate.

	WEIGHT OF SAMPLES AS % SOLIDS			
NO. OF DAYS	21-A	21-B	21-C	21-D
0	100.0	100.0	100.0	100.0
3	95.8	96.2	96.0	95.7
4	95.5	95.8	95.8	95.5
7	94.7	94.8	95.0	94.4
12	93.7	93.8	94.3	93.4
16	93.6	93.6	94.3	93.3
18	93.4	93.4	94.2	93.2
OVEN DRIED 4 HOURS AT 110°C	92.0	91.4	93.1	92.0

The loss in weight as a result of heating at 110°C for 4 hours is undoubtedly water loss as a result of molecular condensation because of lessened solubility and increased viscosity effected by such intensive drying in an oven, as was observed long ago, as previously mentioned. Prolonged heating at 100°C effects the same result as does azeotropic distillation at 75-80°C.

PROPYLENE OXIDE-GELATIN REACTION

The reaction of gelatin with propylene oxide could be expected to occur either through its amino groups or through its carboxyl groups to yield substituted propylene glycol derivatives. These derivatives might well have changed glass transition temperatures in the cryogenic regions. A series of reactions of propylene oxide and gelatin was carried out using increasingly drastic conditions to force the reaction. The reaction vessel was a stainless steel Parr bomb provided with a stirrer and an electric heating unit. The reactions carried out are summarized in the table below:

PRESSURE REACTION OF GELATIN AND PROPYLENE OXIDE

SAMPLE NO.	GMS. GELATIN	GMS. WATER	GMS. PROPYLENE OXIDE	REACTION TIME	MAX. P	pH	REACTION T°c	NITROGEN ANHYDROUS BASIS
4934-51	50	500	20	1 hr.	9 lbs.	8	64	17.35
4934-53	50	500	50	4 hrs.	6	8.5	50	17.02
4934-54	100	1000	100	2 hrs.	2	10.	50	17.44
4934-55	75	750	75	2 1/2 hrs.	11	9.5	50	16.75
4934-57	75	750	75	3 hrs.	11	9.7	50	17.06
4934-58	75	750	75	4 hrs.	9	9.85	50	16.43
4934-59	75	750	75	3.5 hrs.	11	9.95	50	17.57
4934-60	75	750	75	3 hrs.	12	10.1	50	17.45
4934-63	75	750	75	2 hrs.	8	11.0	51	16.82
4934-64	75	750	75	1.5 hrs.	7	12.0	52	-
4934-65	75	750	75	3 hrs.	6	9.5 with triethylamine	51	17.30

Inspection of the data showed a drop in pressure at a pH of 10. Attempts to optimize the pH appeared to fail because all other runs failed to indicate a reaction as would have been manifested by a pressure drop. Consequently we concluded that the pH 10 run could not be duplicated and it was assumed that a leak in the head permitted the gas to escape. This is further substantiated by the failure of the nitrogen content to drop as would be expected in the event of propylene oxide addition. As the concluding experiment a trial using more drastic conditions was carried out as follows: 75 gms. of propylene oxide were mixed with 130 gms. of water (solubility = 59 gms. per 100 gms. water). The solution was cooled in an ice bath and added to 75 gms. of Superclear gelatin in a precooled Parr bomb. The gelatin was swelled for 15 minutes, the bomb sealed and heated in a boiling hot water bath for four hours. The internal temperature was 83°C. The gases were vented and the product dried in stainless steel pans. It had no jelly strength and failed to dry. The gelatin was apparently degraded and the oxide converted to the glycol. From this final experiment using propylene oxide, we concluded that further work with propylene oxide was not warranted.

CHANGES IN THE THICKNESS-WIDTH RATIO

In an effort to increase the sensitivity of our torsion pendulum determinations, we increased the thickness width ratio of our test specimens. We have changed the ratio by changing the thickness and leaving the width constant. Thus, two films, prepared from 25 gms of gelatin and from 75 gms of gelatin and water were spread over the same area using our cast iron ring mold. After conditioning, both films were cut into 1 x 6 cm specimens in duplicate for evaluation. It was found that if the film thickness is double for our regular specimens of one centimeter and thirty mil thickness, the period on the torsion pendulum is unfavorable in that the specimen is too stiff in comparison with the inertial discs available. Consequently, the size of our standard specimen has been changed to .7 cm. by 6.0 cm.

GLYCEROLCHLOROHYDRIN AND GELATIN

The reaction of gelatin with glycerolchlorohydrin was initially thought to be straight forward to yield the glycerol residue introduced onto the amino groups of gelatin.

