THE COMPOSITION AND STRUCTURE OF PROTEINS
(A REVIEW)

AND

THE STRUCTURE OF GELATIN AND THE EFFECT OF UREA AND RELATED SUBSTANCES, AND SOME ELECTROLYTES ON THE RATE OF GELATION OF GELATIN SOLUTIONS

BY

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FORWARD

The experimental work reported in the coming pages was contemplated by Prof. W.F.H.M. Mommaerts in an effort to find the forces that act in muscle contraction. Parallel to his work on myosin and muscle contraction, this work was run to find out the importance of the resonance bond in proteins. Gelatin was taken as an example, which may be an arbitrary choice, yet it possessed the property of gelation which is a convenient characteristic to handle. In his stay at Istambul Universitesi in Istanbul in 1945 he observed that urea inhibited the gelation of gelatin sols. This effect could be due to the resonating structure of urea similar ot that of peptides. It might be, then, that urea is attacking the resonance bond in proteins in some of which causing denaturation. With this in mind the experiments were planned and executed.

I am much indebted to Prof. Mommaerts for his valuable suggestions and care. I am also indebted to Prof. S. Kerr of the Biochemistry Department and Prof. W. West of the Chemistry Department for making it possible for me to carry on the work, to Mr. Saradarian and the rest of the Staff of the Biochemistry Department for facilitating the work in their department, to Mr. David Azrak of the Glass Blowing Department for making the unavailable glass equipment, and to Mr. H. Rubeiz, Secretary of the Chemistry Department for typing the report.

PART ONE

THE COMPOSITION AND STRUCUTRE OF PROTEINS

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PART ONE

THE COMPOSITION AND STRUCTURE OF PROTEINS

INTRODUCTION

The story of Proteins goes back in its beginning to prehistoric ages. Some protein substances were known thousands of years ago. History has recorded the manufacture of glue from skins, cheese from milk; the tanning of hides; the hardening of eggs by boiling and by baking; the distillation of horn for ammonia and the clarification of turbid solutions by heat coagulation of a protein dissolved in them; all of which are examples of proteins and their properties. As laboratory materials for investigation, proteins are not new entries in modern chemical research; for as early as 1839 the Dutch chemist, Mulder(1)*, stressed the importance of proteins in the constitution of the cell protoplasm. Since then it became recognized that proteins are the essential solid constituents of animal and plant tissue. No wonder, then, that this class of compounds be called "proteins" after the Greek word "protos" which means first.

From the beginning investigations on proteins followed no planned general system. Working on apparently amorphous compounds was no easy enterprise, while proteins addéd many more difficulties to the scientists. The lability to heat and many chemical substances as well as their difficult preparation made proteins least attractive to investigators. Proteins could not be identified from one another through their chemical properties, and it is mainly through physical means like solubility that proteins could be identified from one another. Then what property distinguishes them as proteins, a distinct class of chemical compounds? The answer to this question had to be sought in the chemical composition and structure. Four elements are always present in proportions that vary only slightly in different proteins. These elements are Carbon, Hydrogen, Oxygen, and Nitrogen, while Sulfur, Phosphorus, and traces of other elements may be found. Generally, proteins are of high molecular weights. Substances with some claim on the name 'protein' may have molecular weights between a few thousands and some hundred millions. Typical proteins usually fall within the limits of 17000 (more often 34000) and a few millions. On complete hydrolysis with acids and bases proteins yield a number of amino acids in various properties.

In recent years the physiological importance of proteins attracted attention, while their use and industrial importance pushed them further into the foreground of research. Increasing knowledge brought with it the necessity of a classification of the subject; the preparation and identification of proteins; the ascertainment of the purity of protein samples and preparations and its identity with the natural substance in the animal or plant tissue; the identification of its composition and structure, and the investigation of the mechanism of its physiological activity. The isolation of viruses as

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* Cited from Bodansky, "Introduction to Physilogical Chem.",1938, p.2

homogenous crystalline protein substances that continue to possess the virus activity and reproductivity as well as the discovery of the importance of engymes in metabolism increased the response of scientists toward a serious active approach to the field of protein chemistry.

In this thesis no attempt shall be made to review the classification of proteins or methods of preparation and purification as both subjects are beyond the scope of the paper. Instead, the protein molecules shall be pictured as viewed in modern literature. More importance shall be given to fibrous proteins, and in the second part of the thesis the discussion will be devoted to gelatin giving a review of what is known about it of importance to its structure studies as well as a report on the experimental work done by the writer under the guidance of Professor W.F.H.M. Mommaerts of the Biochemistry Department in the American University of Beirut, the work being on the effect of a number of chemical substances on the gelation property of gelatin.

CONSTITUTION OF PROTEINS

Composition of the Protein molecule :-

The hydrolysis of protein substances yield a number of optically active α-amino-acids or imino-acids. Leucine and glycine were isolated from protein hydrolysates in 1819 and 1820 by Proust (2)* and Braconnot (3)*. Cystine was described by Wollaston in 1810 (4)* and isolated from proteins about 80 years later. No less than 28+ amino-acids have since been isolated from proteins. They include Mono-amino-monocarboxylic, diamino-monocarboxylic, monoamino-dicarboxylic, hydroxy-amino, and sulfur or phosphorus containing amino-acids. All of the discovered amino-acids are optically active compounds when isolated after the careful acid hydrolysis of the proteins. They exhibit levo-rotation of polarized light.

Simple proteins yield on hydrolysis only amino or imino-acids, while the more complex proteins may contain carbohydrates, fats, nucleic acids or a prosthetic group like heme.

It is apparent that the elementary composition of a protein substance is dependent on the kind of its amino-acid contents and their relative availability in the protein as well as the nature of the prosthetic group in conjugation with the protein molecule. In the simple unconjugated proteins we always find C, H, O, N, and S. The percentages of these element do not vary to a large extent in the different proteins as may be observed in the following example according to Osborne (27) in

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* Cited from: Cohn and Edsall, 'Proteins, amino-acids and Peptides' 1943, p. 338

\+ Cited from: M. Steel, 'Biological and Clinical Chemistry', 1937 p. 99

1902 cited from Cohn and Edsall, "Proteins, Amino acids, and Peptides", 1943, p. 341.

Composition

Protein	C	H	N	O	S	P
Fibrin	52.7	6.8	16.9	22.5	1.1	-
Edestin	51.5	7.0	18.7	21.9	0.9	-
Casein	53.5	7.0	15.8	22.4	0.8	0.9

Formula

Protein	C	H	N	O	S	P	mol. wt.
Fibrin	645	1004	178	207	5	-	14708
Edestin	624	1021	193	199	4	-	14523
Casein	708	1130	180	224	4	4	15982

Preparations of pure proteins lacked enough evidence of their molecular homogenity; so were methods of estimating amino-acids of protein hydrolysate unreliable. While the homogenity of many protein preparations remain doubtful and a few of the amino-acids could be estimated to within 3% error, theories on the strucutre of protein molecules have already been advanced depending on the more reliable data.

<u>The Peptide Theory (Hofmeister and Fischer)</u> :-

The hypothesis on protein structure that is compatible with most of our knowledge about proteins is that arrived at simultaneously by Hofmeister (5)* and Fischer (6)* in 1902. It supposes that the amino-acids are bound together in long chains through their α-amino and carboxyl groups by peptide links. In their hypothesis they rely upon such experimental facts :-

1. Osmotic pressure measurements and ultracentrifugation (in some special cases investigations of viscosity, e.g., Mommaerts, Arkiv för Kemi (1945) 19 A No 17) picture a very large molecule. In many cases the molecular weights correspond to hundreds and thousands of amino-acid residues in a single protein molecule.

2. The available acid and basic groups in a protein molecule

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* Cited from C.Schmidt, 'The Chemistry of Amino-aicds and Proteins', 1938, p. 301.

can be estimated by titrometric methods. Results give too low a value to account for all the amino-acid residues present in the protein, showing that -COOH and -NH_2 groups are involved in the condensation .

3. On the hydrolysis of protein substances equivalent quantities of acid and basic groups are liberated.

4. Proteins give a biuret reaction which is given only by compounds that contain the peptide bond.

5. Peptide union is encountered in nature, e.g. Hippuric acid, $C_6H_5.CONH.CH_2.COOH$, so that it is not unreasonable to suppose that it occurs also in the biosynthesis of proteins.

6. Synthetic peptide and poly-peptides have been synthesized and can be hydrolyzed by the specific action enzymes.

Before elaborating the peptide theory of Protein Structure into its modern form a review of the chemical characteristics of the proteins will be introduced because they are the outward evidence of the structure of protein molecules.

The Chemical Characteristics of Proteins.

The Free Basic Groups:- It has been mentioned in a previous place that the hydrolysate of many proteins contain amino-acids that are decidedly basic. These are arginine, histidine and lysine, containing a terminal guanidino, imidazole, and ϵ- amino groups respectively.

(a) Arginine:- There is evidence that the guanidino group of arginine in proteins is not bound in peptide linkage with other amino-acids but is found free or bound with a prosthetic group in salt linkage with acid groups from within or foreign to the protein molecule. The evidence is taken from the following experiments (7)*.

1. Nitration of the protein produces a nitro-arginine similar to that obtained by the nitration of free arginine.

2. The strongly basic prtein, clupein, has an acid combining capacity equivalent to the total amount of free arginine obtained after hydrolysis.