The problem seemed essentially to be one of removing the protons from the amino groups of gelatin so that the active chlorine of glycerolchlorohydrin could split off with one of the hydrogens in the form of hydrogen chloride resulting in a C-N bond between the glyceryl residue and the gelatin. The presence of an alkali to remove the proton and to neutralize the hydrogen chloride was deemed necessary.

In our initial experiment we used the mild alkalinity of sodium bicarbonate. Subsequently we used sodium carbonate and finally sodium hydroxide. The results were as follows:

LAB. NO.	ALKALI USED	pH	% NITROGEN ANHYDROUS BASIS
4934-70	Sodium bicarbonate	8.7	18.24%
4934-71-2	Sodium carbonate	10.0	17.85%
4974-8	Sodium hydroxide	11.8	17.63%

The nitrogen values were so close to that of gelatin itself that the extent of the reaction was negligible, therefore we concluded that the glycerolchlorohydrin had not reacted to yield the modified gelatin.

GLYCIDYLMETHACRYLATE AND GELATIN

The availability of glycidylmethacrylate with its two reactive centers suggested that it might be useful in altering the properties of gelatin. Through its epoxy function it should be capable of reacting with gelatin through the carboxyl groups and through the double bond of its methacrylate residue it should be capable of homopolymerization. With these thoughts in mind we have reacted

25 gms of gelatin dissolved in 200 gms of water with 3.4 gms of glycidyl methacrylate. After stirring for fifteen minutes at 45°C, the mixture was poured onto a Teflon surface to form a film. The film required four days to dry. The dry film was divided and one half was cut into 7 x 100 mm specimens while the other was heat treated at 60°C for two hours and then cut into 7 x 100 mm specimens. The samples were designated as 4974-16A and 4974-16B respectively. They were evaluated on the torsional pendulum, and the glass transition temperatures were about -80°C and -76°C. Even though the Tg were slightly lower, the change between the two samples was not significant.

BENZOYLATION OF GELATIN

The introduction of benzoyl groups into the gelatin molecule could be expected to change the properties of gelatin. In order to test this hypothesis, we have treated gelatin with benzoyl chloride in the presence of alkali in a classical Schotten-Baumann reaction. The amount of benzoyl chloride used was 200% in excess over the free amino groups present. The reaction product was cast into film and dialyzed three days in running water and cast into a film for evaluation in the pendulum. (4974-17). This sample after drying over phosphorus pentoxide showed an apparent Tg of -130°C. (It is intended to run a confirmation experiment, since this was not duplicated).

LAUROYLATED GELATIN

The introduction of a long chain acyl group such as the lauroyl group should effect gelatin markedly. To test this idea we have prepared lauroyl gelatin from lauroyl chloride, gelatin and sodium hydroxide. A film was formed from the reaction product and evaluated as 4974-32. This sample on being dried over phosphorus pentoxide had a Tg of -60°C which was taken not to indicate any change.

ETHOXYLATION OF GELATIN

A 10% solution of gelatin was treated with gaseous ethylene oxide at 50°C at a pH of 9 with mechanical agitation for about 2 hours. After adjusting the pH to 6-7, the mix was chilled, cut into strips and dialyzed for two days. The final product was cast into a film for evaluation (4974-20). The nitrogen content was found to be 17.93%. This indicated that very little ethoxylation had taken place. The sample on evaluation on the torsional pendulum had a glass transition temperature of about -60°C which signified no change in Tg from that of gelatin, and thus substantiated the conclusion based on the high nitrogen analysis.

MOLECULAR SIZE OF GELATINS

The effect of molecular size upon the glass transition temperature was evaluated by running three gelatin samples (100, 200 and 300 gms. jelly strength) on the torsion pendulum. The higher the molecular weight or jelly strength, the lower the Tg obtained. This indicated that collagen itself might exhibit a lower Tg and to this end experiments using solvent extracted chamois and acetone dehydrated limed hide substance have been carried out along with collagen-gelatin laminates.

COLLAGEN FILMS

As an example of a collagen film, our first specimen was a sample of chamois skin extracted in a Sohxlet extractor using ethyl ether for several hours. After drying overnight, a 7 x 100 mm specimen was cut and desiccated over phosphorus pentoxide. Three test strips cut from one location were identified as 4974-43-A. A second set from a different location were marked 4974-43-B. Due to lack of rigidity in these samples, it was decided that they should be desiccated over phosphorus pentoxide over an extended period; so that they would become suitable for evaluation on the torsional pendulum. However, these samples could not be evaluated on the torsion pendulum since they failed to develop any rigidity or any restorative torque in the apparatus.

Similarly, the dehydrated limed hide substance was split with a knife to yield specimens about one mm thick and 7 x 100 mm in the other dimensions. These too were dried over phosphorus pentoxide prior to evaluation on the torsional pendulum. This sample showed an apparent Tg of about -80°C.

Another form of collagen films are laminates made up of thin collagen films laminated together with gelatin. Collagen films available commercially as synthetic sausage casings were dialyzed in running water to remove water soluble constituents such as the glycerol plasticizer and then extracted repeatedly with acetone to remove any fat. Three films were remoistened and patted damp dry with a towel and interleaved with 2 mil thick gelatin films. The sandwich was pressed at 200 psig at 40-42°C for 5 minutes. A thicker specimen was prepared from four collagen films and three 3 mil gelatin films at 125°F and 200 psig for 30 minutes. Another film was made similarly except it was pressed at 400 psig. All samples were cut before they were completely dry for evaluation on the torsion pendulum. However, these samples de-laminated while being equilibrated over phosphorus pentoxide prior to dynamic mechanical evaluation, and it is thus questionable how much might be learned by such evaluation.

A second form of collagen film used was that obtained from synthetic frankfurter casings made from collagen. Since these films are only about 1 mil thick, we required twenty pieces to obtain the desired thickness of twenty mils. The dry films were coated with a 10% gelatin solution using a paint brush as an applicator. The stack of films was wrapped in siliconized paper and pressed at about 400 psig and 125°F. While still damp the usual 7 x 100 mm specimens were cut out (4974-45A). Even though the problem of delamination still endangered the evaluation of these two samples on the torsional pendulum, a trial evaluation was carried out on the pendulum. De-lamination occured during this evaluation, although it has not been determined at what point. An apparent Tg of -54°C was observed.

OXYPOLYGELATIN

The use of gelatin as a blood plasma extender has received consideration but has suffered from the drawback that gelatin solutions gel too readily at body temperatures.

Reduction of the gelling tendency by controlled degradation of the gelatin has the drawback that the molecular sizes are reduced to an extent that causes them to be eliminated too rapidly from the blood stream. To overcome these problems, oxypolygelatin was developed.

Working according to U. S. Patent 2,591,133 we dissolved 80 gms of gelatin in 1,560 gms of water adjusted to pH to 6.8. The temperature was raised to 70°C, using a water bath, and 22.5 ml of 3% glyoxal and sufficient dilute sodium hydroxide to neutralize any acid in the aldehyde. The mixture was held at 70°C with stirring for 50 minutes. Finally added 5.1 ml of 30% hydrogen peroxide and held for one hour. The product remained liquid at room temperature. A film was formed readily by pouring the required amount of solution into a Teflon lined frying pan and drying in an unheated wind tunnel (4974-28). This sample after drying over phosphorus pentoxide had a Tg of -58°C.

HETEROGENEOUS FILMS OF GELATIN

The possibility that small quantities of synthetic polymers in gelatin films could confer desirable properties on to the film has not been overlooked. The synthetic polymers considered are of widely varying characteristics; viz., polyacrylamide, a water soluble polymer having great thickening power, polyvinyl alcohol, being less soluble in water and less compatible with gelatin and polyvinyl acetate which is water insoluble but emulsifies readily. The preparation of all three films was similar and is given in the example below:

20 gms of gelatin was swelled in 100 gms water, 200 mg

of polyacrylamide (Separan NP 20) dissolved in 20 ml of water was added to the melted gelatin solution and stirred at 40°C for one hour using a magnetic stirrer and then cast into films.

The table below summarizes the composition of the three films:

LAB. NO.	POLYACRYLAMIDE SEPARAN NP 20	POLYVINYL ALCOHOL ELVANOL 52-22	POLYVINYL ACETATE EMULSION 50%	GELATIN g	WATER g
4974-27-A	0.2 g			20	120
4974-27-B		0.2 g		20	120
4974-27-C			0.4 g	20	100

All films were dried and cut into 7 x 100 mm specimens for evalutation on the pendulum.

These samples were evaluated on the torsional pendulum and these samples evaluated manifest slightly lower Tg of about -80°C, -74°C, -72°C, respectively.

CONCLUSIONS

From the experiments reported here it is concluded that very desireable degrees of impermeability can be obtained with gelatin films. However, it must also be concluded that few, if any, of the chemical modifications described led to any significant improvements in the cryogenic flexibility of these films. The great flexibility retained by some collagen films suggests that structural differences between collagen and gelatin may be more vital than chemical differences in determining this flexibility. Attempts to wed the impermeability of gelatin films to the flexibility of collagen structures by physical combination have thus far been unsuccessful.