3. Unhydrolyzed protein gives a positive Sakaguchi test (8)*.

(b) Histidine:- It is generally accepted that the imidazole ring of histidine is uncombined along the polypeptide chain (7)**, through in glycoproteins the polysaccaride may be joined to the peptide chain through the imidazole ring (9)**

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* Cited from: ibid. p. 284

** ibid. p. 283.

(c) Lysine and Other Free Amino Groups:- If a lysine containigh protein is treated with nitrous acid, no lysine can be obtained from the hydrolysate of the deaminized protein. If a quantitative deamination is carried out in a Van-Slyke apparatus for 25 min., half as much nitrogen is liberated than by the lysine fraction of the acid hydrolysate of the protein when deaminized by the same method (10).

Prtoeins that contain lysine residues react with phenyl-isocyanate (11) to form the phenyl ureamino-compound

$$C_6H_5.NH.CO.NH.CH_2.CH_2.CH_2.CH_2.CH(NH_2).COOH$$

while proteins like zein which ~~do not~~ contain no lysine residues do not. If gelatin is benzenesulfonated, then hydrolyzed with formic and sulfuric acids, at least 50% of the originally free amino groups can be accounted for by the isolation of ϵ-aminobenzine - sulfonyl-d-lysine (12). In these experiments we have good evidence that the ϵ-amino groups of the lysine residues of proteins do not form a part of the peptide chain.

Unless the basic end of the peptide chain is a proline or hydroxyproline as is the case in the protein zein (11), we should expect a free α-amine group at one end of each peptide chain. The Number of these amino groups to the total number of free amino-groups depends on the length of the peptide chain and the number of lysine residues in each chain. In gelatin only $\frac{1}{8}$% of the total free amino groups can be assigned to α-amino groups (12).

The Free Acidic Groups:-

Carboxyl groups:- Though the amount of dicarboxylic acids that can be isolated from protein hydrolysate is large, the protein is only weakly acidic. This is because the majority of the carboxyl groups not in the peptide chain, like the other carboxyl of aspartic, glutamic, and hydroxy-glutamic acid residues, are found as amides that split ammonia on hydrolysis. In some proteins the ratio of NH_3 - N to carboxylic acid - N is close to 1:1. In fact an investigation of 15 proteins (15) * shows that in 6 of them the deviation from the 1:1 ratio was 10%, less than 50% in another 7 and in 2 proteins 80 and 140% respectively to show that the amount of free carboxyl groups vary widely among different proteins.

It should be noted that the phenolic hydroxyl groups of the residual amino-acids have base binding properties in the presence of a strong alkali though it is probable that under physiological conditions they behave as neutral groups.

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* Ibid. p. 286.

Alcoholic Hydroxyl Groups

Four amino acids are definitely known to conatin a Beta-hydroxyl group. These are serine, hydroxy-proline, Beta-hydroxy-glutamic acid and Beta-hydroxy-α-amino-n-butyric acid. Their hydroxyls may exist free or esterified with phosphoric acid or in ester or ether links with other amino-acids.

Sulfur

Of the four known sulfur containing amino-acids; cystine, cysteine, djenkolic acid, and methionine; more is known of -ture cystine and cysteine concerning their influence on the strucute and physiological activity of proteins. The availability of -SH groups in proteins can be tested for by sodium nitro-prusside ($Na_2Fe(CN)_5NO.2H_2O$). It is observed that while some natural proteins give a positive test, others give the test only after denaturation and others only after reduction with cyanide. Certain proteins like globin and brain proteins do not give the free -SH at all. Hence it is observed that in some proteins the -SH groups are masked to be liberated only after denaturation of the protein. In some proteins they are found as -S.S- groups which liberate -SH on reduction. A great deal of work has been reported on the importance and the role of the -SH groups in protein denaturation, activation and inactivation which cannot be included here. It is possible that the disulfide group can form a bridge between two chemically similar or dissimilar polypeptide chains, or between two part of a folded chain.

Phosphorus:-

Phosphorus may occur in some proteins as casein where it may be present in the phosphoric esters of hydroxyamino-acids.

Nucleoproteins contain a large amount of phosphorus which belong mainly to the nucleic acid fraction where it is phosphoric acid residues.

Lipoproteins like lecithovitellin of egg yolk contain a fatty acid fraction which is unstable toward alcohol (18)*. This fraction is called a phosphatid and is a combined glycerol, fatty acids, phosphoric acid and a nitrogen base.

Sugars, Polyuronic Acids,

These are found in a variety of proteins like mucoids, mucins, glucido proteins, etc.

The Arrangement of Amino Acids in the Polypetide Chain.

The Bergman and Niemann Periodicity Theory:- On the basis of amalytical data of their choice, Bergman and Niemann arrived at certain simple numerical relations that hold between the

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* Ibid. p. 292 (Ref. 85).

amino acids in the proteins they investigated. They claim that the total number of the amino-acids in a polypeptide is expressible by the formula $2^m \times 3^n$ where m and n are integers or equal to zero; and the number of residues of any amino-acid is $2^m \times 3^n$ where m and n are integers or equal to zero. To this theory the fibrous proteins respond quite well and examples can be found in the writings of Bergman and Niemann (23, 24, 25, 26). Bergman and Niemann suggest, also, that the response to their formula implies a periodicity in the arrangement of the amino-acid residues along the polypeptide chain. This periodicity does not necessarily hold true even if the formula $2^m \times 3^n$ for each amino-acid residue is correct. The long periodic spacings revealed by X-ray diffraction pictures of proteins renders a periodicity hypothesis attractive. In considering such or any other hypothesis it should be remembered that, so far, the data on the amino-acid content of proteins are only approximately correct and the proteins under investigation may not be homogeneous molecular or polypeptide species. This does not invalidate the Bergman and Niemann hypotheses, it may, even, lend support to it. All that can be said about its correctness now is that it has been born immaturely, so cannot yet be acclaimed universally correct, but remains useful in the study of protein structure. Kratky iodinated silk fibroin, introducing heavy centers of di-iodotyrosine along the polypetide chain.X-ray pictures show remarkable periodicity of the newly made heavy centres. The periodicity found however, did not correspond accurately to what was to be expected from the tyrosine contents. This discrepancy, it is true, can be explained by additional hypotheses. On the other hand, the result does not form an argument in favour of Bergmann and Niemann hypothesis in its simplest form. Guanidino groups of the arginine residues do not give a quantitative Sakaguchi reaction. According to Roche and Morgue (13)* this is due to proximity of the guanidine group to such functional groups as -OH, -S.S-, and -SMe. along the peptide chain. If this is true, it would be suggestive of an orderly distribution of the arginine residues with respect to the above mentioned active groups containing amino-acids along the protein molecule. This observation lends, in this form, support to the hypothesis of Bergmann and Niemann.

Protein Structure from X-ray Diffraction Pictures:

The Structure of polypeptide chains :- Diagram, No. 1, for the structure of a fully extended polypetide chain has been proposed by Corey (27) on the basis of X-ray studies on glycine (28), alanine (29), and diketopiperazine (30) crystals. The bond angles about the carbonyl carbon and the nitrogen atoms are assumed to be 120° as in diketo-piperazine. Around the α-carbon atoms, the bounds are assumed to be arranged in nearly tetrahedral fashion, except that the C-C-N bond angle is taken as 116°, a mean- mean of the values found for

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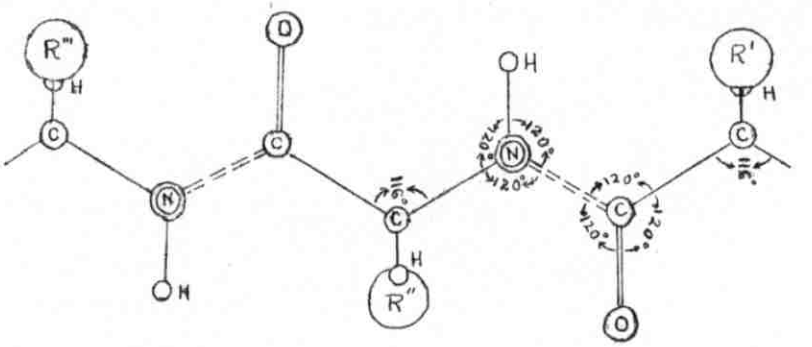
* Cited from : Brit. Ch. and Phys. Abst. 1946, A II, 628.

glycine (112°) and diketopiperrazine (120°). The fully extended chain is coplaner with -C=O oxygen atom, and the -NH hydrogen atoms are in the same plane of the chain. The distance along the chain corresponding to one amino-acid residue is 3.67 Å. Since the spacial configuration of the groups around the α-carbon atom on all amino-acids is the same, the side chains, R', R", R"', etc. Attached to the α-carbon atoms point alternately, first (R' , R"') to the left and upwards from the plane of chain. Next (R") to the right and downward from the plane of the chain.



Diag. 1

With X-ray diffraction data it is possible to compare Corey's model with actual protein crystals or fibers, which have micro crystalline micels. In such a study by the X-rays proteins fall into two main groups, the fibrous proteins and the globular proteins. The fibrous proteins, e.g., silk fibroin, keratin, myosin and collagen, have X-ray pictures that are not, in most cases, well defined and indicate a long molecule with a short crossectional axis. Globular proteins give more detailed X-ray pictures indicating a finer crystalline strucutre with an ellipsoidal molecule which may be fat or thin depending upon the protein in question. Several ways have been developed to contrust models from X-rays pictures and the present position remains disputable. Since we are concerned mainly with gelatin, the topic of the second part of the report, the discussion will be limited to the fibrous proteins to which gelatin belongs as well as some globular proteins after denaturation by urea (31).