Early in the program described, it was decided to standardize the gelatin used. This decision was not tested until late in the program, when it seemed that the chemical modification studies were not leading to increased knowledge of mechanisms for improving cryogenic flexibility. It was felt that the use of gelatins of varying molecular weights could provide a method of looking at the effects of crosslink density in an inverse manner. This conclusion was reached for the following reasons. We currently accept the structure of collagen to be a cable-like molecule consisting of three strands of "parent gelatin" (Veis, "Macromolecular Chemistry of Gelatin", Chap. 1, esp. p. 42-43, Academic Press, New York, 1964). This structure is stabilized by the hydrogen bonds associated with the peptide bonds, which normally lead to helix formation in those protein molecules characterized by that structure. Helix formation is not possible in collagen and gelatin because of the high relative amounts of the iminoacids, proline and hydroxy proline. The process of gelatin production is a melting out of the stabilizing hydrogen bonds to give single-strands of "parent gelatin" accompanied, unfortunately, by some hydrolysis of the "parent gelatin" strands.

In solution, gelation behaves like a randomly coiled polymer, and the process of gelation of gelatin solutions is a random reformation of the "melted out" hydrogen bonds. It was thus expected that the density of cross-links along a strand would be independent of the length of the strand, and that the longer strands present in higher molecular weight gelatin would give an effect which in another material might be taken as a manifestation of increased cross-link density. The samples testing this effect showed a lowering of T_g with increasing molecular weight. This may result from the fact that lower molecular weight gelatins, by virtue of the process of their preparation, are characterized by a less homogenous molecular weight distribution. The matrix provided by the gelation of a network of larger gelatin molecules can thus be tightened by a filling in with smaller molecules. This process is less possible in a gelatin consisting more nearly of large molecules only. Therefore, increased flexibility may result in films prepared from higher molecular weight gelatin.

While the flexibility of collageneous materials at low temperatures may be related to the fact that the "parent gelatin" chains are intact, and thus the material has a higher molecular weight, it seems more likely due to the specific structure of collagen. This, coupled with the observations that cross-linking does not increase cryogenic flexibility, leads to the conclusion that a chemical process for increasing the molecular weight of the gelatin will not prove to be a uniformly reliable procedure for increasing cryogenic flexibility.

The reaction product with benzoyl chloride, which seems to show such a substantial change in T_g, must be taken as an artifact for the purposes of this report, since it has not been re-confirmed or verified. Thus, it must be concluded that, while gelatin films show excellent impermeability to gases, their use will be limited by their less than optimal cryogenic flexibility, and that this work has not provided vital insight toward the substantial improvement of the cryogenic flexibility.

CHARACTERISTICS OF GELATIN FILMS

The tremendous film forming tendencies of gelatin as well as its derivatives explain many of its uses. In the process of manufacture mildly acidic conditions coupled with highly efficient filtration produces a product almost entirely free from traces of fat, or particulate contaminants. This explains the ease of producing films free from pinholes.

As a mixture of hydrolysis products derived from collagen, commercial gelatin contains a distribution of polypeptide molecules which has been found by Williams, Saunders and Cicirelli (J. Phys. Chem. 58, 774 (1954) to be adequately approximated as a Lansing-Kraemer (J. Am. Chem. Soc. 57, 1369 (1935) distribution with a mode of about 10,000 and a non-uniformity coefficient of about 1.36.

The isoelectric point at which turbidity and foaming tendency are maximum is about 8.5 for acid cured gelatin as used in this study. By working in a pH range of 4 to 7 we have been able to minimize gas permeability. The reversibility of gelation has facilitated the pouring of films and the control of their size and thickness. Since it can serve as its own membrane, dialyses to remove soluble impurities poses no problem. Many salts exhibit pronounced effects on the rate of gelation and the strength of the gel. Soluble sulfates, malates and citrates increase the rate of gelation at any given concentration whereas chlorides, nitrates, and thiocyanates have a reverse effect. Organic compounds that retard or inhibit gelation are urea, thiourea, alkali naphthalene sulfonates, chloral hydrate and ethyl ether.

Water is the only true solvent for gelatin but with a normal moisture content of 5 to 8% it can be dissolved in mixtures of glacial acetic and phosphoric acids. If the moisture content is in the 10 to 20% range then a number of polar solvents may be used including glycerol, the glycols, polyglycols, N-methyl pyrrolidone, dimethyl formamide, dimethyl sulfoxide, sorbitol and lower aliphatic and acetylenic alcohols. It has been shown in the course of this work that clear flexible films of gelatin containing high levels of nonvolatile polar plasticizers provide an excellent barrier against such gases as nitrogen, helium, hydrogen and particularly oxygen. Gelatin is approximately 5 times more impermeable to oxygen than polyvinylidene chloride (Saran).