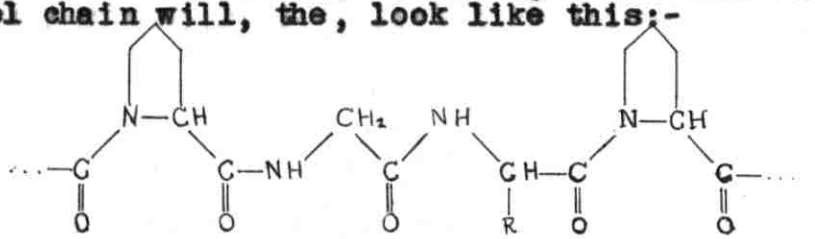
A number of fireous proteins obtained from different tissues have been examined; but all appear to belong to one or the other of two groups.

(1) The keratin-myosin group (2) The collagen group. Silk fibroin belongs to the first group and has been given much attention in X-ray studies. Silk fibres cannot be reversibly stretched over a long distance so its molecules represent an almost completely stretched form and should, therefore, compare well in structure with Corey's model if the latter is correct. For fibroin a marked periodicity along the fibre axis is found at a spacing of 7.0 ± 0.2 Å which is double the lengh (3.5 Å) of an amino-acid residue,

suggesting a repeating unit of 2-amino-acids along that axis. Since 3.5 Å is almost the spacing for a completely stretched model, hence the X-ray picture is in agreement with the non-elastic stretching property of silk fibroin. Any irreversible plastic, stretching of the fibre would, then, mean a displacement in the fibre of the polypeptide chains with respect to one another.

The fibrous protein, keratin of wool, hair, feather, etc, also gives well defined fibre diagrams. The tips of quill feather give the best diagram that excell in complexity and clarity many globular proteins and indicate the high state of orderly crystalline arrangement (32). Hair keratin stretches reversibly when wet or when in dilute alkali. So two forms of keratin can be distinguished. Astbury calls them α- and Beta-keratin. The fully extended keratin which would resemble silk fibroin is the Beta-form, while the natural unstretched form is the α-keratin. Since the stretching is reversible from α- to Beta- no sliding displacement of polypeptide chains with one another occurs. On changing Beta-keratin into the α-form the folding seems to take place on the plane of the chain for the side chain spacings do not change on stretching.

Collagen of connective tissue, tendons, cartilage and other tissue represents a ~~fibrb~~ fibrous protein which is built on a plan different apparently from the plan of the keratin-myosin group. Chemically collagen (and gelatin) has a high proline and hydroxyproline content amounting to a little less than third, while glycine numbers about another third of the total amino-acid residues of the protein. The principal spacing along the fibre-axis is about 2.86 Å which appears to correspond to the average length of each amino-acid residue along the fibre-axis. The back bone spacing (between the polypeptide chains) is very diffuse and amounts to about 4.4 Å, and the side chain spacing in wet gelatin is about 11.5 Å, Although collagen fibres are quite inextensible, the 2.86 Å spacing is much smaller than the 3.4 Å of the Beta-keratin. Astbury (33)* proposes the model :- P-G-R-PG-R- P-G-R- etc. where P (with the exception of one residue in eighteen) stands for proline or hydroxyproline, G for glycine and R for one or another of the ream remaining amino-acid residues. The model chain will, the, look like this:-



The total length of P, G, and R. along the chain axis is 8.55 Å (by calculation). This value, divided by three, gives

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* Cited from : Cohn and Edsall; 'Proteins, Amino-acids and Peptides'; 1943, p. 327.

2.85 Å per residue which is in excellent agreement with the observed value, 2.86 Å. This model still awaits further tests though it successfully accounts for the proline and glycine contents of gelatin.

Summing up the situation, proteins may be of a molecular structure that follow the model constructed in the following manner :- The protein molecule is composed of long polypeptide chains formed by the peptide linking of α-amino-acids. These chains are usually extended or partially extended. They aggregate in bundles in positions parallel to one another, appearing as an orderly pile of plates or planes of chains running parallel to one another. These planes may actually be of very small dimensions, through twisting of the chains. The whole crystalline pattern may be ill developped, in accordance with the diffuse character of the X-ray patterns. The distance between the amino-acid residues is small and differs in proteins being 2.86 Å in collagens and 3.5 Å in silk fibroin. The backbone spacing on the same plane is 4.4 Å in collagens and 4.65 Å in keratins. This spacing contains the carbonyl oxygens and the -NH groups, so it is supposed that the chains are bound together in that plane by a reasonating structure of the two groups, sometimes called hydrogen bond. Between the plates of the pile lie the side chains of the amino-acids in a spacing that averages 10 Å. Along the polypeptide chain the side chains of the residual amino-acids point alternately downward and upward. In the pile the plates would be bound with one another by the functional groups of the side chains, ionizable or not as the case may be. By an orderly distribution of the side chains two planes are produced one of which can be hydrophylic and the other hydrophobic. The remaining two sides of the rectangular cube, formed by the polypeptide bundle, contain the $-NH_2$- and -COOH group at the ends of the polypetide chains. These may combine with one another to lengthen the chain and the combination may be ionic, when it is weak and easily hydrolyzed, or may be a peptide link which is strong. The protein would be hydrated or wet by entry of water molecules into the side chain spacings ionizing its ionizable groups and hydrolyzing chain ends as well, thus finally disaggregating the protein molecules (not the polypeptide chains) and bringing them into solution.

The Value of X-ray diffraction pictures is presently very difficult to judge though it is hoped to increase in importance and usefulness in the future. It should be remembered that the models constructed after X-ray data are interpretations of very few diffuse points in the X-ray diffraction pictures which also change with the methods of taking pictures of the crystals. The interpretations are made by guessing what the sturcture or origin of the spot on the picture is, then computing from the proposed sturcture what the position and intensity of the spot should be. If there is reasonable agreement between the observed and computed values, the approximate assumed structure is modified and refined to give better

agreement. This approach has proved difficult in proteins as not enough is known about the stereo-Chemistry of the protein molecules to permit an intelligent guess.

Proteolytic enzymes and Protein Structure :-

Before ending this section it is noteworthy to mention the use of enzymes in the field of protein structure. Enzymes appear to have specific action on substrates. They would, then, be expected to be useful in determining links in proteins by analyzing the products of proteolysis. But proteolytic enzymes can synthesize as well as hydrolyze, so doubt can be thrown on whether the hydrolysis products are not secondary products of synthesis from the primary hydrolysis, etc. A greater knowledge of the specificity of enzyme action to substrate condition may yield in the future important information about protein structure.

CONCLUSION :- As is the case in all branches of organic chemistry, the best test for the validity of a molecular structure hypotheses is the complete synthesis of the molecule by well understood methods. In protein chemistry this is far from realization due to the complexity of its molecules and their lability and optical acitivity.

In recent years the effect of substances on proteins has been yielding good information useful in structure studies. The second part of ~~the~~ this report deals with and example of a specific case, the study of the effect of urea and related compounds on the power of gelatina of gelatin solutions and the relation of this effect on the structure of gelatin molecules in aqueous solutions at different hydrogen ion concentrations.

PART II

THE STRUCTURE OF GELATIN ; AND THE EFFECT OF UREA AND RELATED SUBSTANCES , AND SOME ELECTROLYTES ON THE RATE OF GELATION OF GELATIN SOLUTIONS.

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INTRODUCTION

The Preparation of Gelatin :

Gelatin is prepared from bones, cartilage, hides and tendons of animals by boiling with water to dissolve the collagens. Prolonged boiling will not only change the collagen into gelatin but will eventually change it into glue. The process of boiling brings about a partial hydrolysis of collagen into gelatin. Since the property of gelatin depends on its history, no two gelatin samples may be considered the same substance. For this reason all the experiments made by the writer were on a gelatin stock of the produce of Merck Darmstadt, Germany.

The Properties of Gelatin :

Physical Properties:- In the dry form gelatin is obtained in glassy sheets or coarse powder. In solution it is colloidal producing sols and gels at sufficiently ligh concentrations. Its solubility is least at isoelectric pH 4.7. The solid gelatin can absorb many times its weight of water while remaining form. Good grades of gelatin have a light pale yellow colour which becomes dark in poorer grades.

Gelation:- Gelatin sols gel on cooling, and the higher the conc. of the sol the quicker its sets and the firmer the gel is. The time it take the sol to gel at a certain temperature is not a simple proportionality with the gelatin concentration. Feldman reports* (14) that the heat envolved during the gelation of the sols decreases when the samples are repeatedly warmed to the sol and cooled to gel. This may be a third of the original value during the sixth cooling. Hence, in gelation experiments the procedure of for preparing the sols should be standardized with minimum melting of the stock solution.

The time of gelation of the sol is also dependent upon its pH, the shortest gelation time being given at about the isoelectric point, so pH. control should be observed in gelation experiments. This difficulty was solved by the writer on the expence of the first difficulty, repeated melting. It was found that the pH of the sols changes appreciably with temperature, so the temperature at the pH determination should be controlled and standardized. In practice that is difficult to observe and as a consequence appreciable experimental error is introduced.

Viscosity: Variables in the viscosity of gelatin sols are too many. On changing the temperature of a sol the

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* Br. Ch. And Phy. Abstracts, 1942, I 59.

viscosity of the solution does not come to an equilibrium quickly especially when the temperature drops. So in viscosity measurments standard conditions should be maintained.

Absorption spectrum : Gelatin is found to give an absorption band spectrum between 2500 and 2300 Å. A true light absorption is difficult to obtain because of the diffuse nature of the dispersed light. The work of Custers et.al. (16)* show that the light absorption by the gel and sol is almost the same except that the peak 2500 Å is lower in the gel state. In the range of pH 2.5 to 8.6 the true light absorption is independent of pH, while diffuse dispersion reaches a maximum at the isoelectric point. According to Custer et.al. the light absorption by gelatin is independent of the -COOH and $-NH_2$ functional groups of the protein.

Refractive index : The transformation of the sol into gel produces an alteration in the refractive index N of gelatin. N for isoelectric gelatin is found to decrease(+) on standing. This is due to the formation of a flocculent precipitate that forms slowly and separates. The refraction depends, also, on the history of the gelatin, and upon its innization.

Light scattering : Light scattering by gelatin (Tyndall effect) has been well investigated. Dhéré and Gorglewski found pruified gelatin solutions turbid at below 30°C. and isoelectric pH. Raising the temperature or adding base or acid diminished the turbidity. The turbidity is maximum at 2.5% gelatin concentration. The variables in this light scattering, then, are temperature, conc. of gelatin and the pH of the solution. As a consequesnce, it can be used to determine the isoelectric pH. of the gelatin sample. On mixing gelatins of different maxima ~~pHs~~, one variety usually shows predominance over the other.

The increase in turbidity of gelatin solutions by lowering their temperatures is limited to the pH range 4.5 to 7.5 for a sample of gelatin. This turbidity, is, then, not linked directly with the phenomenon of gelation as the latter can occur beyond the pH range given above. The effect on turbidity produced by H^+ and OH^- is also produced, though less effectively by neutral salts and certain nonelectrolytes such as alcohol, urea and amino-acids.

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* Cited from : C.Schmidt, Chemistry of Amino-acids and Proteins 1938, p.560

(+) " " C.Schmidt, Chemistry of Amino-acids and Proteins 1938, p.563

Krishnamurti (17) carried out a study of the relation of temperature and concentration of gelatin with light scattering. Light scattering by 2% sol is not changed on cooling from 50° to 25°C., but below 25° there is a steady increase in turbidity. The rate of change of scattering is not the same as when the gel is warmed. On cooling the 2% sol the abrupt change in scattering occurs between 25° and 10° while on melting the 2% gel the same reversed change occurs between 18° and 28°, which is an expression of the time lag in the rate of change of light scattering. The light scattering by the sol increases in intensity to a maximum at 2% gelatin beyond which there is no increase with increasing concentration. In the gel state beyond the 2% there is a decrease in intensity of scattered light.

Optical Rotation : In its optical rotation property gelatin is not a good example of proteins being unique in its behaviour and does not show the variations with pH. common to other proteins. According to Kraemer and Fanselow (19) the change in rotation produced by pH. cannot be correlated with influences arising from dissociation. Their work as well as that of Smith (20) indicate a strong parallelism between the changes in optical rotation and sol-gel transformation. At above 35°the specific rotation is constant and independent of concentration, pH. , and temperature of gelatin in the presence of some electrolytes. Cooling to 30°produces a large increase in the levo-rotation of gelatin, which continues for several days at that temperature until it attains a constant value. Below 15°a further temperature lowering produces slight changes in rotation. In the opinion of Smith the changes in the region of 30° to 15°are due to mutarotation, the phenomenon being reversible by changing the temperature. This behaviour of gelatin led Smith to postulate the existance of two forms of gelatin that are in thermal equilibrium with one another, one existing in the sol, the other in the gel and each being characterized by a distinct specific rotation value. The sol form should be unstable below 30°and the gel form above 15°. Since the course of the change in the rotation followed a second order reaction, he assumed that the sol molecules (think of) associate into gel molecules. It is not needed to forms of gelatin as two chemical substances, for the optical rotation of a compound depends on the presence of other polar compounds. So simple association or dissociation of the gelatin molecules or its polypeptide chains will probably influence the rotation.

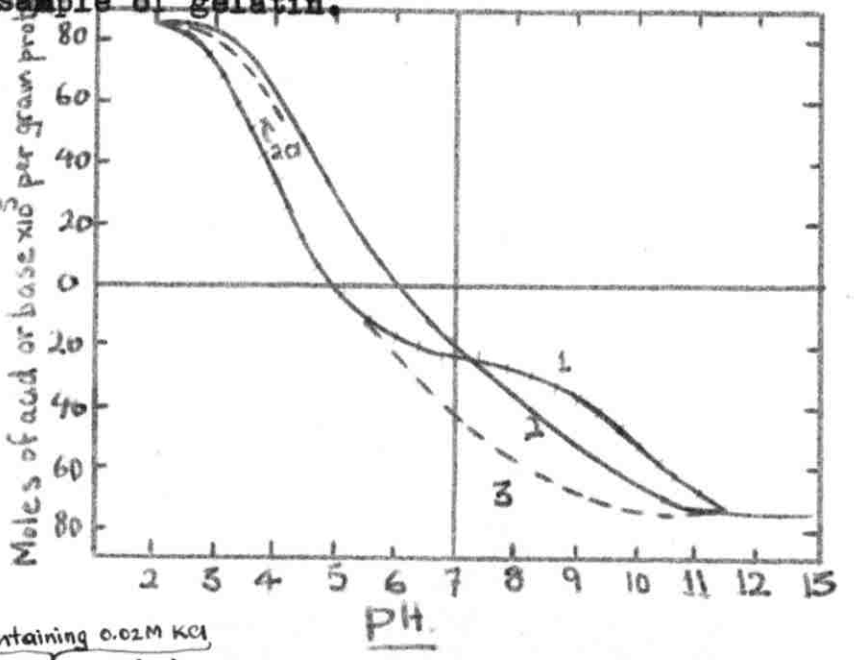
Rotary Dispersion : Carpenter and Lovelace (21) made the most complete study on the influence of neutral salts and urea on the rotary dispersion of gelatin. There is relation between the rotary dispersion and the absorption spectrum of gelatin. in its simplest form this relation is controlled by a single term Drude equation which locates 2200 Å as the wavelength of the gelatin absorption spectrum. At 40°the dispersion constants bear a linear relation to the concentration of the halide salts or urea. At 0.5°the relation gives a curve composed of two parallel lines that meet in a form indicative of association or dissociation. From this Carpenter et. al. conclude that the transformation embodied in the change involve associa-

tion or dissociation. Estimation of the number of optically active components in gelatin indicated two which is a confirmation of their theory. L. pauling* remarks, ' if the changes in rotation depend upon an equilibrium between a state of association and that of dissociation, then the same effect as brought about by temperature and halides should be brought about by changing the concentration of gelatin. It is more likely that one gelatin is in two tautomeric forms which have different optical properties. '

Agents that inhibit levo-rotation of gelatin also impairs its gelation reversibly or irreversibly. Heating gelatin at 140° for a few minutes not only destroys mutarotation irreversibly but also destroys the power of gelatin to form gels irrepairably. The influence of salts was investigated by Carpenter and his coworkers The salts of weak organic acids have a little influence while the neutral salts followed the Hofmeister series ($CNS^- > I^- > Br^- > Cl^-$) in their ~~influence~~ capacity to lower rotation. Their effect as well as that of urea is paralleled by a similar inhibition of gelation, their effect being reversible, i.e., on their removal by dialysis the levo-rotation is recovered.

Chemical Properties of Gelatin :

Like all naturally occuring proteins, gelatin is amphoteric as evidenced by its acid and base binding property. As it is composed of acidic and basic amino-acids, the titration curve for gelatin, illust. I , corresponds to that of a mixture of the amino acids that make it. Illustration II gives the amino-acid content of a sample of gelatin.



Illust. I :(1) The titration curve of gelatin in water, (2) in 80% ethanol, (2a) is derived for 2 without KCl, (3) in 1% formaldehyde slution. Reproduced from Cohn and Edsall,'Proteins, Amino-acids, and Peptides',1943, p.493, based on data of Lichtenstein, I., Biochem. Zeit., 303, 20 (L940) in Webers laboratory.

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* Cited from Schmidt,'The Chemistry of Amino-acids and Proteins' L938, p.593,in a personal communication to the book's auther.

Amino-acid	Mol. Wt.	% of amino- acid residue
Glycine	57.03	19.38
Alanine	71.04	6.94
Serine	87.08	2.73
Threonine	101.10	1.19
Leucine, and iso-	113.08	6.12
Proline	97.08	16.62
Oxyproline	113.08	12.42
Phenyl- alanine	147.08	1.25
Cystine	222.18	0.17
Histidine	137.08	2.60
Arginine	156.08	7.78
Lysine	128.08	5.19
Aspartic acid	115.08	2.94
Glutamic acid	129.08	1.70
Glutamine	128.06	3.44
		Total 90.47 %

Illustration LL , Amino-acid content of gelatin. reproduced from Cohn and Edsall,'Proteins, Amino-acids,and Peptides', 1943, p. 368.