Gelatin may exist in two forms which are reversible with changes in temperature. These changes are manifested by drying films at 20°C and 50°C and noting changes in tensile strength. This has been shown also in this study by heat treatment at 101°C whereby gas permeability was lowered approximately 60% and tensile strength decreased 8%. Irradiation with ultraviolet light effected less improvement in gas impermeability but greater loss in tensile strength. Subjecting films to liquid nitrogen temperatures decreased impermeability to gases but also lowered the tensile strength.

The ratio of gel strength to viscosity is dependent upon the curing medium to which the collagen material is subject. In the case of acid cured stock from which the gelatin used in this study was derived a gel strength (Bloom) of 300 gms. is accompanied by a viscosity of 60-65 millipoises as measured at a concentration of 7.15 gms. gelatin in 100 cc water. In contrast to this Jelly-Viscosity ratio of 5 to 1 an alkaline cured stock would yield a gelatin of the approximately same gel strength but a viscosity of 100 or a ratio of 3 to 1. The acid cured gelatin is much preferred for casting in films because it spreads easily, discharges entrapped air rapidly and sets to a gel rapidly thereby permitting rehandling.

The very high gel strength of acid cure gelatin permits efficient dialysis to remove water soluble substances. No additional membrane is required and the aqueous gels hold up well even at 2% concentration at 10°C. This has made the purification of modified gelatins relatively simple.

The following table shows certain optimum physical properties as determined for those gelatin films which appeared to offer the most promise:

OPTIMUM FILM FORMULATION FOR EACH CLASS OF FILMS

No.	KIND OF GELATIN	AMOUNT AND KIND OF PLASTICIZER	AMOUNT AND KIND OF CROSSLINKING AGENT	He TRANSMISSION cc/100 in.2/ 24 hrs./atm.	TENSILE STRENGT LBS./ SQ. IN.
1	ordinary	5% Glycerol	0.5% formaldehyde	10.07	5100
2	ordinary	5% "	0.7% Glyoxal	9.46	4930
3	ordinary	5% "	0.7% "	10.7	3710
4	ordinary	17% "	none	8.05	6230
5	cyanamidated	14% "	none	12.32	4660

COMMENTS:

If maximum gas impermeability with maximum strength is desired, then number 4 is the formulation of choice. If it is permissible to sacrifice some of the impermeability of the film for the sake of greater water resistance or insolubility, then either Nos. 1 or 2 is the best choice depending upon which of the two tested properties is the more desired.

GLASS TRANSITION TEMPERATURES

NO.	TYPE OF GELATIN	PLASTICIZER	T_g
4934-23-A	Deaminated	None	-60°C
4934-27-A-1	Neutral Gelatin	Glycidol(20%)	-60°C
4934-29-A	Superclear	Glycidol(10%)	-60°C
4934-29-D	Superclear	Glycidol(10%)heated	-60°C
4974-32	Lauroylated	None	-60°C
	Split Hide Substance	-	-80°C(apparent)
4974-27-A	Superclear	1% Polyacrylamide	-80°C
2974-27-B	"	1% Polyvinyl alcohol	-74°C
2974-27-C	"	1% Polyvinylacetate	-72°C

Due to the excellent reproducibility of the methods used to determine strength and permeability of film made from gelatin or its derivatives, we have concluded that we have a raw material from which films can be cast having a predetermined permeability.

Due to the distinct feasibility of making derivatives, with ultimate purification by dialysis and with or without the incorporation of foreign materials, we are confident that many films can be developed which could be designed for incorporation into artificial kidneys or other mechanical substitutes for vital organs.

The probability of success in such an endeavor is increased by the following techniques which have been evaluated in a most casual way but have not been studied adequately.

a. Preparation of film materials from coascervates inside and outside the critical range. Such blends or mixtures could be developed using gelatin or its derivatives with polyvinyl alcohol, natural gums (acacia, locust bean, carraghєen, etc.) starch and its derivatives.

b. Since techniques are known for introducing a vinyl substituent into the gelatin molecules, the whole field of graft polymerization is available for study.

c. The incorporation of inert materials offers another large field of study. Microcrystalline cellulose, asbestos and collagen have variable compatibilities with gelatin to say nothing of such colloidal dispersions as silver iodide, gold, hydrated clays and the like.

d. It is believed that films can be designed to carry an electric current up to certain limits.

Few materials offer such versatility, availability and economy as does gelatin and its fibrous precursor, collagen. Susceptibility to chemical and biological reactions may provide many advantages as well as disadvantages.

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