The Structure of Gelatin Molecules :

A number of investigators have shown that gelatin is not a homogeneous protein. It represents a mixture of degradation products of collagen. Its properties are dependent on its history. Mosimann and Signer*(24) in the Svedberg institute report two gelatin fractions of mol. wt. 89,000 and 16,000 characterized by the determination of sedimentation constants in the ultracentrifuge. The former (HF1) consists of lattice type molecules and the latter (VI)of short straight chain or branched chain type molecules. After fractionation (HF1) still showed polydispersion while (VI) appears quite uniform. It appears, then, that a hypothesis on the structure of gelatin is difficult to formulate. So far no one hypothesis could explain all the facts about gelatin. It should also be remembered that not the same gelatin was used in all the experiments from which data was obtained, e.g.,the isoelectric point of various gelatins range from pH. 4.4 to 8. The situation can be summarized in the following:

Gelatin appears to be composed of long and short fibrillar molecules of molecular weights ranging from 16200 or less to 89000 or more. These molecular species seem to have different characteristics and each of them of unknown chemical composition. It is not possible to say if at any particular state of gelatin they are combined with one another. Two kinds of molecules are claimed to exist one being the product of association or dissociation of the other. It is also claimed that, optically, there is no difference between the molecular species that can undergo mutarotation, one form stable above 30°and the other below 18°, while

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* Cited from Br. Ch. and Phy. Abst., 1945, III, 316.

any substance that produces mutarotation will disturb the balance between the sol and gel form states. Little is known of the reasons of gelation. It does not seem to be binding of water, though water may be bound in gelation. Busse* has an explanation for gel fomation in general. In order that a system may have elastic proerties it should have, he claims, (a) long fibrous molecules,(b) weak or uniform cohesive forces around the fibers, (c) an interlacing of the fibers, (d) and a means of storing up free energy when the fibers are distorted. X-ray investigations show that gelatin gels consist of miscelles, built up from long polypeptide chains which are loose at the ends, but bound three dimensionally at the middle. The loose ends form the means of attachment between miscelle and miscelle. The opacity produced by cooling gelatin sols at the iso-electric point is not directly related to gelation nor are the reasons of its formation known. The experiments reported in the section,'Experimental', indicate that some substances inhibit, others promote, and glucose has no effect on gelation. As gelation is tied up with rotary dispersion it would be interesting to compare the effect on dispersion caused by these compounds and their influence on gelation. In the process of gelation heat is evolved showing that the gel is thermodynamically more stable than the sol. In my opinion gelation can be thought of in the following manner:-

In the solid state gelatin gives a very diffuse X-ray picture with few clear spots in it. It means that there are in the solid many lattice ceters. These centers are not orderly bound to one another; instead alot of branching, twisting and interlacing may exist. The lattice centers are composed of aggregates of long polypeptide chains that are bound by residual valencies. If the solid gelatin is soaked in water , a liquid of high dielectric, constant, the ionizable valencies are broken up or hydrolyzed, and the miscelles are broken apart. when the amount of polar liquid solvent is large, the dispersion is complete and the gelatin is dissolved. When the residual valency bonds become weak but still hinder the free movement of the miscelles,i.e. the flow of the solution, a gel is obtained. The addition of salts may ionize or associate the gelatin miscelles producing an inhibition or promotion of gelation but the phenomenon of salting out and salting in is not well understood. Since the residual valencies include the possibility of hydrogen resonance bonds at one of the planes of the miscelles lattice, it would be expected that small molecules of similar resonating structures can associate with the polypeptide chains at the site of the resonance bonds. This would weaken the bonds so that when the concentration of the small molecules like urea is high enough the gelation is completedy inhibited. This condition is found true for a number of water soluble compounds of high resonance energies like urea, allyl-urea, thio-urea, thioacetamide, guanidine-HCl, s-diphenyl-guanidine hydrochloride, etc. Thus resonating compounds influence gelatin sol-gel transformation probably by hindering the resonance bond formation between the aggregates of the polypeptide chains,

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* Cited from : C.Schmidt, 'Chemistry of Amino-acids and Proteins' p.449 (1938)

while electrolytes and polar solvents will hinder and in some cases promote the acid-base bond formation.

It should not be very difficult to verify this opinion. X-ray diffraction pictures of gels containing urea and related compounds should show a more diffuse and wider resonance bond spacing while the inhibiting electrolytes should show wider side chain spacings between the polypeptide chains.

Experiments to show the inportance of the residual valency bonds and more particularly the resonance in gelation of gelatin were planned. The inferences from the results of the performed experiments have already been discussed in the preceding paragraphs and a discussion of the experimental part follows.

EXPERIMENTAL

Methods, Apparatus, Equipment, and Preparation of Solutions.

Gelatin stock solutions were prepared in 10% concentration in buffered distilled water and titrated to the desired pH value by the components of the buffer used. The buffers were either a mixture of 0.2 M Na Acetate + 0.2 M Acteic acid, or 0.2 M Na_2HPO_4 + 0.1 M Citric acid, or 0.2 M Na_2HPO_4 + 0.1 M NaOH depending upon the pH value required as indicated in the tables at the end of the report. To avoid excessive wastage a small sample of gelatin would be titrated with the components of the buffer to the desired pH, the amount of the component to produce the pH is calculated for the quanttity of gelatin to be used in the stock solution, added to the gelatin, and the volume of the solution was made to 10% gelatin concentration by the buffer, and the pH value of the solution checked. Gelatin was melted the least number of times and excessive heating and rise in temperature was avoided. Since the pH value for gelatin solutions changed with temperature which could not be well controlled, about 30°C. was the average temperature at which its pH was determined.

The substances used for finding their influence on gelation were used in solution form in the presence of buffer at equal concentration to that in gelatin. All dilutions were made with the same Buffer solution. Where buffering is not indicated it was not used in any of the solutions which were brought to the desired pH by the acid or base component of the electrolyte used in the experiment.

The pH determinations were made by a glass electrode against a calomel electrode of a Beckman pH meter," Beckman instruments, model G." and the readings are accurate to the first decimal, the second being an approximation. The instrument has a temperature compensator and the temperature at which the readings were made were not recorded but for other than the gelatin stock solution was ususally between 18° and 23°C. The instrument is adapted to determinations of very small quantities and in some determinations ½ cc. was used.

Since some of the reagents were in short supply and the solutions had to be at high concentrations, the volumes of solutions used in the gelation tests were necessarily small. For the purpose of volume measurements capillary pipettes were specially constructed and caliberated at 0.05 cc. graduations with total capacity of 0.6 cc., 0.2 cc., and 0.1 cc.. 2 cc. and 4 cc. beakers were made for the pH determinations and glass tubes of equal dimensions diameter and make. were used for gelation.

In the gelation experiments all the samples for testing were kept in a water bath at 37°C for about 30 minutes and then immersed in the cooling bath kept at 10°C. The accuracy of the heating bath was unnecessarily high ± 0.005 °C and the accuracy of the cooling bath was ± 0.08°C. The heating bath was a Fischer Scientific Company model with an ether-mercury thermostat. The cooling bath was constructed in the laboratory out of reclaimed meterial. Cooling was produced by immersing in the water a cylinder containing chipped ice and the heating was by a 125 W. heating knife. A thermostat immersed in the bath kept the temperature at 10° by balancing the heating with the cooling. The thermostat was like the heating bath one but contained ethyl chloride instead of ether. It was connected to a 7 volt source of current and to the coil of an electric bell. In the electric bell the 7 volt current was made to run through the coil separately and the heating current circuit passed thru the arm of the bell. In this arrangement the bell acted as a relay closing and openning the heating circuit by the lowering or rising, respectively, of the mercury column in the thermostat.

As gelation is not a sudden change of state especially in the apparently dilute gelatin solutions where the gel never becomes firm, the sol was considered changed to a gel when it did not flow any more in the tube. A stop watch was used to measure the time in minutes and seconds its takes the sol to become gel on cooling.

Experiments and results

At the beginning of the work no systematic study could be immediately made. The influence of temperature changes and pH changes had to be found and evaluated and then regulated. The discovery that a new compound was reactive one way or the other made place for more experiments or the testing of new reagent compounds. Some of the chemicals arrived later in the course of the investigations and some others were found effective later too as in the case of sulfates, Thioacetamide and s-diphenyl-guanidine.HCl. In the case of Thio-acetamide, Mr. Heller, working in the same laboratory, found it quite effective in lowering the viscosity of actomyosin solutions. This suggested its use with gelatin, and it came out to be highly prohibitive of gelation comparable with the strongly effective Thiourea and guanidine hydrochloride. At one stage in the development of the experimental methods viscosity was hoped to be an easier problem to tackle and the experiments would involve less errors than the gelation experiments. The idea was experimentally investigated. An Ostwald capillary pipette was used for the viscosity determinations, and a trial was made with solutions of urea containing different percentages

of gelatin to obtain the more suitable concentrations. The solutions were kept in the ice box and when used were heated at 37° for 30 min. and their viscosity determined at 23°C. At this temperature the viscosity was not constant; there was a time lag with rate of cooling, and the extent of the lag differed in the different solutions. To get consistent viscosity measurements an arbitrary set of conditions had, therefore, to be chosen and being arbitrary the situation with viscosity was not better than that with gelation errors. Furthermore, with gelation experiments there was the technical practice gained in several months of investigations; also in viscosity experiments large volumes of solutions involving large quantities of scarce reagents made the viscosity investigations undesirable.

The factors involved in affecting the gelation time were found to be (a) the previous history of the gelatin solutions (b) the accuracy in preparing the solutions and in volumetry measurements (c) the pH of the solutions had to be the same for each investigation of the effect of concentration of the reagent on rate of gelation (d) the constancy of the temperature of the cooling bath and (e) the definition of the gel state, i.e. when the solution can be considered a gel. Other minor factors were also previously considered. Many experiments, whose results are not recorded in this paper, were performed to control the five factors mentioned above. The procedure was then standardized and the methods used were explained in the preceding section.

It was contemplated to study a large number of urea and related compounds. Unfortunately, a number of them were insoluble or slightly so. Of these it is possible to mention acetyl methyl urea, methyl iso-thiourea sulfate, thio-semicarbazide and several others. A few were soluble only in acid medium and their use was limited to that condition. Semicarbazide HCl showed a definite and marked prohibitive effect on the rate of gelation; but as it is acidic and the pH of its solutions cannot be controlled without unsurmountable difficulties, it was dropped out of the list of substances to be investigated. The list of substances investigated includes :-

Urea, Methyl urea, allyl urea, thiourea, guanidine-HCl, s-diphenylguanidine HCl, acetamide, thioacetamide, glycine, alanine, KCl, KNO_3, K_2SO_4, Na_2SO_4, $MgCl_2$.

The effect of those compounds on the rate of gelation of 5% gelation sols, in some cases 3% gelatin sols, with or without buffer was studied at the pH values 4.7, being supposed to be the isoelectric point of gelatin, 8.0, 11.5, 3.45 and in some cases about 2.7 instead of 3.45. The tables at the end of the report give a complete record of these experiments and their results. The diagrams of curves (after) preceding the charts represent the results of the experiments expressed in cartegian coordinates and graphical lines. Each curve is labled with the name of the reagent whose effect on the rate of gelation it represents, also the no. of the chart from which data used for constructing the curve is

mentioned. In some of the diagrams a gelatin curve is drawn. These gelatin curves are for the time of gelation of varying concentration of its solutions with no reagent but buffer added to it. It will be noticed that the curves of the rate of gelatin are drawn for the time of gelation against the molar concentration of the added reagent.

The influence of the compounds, considered, on gelation can be classified into three classes (a) inhibitors, (b) promoters (c) neutral. The following is a list of the compounds used in buffered solutions in the order of their decreasing inhibitive effect, and reference is made to the number of the diagram in which the curves for the mentioned compounds appear for closer comparision.

pH 4.7, Acetate buffer. diag.2, 5% gelatin:-

s- diphenyl guanidine HCl, Thiourea, Guanidine HCl, Allylurea, urea, KCl and Methyl urea, acetamide (KCl is more inhibitive than methyl urea above 1.34 molar cons.

pH 3.45, Citric acid - Na_2Phosphate buffer, diag.3, 5% gelatin:

s-diphenylguanidine HCl, Thiourea, Thioacetamide, Guanidine. HCl, Allylurea, Urea and Methyl urea, Acetamide and KCl (Urea is more inhibitive than methylurea above 0.6M. concentration, and KCl is more inhibitive than acetamide above 1.0M. concentration.

pH 8.0, Citric acid - Na_2Phosphate buffer, diag.4,5% Gelatin:

Thiourea, Thioacetamide, guanidine HCl, Allylurea, Urea, Methyl urea, KCl, Acetamide. (Urea is more inhibitive above 0.6M. conc. than Methylurea as at pH 5.45) Glycine promotes gelation.

pH 11.5, NaOH - Na_2HPO_4 buffer; diag.5, 5% Gelatin:-

Thiourea, Guanidine HCl, Urea, KCl.

What follows is a discussion of the results obtained in the gelation experiments. Because the rate of gelation of gelatin solution changes with concentration and hydrogen ion concentration as evidenced by the gelatin curves in diagrams 2,3,4,and5. etc., a comparison of the effect of the compounds under investigation cannot be directly compared at different pH. values. A method had to be developed and the easiest and apparently justifiable method will be explained and used. The explanation shall be by an example taken from diagram 2. and appears in diagram 13. In diagram 2 appears a gelatin curve which stands for the change in the time of gelation for a change in the gelatin concentration. In the same diagram the Allylurea curve indicates the change in the rate of gelation of a 5% gelatin solution for a change in the quantity of allylurea added to that solution. It will be noticed that while untreated 5% gelatin gels at 10°C in 75 seconds, the same solution but containing allylurea gels at more than 75 seconds. Eventually the addition of more allylurea will

completely inhibit gelation and the curve becomes paralled to the time axis. The same effect as produced by allylurea and other inhibiting agents is produced by decreasing the concentration of gelatin solution. It is therefore assumed that the allylurea decreases the concentration of gelatin capable of forming a gel. This is not necessarily so because in reality the gelatin molecules may not be divided into aggregating and completely disaggregating forms but the effect of allylurea may be to weaken some bonds that act in aggregation which is probably the case. Since in both explanations the statistical result as to the inhibiting power remains the same the first assumption is made use of. To show that the damage done to the gelatinn is dependent on the amount of reagent and not the concentration of gelation used in the solutions a trial with thiorurea, guanidine.HCl, and KCl was made at pH 3.45 in which a molar conc. of these reagents was chosen, kept constant and the concentration of the gelatin changed. The results appear in diagram8. A gelatin curve was made and a curve transformation was made on each of the curves of the three compounds. The new curves are shown in diagram 9. as an example of how curves 9 were constructed, we take Thiourea curve. At 9% gelatin the solution containing thiourea behaved as though it really was of 6.5% gelatin concentration. It means that the thiourea 0.2 M., effected an apparent 2.4 drop in the percentage of gelatin in solution. The same is made for other gelatin concentrations and a curve is drawn, diag. 9, for the apparent drop in gelatin conc. by 0.2 M. thiourea against the actual gelatin concentration. If the effect of thiourea is independent of excess gelatin a straight line curve should be obtained, that is almost true especially at concentrations of less than 6% gelatin. Above this concentrations other factors may also be effecting the gelation. Notably the fact that the contribution of the non-gelating gelatin to the viscosity of the system cannot be neglected, especially at higher concentrations. This increase in viscosity would promote the early setting of the gel. The assumption that the effect of thiourea, guanidine.HCl, and KCl in apparently diminishing the concentration of gelatin is independent of excess gelatin concentration and depends upon their concentration is almost correct and is also probably so for other compounds. Returning to the Allylurea curve of diagram 2, a curve transformation can therefore be safely made for it and in which the molar concentration of the Allylurea is drawn against the apparent decrease in gelatin concentration and the new curve appears in diagram 13. This curve is important because the real gelatin concentration is eliminated and it represents the disaggregating influende allylurea has on the gelatin. Similar curves can be drawn for other pH values and the effect of pH on the disaggregating activity of Allylurea can thus be studied. Such curves were constructed for all the reagents appearing in diagrams 2,3,4,and 5. and are shown in diagrams 13, 14, 15, and 16 with the exception of the gelation promoting amino-acid glycine. A discussion of each of the reagent compounds will now be made separetely.

Urea :- was investigated at pH 4.7, 3.45, 8.0, 11.5. It was prepared in concentrated solution for each pH value and the results appear in charts 2, 16, 31, and 41 and as curves in diagrams 2, 3, 4, and 5. It is noticeble that the approximate minimum requirement to completly inhibit the gelation of 5% gelatin is 1.6 M. at pH 4.7, 1.2M at pH 3.45, 1.4M at pH 8.0 and 0.45 M. at pH 11.5. Diagrams 13, 14, 15, and 16 contain the curves of apparent change in gelatin concentration with increase of urea concentration at the four pH values. A comparison of these curves indicates an apparently irregular change with pH with pH 3.45 the most reactive medium other than pH 11.5 whose curves are not complete for any important change of concentration.

Methyl urea:- charts 3, 17, 32, investigated at pH 4.7, 3.45, 8.0 Activity is greatest at pH 3.45 with the other two pH values almost similar.

Allyl-Urea :- charts 4, 18, 33, investigated at pH 4.7 3.45, and 8.0 Activity is greatest as pH 3.45 and similar at other acidities mentioned.

Thiourea:- charts 5, 19, 34, 42, investigated at pH 4.7, 3.45, 8.0, 11.5 and found more inhibition at pH 3.45 though the tendency at pH 11.5 is toward a greater inhibition.

Guanidine.HCl:- charts 6, 20, 35, 43, investigated at Ph 4.7, 3,45, 8.0, 11.5 and found least active at the akaline pHs and most active at pH 4.7.

s-diphenyl guanidine.HCl:- charts 7, 21. investigated at pH 4.7, 3.45. at the other pH values s-diphenyl guanidine is insoluble and could not be tested. Its inhibiting activity was greater at the isoelectric pH unlike the first 4 compounds. It should be remembered that it is a salt and in behaviour reacts like its relative guanidine hydrochloride another neutral salt.

Acetamide :- charts 8, 22, 36, at pH 4.7, 3.45, 8.0. It is definitely more reactive at the acid sids of the isoelectric pH and its reactivity at pH 8, and 4.7 is almost the same.

Thioacetamide:- charts 23, 37 at pH 3.45 and 8.0. Again a slightly greater reactivity appears at pH 3.45 especially at the higher concentrations.

KCl :- charts 9, 24, 38, and 44. Another salt, investigated at pH 4.7, 3.45, 8.0 and 11.5, again has a higher reactivity at the gelatin isoelectric pH 4.7. See also chart 47 a,b,c, for the tests without buffer .

KNO_3 :- chart 48, a,b,c. for pH 4.8, 2.6 and 11.65. KNO_3 has an inhibitive effect on gelation at all three pH values, and is very much more inhibitive than KCl, and $MgCl_2$ at all the pH values investigated. Its solutions were prepared to the desired pH without buffer. Instead nitric acid and KOH were used to adjust the pH values.

K_2SO_4 :- charts 49 a, b, c. without buffers at pH 4.7, 2.8 at 11.7 also charts 12 for pH 4.7 and charts 28 a,b for pH 3.45 with Citric acid -Na_2HPO_4 buffer. When no buffer was used the pH of the solutions (charts 49 a, b, and c.) were adjusted with H_2SO_4 and KOH solutions. Tests were made with 5% and 3% gelatin. Results showed marked promotion of gelation. At pH 4.7 where no buffer was used and the gelatin concentration was 3% the promotion was suddenly reversed at 0.25 M. K_2SO_4, but before the time of gelatinn of 3% pure gelatin solution was reached a salting out of the gelatin occured. Salting out was easier at the acid side of the pH and was always accompanied by turbidity before the salting out stage occurred.

Na_2SO_4 : charts 52 a,b,c. at pH 4.7, 2.8, 11.3 - 11.68. Its solutions were not buffered and were brought to the required pH with NaOH and H_2SO_4. High concentrations of Na_2SO_4 could not be obtained because of its low solubility. It was promoted the gelation at all 3 pH values.

$MgSO_4.7H_2O$: charts 51 a,b. at pH 4.7 and 2.8. Being insoluble in alkaline solutions it was not studied above pH 4.7. $MgSO_4$ was found to change appreciably the pH of buffers or gelatin solutions. It was, therefore, necessary to avoid the use of buffers and as a result a number of slats were investigated without buffers. Like K_2SO_4 and Na_2SO_4, $MgSO_4$ promotes the gelation of 3% gelatin sols.

$MgCl_2.6H_2O$:- Charts 50 a, and b. at Ph 4.7 and 2.75 was used with no buffers. It was found inhibitive towards gelatin gelation at both pH values but less so than KNO_3 as previously indicated.

Glycine : at pH 8 in citric acid -Na_2HPO_4 buffer (diag. 4) glycine proved to be a gelation promoting agent. This property was retained at pH 11.5 in Na_2HPO_4 even thout at this pH it should be present as the sodium salt, yet At 0.5M. conc. at pH 11.5 (diag. 5) its gelation curve has a minimum showing maximum promtion after which its power decreased. This property of glycine, an amino-acid, made an investigation of of other amino-acids important though limited by their solubility. At pH 4.7 glycine is almost isoelectric and is therefore, zwitterionic. By pH 3.45 it had started to be a positive ion and is less promting (by comparison of its curves in diagram 6 and 7 with respect to the apparent increase in gelatin concentration)

Alanine :- what was said about glycine at pH 3.45 and 4.7 can be equally said about alanine at the same pH values. Refer to diagram 6 and 7.

Glucose :- was the only compound tested and found unreactive i.e., had practically no effect on the rate of gelation (it had only very slight promoting effect and has to do probably with increase in viscosity bringing an early state of gelation.) Refer to diagrams 6 and 7 or charts 11 and 29 at pH 3.45 and 4.7 in the presence of citric acid -Na_2HPO_4

buffer.

In the hope of finding the importance of the functional groups of gelatin in the process of gelatinn and their reàation to the effect of the previously discussed compounds, it was desirable to obtain chemical variations of the gelatin that has gelation properties in order to find the effect of the compounds on them. A removal of the amino-groups was the first to be thought of. Free amino groups exist at the end of polypeptide chains, in the lysine residue and in the arginine residue where it is a guanidino group. Gelatin was deaminized with $NaNO_2$ and Acetic acid following the method of Blasel and Metula (22) but for the product the Sakaguchi test, modified by Weber (25), was positive indicating that the guanidino radical was not completely destroyed. The deaminized gelation obtained in this manner could be easily salted out at the acid pH by addition of saturated $(NH_4)_2SO_4$ solution, also precipitated with alcohol and acetone. It continued to possess gelation properties that was impaired by urea but its gelation was very poor in alkaline medium. This modification of gelatin was not used in any quantitative determination. An effert was made to destroy the guanidino group. Alexander et.al. (26) report that acetic acid does not produce enough acidity to destroy argining by deamination with nitrous acid. The arginine was changed to ornithine only in a highly acid medium produced by the incprporation of 20% acetic acid and 6% H_2SO_4. It was tried to apply this to gelatin and the pH was lowered to 2,1 and even 0.5. The Weber modification of Sakaguchi test was still positive after 16 hours of deamination and eventually small amounts of yellow substance precipitated and nothing could be salted out. The yellow precipitate was not a gel nor had gelation properties. This reaction needs more investigation and may be a valuable procees. The Sakaguchi reaction was then tried with NaOBr instead of NaOCl and by the time the Sakaguchi test was nearing to negative a precipitate was formed that did not gel and was not further investigated salting out with Na_2SO_4 yielded no valuable products and so both attempts at complete deamination failed.

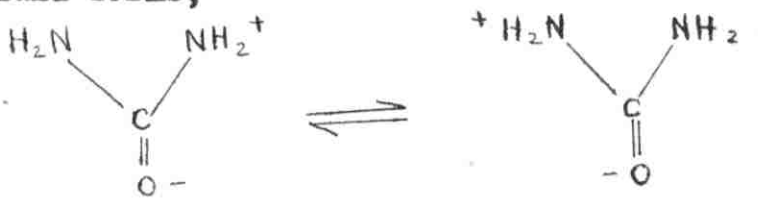
The acetylation of gelatin was then contemplated. Blackburn and Phillips (23) described the methylation and acetylation of a number of insoluble proteins including collagen. The same procedure for acetylation was applied on air dried gelatin coarse powder. The gelatin was covered with acetic anhydride and refluxed for ½ an hour. much swelling occured and so much anhydride was later added to keep the gelatin covered. The product was a deep lemon-orange in colour, insoluble in water, soluble in NaOH in whose solution did not gel at 0°C and showed no signs of apparent increase in viscosity at -6°C. As all the modifications in the gelatin were of little use and because of the limited time, of investigations were stopped that far on this line of the work.

Interpretation of results :

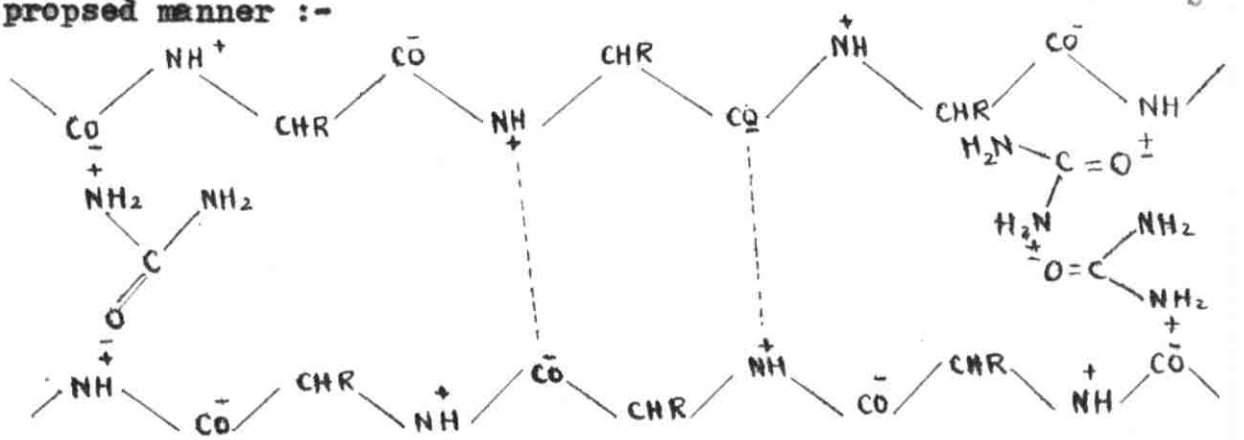
It was been observed that some substances caused inhibition and others caused promotion of the gelation of gelatin

solution s. The inhibition, being produced by a large number of compounds, electrolytes and non-electrolytes, its is not likely that the mechanism of inhibition be the same in all the investigated cases.

It could be understood that inhibition by the neutral slats, KCl, KNO_3, $MgCl_2$ etc. would be brought about by preventing the ionic bonds of gelatin from functioning in aggregating to miscelles together in order to produce an elastic gel. The prevention may be by increasing the ionic diameters of the gelatin ions by the accumulation of the ions of the added electrolyte around the gelation innic centres. The larger ionic diameters would loosen the electrostatic bonds that hold the gelatin aggregates together in the gel. With urea and the other inhibiting non-electrolytes, the mechanisms of inhibition cannot be a loosening of the electrostatic bond. Because urea molecules have a resonating structure giving it a polar character it would be possible think of the effect of urea by a weakening of the H-resonance bonds that may exist inbetween the polypeptide chains as explained in Part. I. Urea could be written structurally in two formula forms,



while the 4.5 Å spacing between the polypeptide chains of a protein are supposed to be regulated by a H-bond which are produced by resonating structures of the peptide chain very similar to that produced by urea and related compounds as well as by the amides. Since the polarity of urea is due to its resonating structures, if the resonance energy is high enough, i,e., its polarity is well pronounced, it can penetrate the 4.5 Å spacing and associate with the polypeptide chains at the site of the H-bonds in the following propsed manner :-



In this way the 4.5 Å spacings will be widened at the site of urea interference where the bonds would be weakened and the strength of the gel would become poorer resulting in complete inhibition of gelation in enough urea concentration.

If the hypotheses given above are true it can be predicted

that whereas H-bonds should not show much dependence on pH, the ionic bonds should have an increasing importance at the isoelectric point of gelatin when the protein is most completely ionized, and its importance is shown by the fastest gelation and firmest gel formed at the isoelectric point.

In comparative investigations at different pH values, use is made of the curve transformations, previously discussed, so that direct comparison becomes possible.

From the phypothesis it would be predicted that the effect of the ions of the electrolyteds should be more pronounced at the isoelectric point as both anion and cation have the chance to inhibit gelation although between pH 4.7 and 8.0 not much change is expected in accordance with titration curve of gelatin, illust. I.

Inhibition by urea should by itself not depend much upon the pH, but if the importance of the ionic bonds decrease at the deviating pH, the relative importance of the Hbonds become greater in producing a gel and therefore inhibition by urea and the related substances would appear to increase. Not much difference is expected between pH 4.7 and pH 8.0 as the ionic state of gelatin is not very much different as revealved by the gelatin titration curve, illustration I.

Enough experimental data are available to test the correctness of the predictions made from the proposed hypothesis. The comparisions of the transformed curves in diag. 13, 14, 15 and 16 are in good agreement with the prediction. It should be rememberd that though guanidine.HCl and its derivative s-diphenylguanidine.HCl have high resonance energies there behaviour would not be expected to be different from other inorganic electrolytes as observed in the experiments.

The results obtained with the salts are in agreement with those obtained by other investigators on the modulus of elasticity of the gels with salts added.* Fluorides and sulfates increase the modulus while chlrides and nitrates decrease it. Fluorides were not tried in the gelatin experiments. The behaviour of sulfates and amino-acids and their salts cannot be explained in detail but it is known that sulfates produce volume contraction in solutions.

In conclusion we find that the effect of urea and related compounds can be predicted and explained on the assumption that they loosen the H-bonds of the protein by associating with it at the site of the same bonds. It is also possible that urea has a freer movement into the polypeptide chains
gelatin

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* Cited from :Schmidt, 'Chemistry of Proteins and the Amino-acids,' 1938, p. 447.

through the side chain spacing 11Å when the ionic bonds loose importance in aggregating the gelatin miscelles at deviations from the isoelectric point.

Experimental Errors:-

In the gelation experiments a large error factor exists. As the time of gelation becomes larger, it becomes difficult to distinguish the moment at which the gelatin mass satisfies the gel definition, because the gel looses its firmness as the gelatin concentration is lowered or as the concentration of the inhibiting substance is increased. This arbitrariness in the end point may be the cause of appreciable error and its extent may not be equal in all the experiments.

The influence of buffer at the concentrations in use was not investigated. Because each stock solution had to be brought to the required pH. by the component solutions of the buffer or component acid or base of the electrolyte in some of the experiments, and since these quantities varied as well as variations in the volumes of the stock solutions used in each test sample, it would be expected to have some errors because of this situation.

As the gelation properties of gelatin solutions diminished by repeated melting, which cannot be avoided, the results for those substances that were tried at the end would be irrelevent with those obtained at the beginning.

It is assumed that the time of gelation of the gelatin without added reagent is the same at all times, while this is not so. This may be the cause of undue curvature of some curves of diagrams 13, 14, 15, and 16 representing the apparent drop in the concentration of gelatin with increase in the concentration of the reagent.

There could have been errors in measuring volumes , especially those of the viscous gelatin solutions. This error would bring about deviations of the experimental points from the smooth curves of the different diagrams representing the time of gelation with change in reagent or gelatin concentration.

The extent of the above mentioned errors, whether they are additive or diminutive, cannot be numerically estimated. By greater practice and more cautious work with more refined methods it might become possible to reduce the errors and eventually the subject quantitatively and mathematically. treated.

Summary Of Part II :-

A short review of the physical and chemical properties of gelatin that are of interest to the study of its molecular structure and gelation was presented. This included a brief account of its preparation, identity, gelation, viscosity, optical rotation, rotary dispersion, and optical dispersion, chemical composition and inferences on its structure and gelation including the conclusions from the experimental section on the effect of urea and related compounds and some electrolytes on gelation. Much of the review was extracted from the book by Carl Shmidt-nn 'The Chemistry of the Amino-Acids and Proteins' as well as various other journals and reviews.

The section 'Experimental dealt with the aim of the experiments , being to find the importance of the different residual bonds in the aggregation of proteins, more especially the resonance bond. Gelatin was taken as an example of proteins and its gelation property investigated in its relation to substances added to it. Urea, a number of its derivatives and related compounds were investigated and their inhibitive effect on gelation was compared at different pH. values. The same was done with the neutral salts KCl, KNO_3, K_2SO_4, Na_2SO_4, $MgSO_4$, and $MgCl_2$. The sulfates promoted gelation while the other salts inhibited it. Glucose had practically no effect on gelation. Alanine, and glycine and its halide and sodium salts promoted gelation at acid, isoelectric, and alkaline pH. The inhibitive reagents were divided into two classes, electrolytes and non-electrolytes. The effect of the former was said to be due to the severing of the ionic bonds that aggregate gelatin and is dependent on the electrostatic condition of the gelatin molecules. the effect of the latter was attributed to their attaking the resonance bond which by itself should be independent of the ionic condition of the gelatin molecules . It is also suggested could be verified in the future , if it is true, by X-ray diffraction pictures which should reveal a widened resonancecbond spacing and the points that correspond to them should appear more diffuse than they normally are.in the presence of the non-electrolyte inhibitors , while they should be unaffected by the electrolyte inhibitors.

Ten sheets of diagrams (Graphs) , Twenty three sheets of tables and a sheet of bibliography follow in respective order.

RATE OF GELATION OF GELATIN SOLUTIONS AT pH.4.7*

No.of sample	cc. gelatin	cc. buffer	Time of gelation ' "	% of gelatin
1.	0.5o	0.5o	2 :15	5.o
2.	0.45	0.55	2 :5o	4.5
3.	0.4o	0.6o	3 :25	4.o
4.	0.35	0.65	5 :oo	3.5
5.	0.3o	0.7o	7 :4o	3.o
6.	0.25	0.75	9 :1o	2.5
7.	0.2o	0.8o	21 :oo	2.o

Solutions:-
Acetate buffer 0.2M pH. 4.7
+ Gelatin 10% in above buffer

Chart No. 1

RATE OF GELATION OF GELATIN SOLUTIONS WITH UREA AT pH. 4.7 *

No.of sample	cc. gelatin	cc. buffer	cc. urea	Mol.of urea	Time of gelation ' "
o.	o.5o	o.5o	o.oo	o.o	2 :15
1.	do.	o.45	o.o5	o.2	3 :oo
2.	do.	o.4o	o.1o	o.4	3 :55
3.	do.	o.35	o.15	o.6	5 :2o
4.	do.	o.3o	o.2o	o.8	7 :25
5.	do.	o.25	o.25	1.o	11 :oo
6.	do.	o.2o	o.3o	1.2	15 :45
7.	do.	o.15	o.35	1.4	22 :15
8.	do.	o.1o	o.4o	1.6	above 3o

Solutions:-
Acetate buffer. 0.2M pH. 4,7
+ Gelatin 10% in
+ above buffer.
Urea 4M in above buffer
All solutions are made to pH. 4.7 .

Chart No. 2

RATE OF GELATION OF GELATIN SOLUTIONS WITH METHYLUREA* at pH. 4.7

No.of sample	cc. gelatin	cc. buffer	cc.Meth-ylurea	Mol.of M-urea	Time of gelation ' "
o.	o.5o	o.5o	o.oo	o.o	2 :15
1.	do.	o.45	o.o5	o.2	2 :55
2.	do.	o.4o	o.1o	o.4	3 :4o
3.	do.	o.35	o.15	o.6	4 :55
4.	do.	o.3o	o.2o	o.8	6 :3o
5.	do.	o.25	o.25	1.o	8 :4o
6.	do.	o.2o	o.3o	1.2	11 :oo
7.	do.	o.15	o.35	1.4	13 :45
8.	do.	o.1o	o.4o	1.6	19 :oo

Solutions:-
Acetate buffer o.2M pH. 4.7 .
+ Gelatin 10% in above buffer.
+ Methylurea 4M in above buffer.

Chart No. 3

* Samples were immersed for ½ hour in a water bath at 37°C. then transferred into a water bath at 10°C. pH of solutions were determined between2o° & 25°C by a glass electrode(Beckman pH.meter)
+ pH. of solutions was corrected to 4.7 by the components of the buffer* (o.2M $NaH_3C_2O_2$ & o.2M $CH_3.COOH$)

RATE OF GELATION OF GELATIN SOLUTIONS AT pH.4.7*

No.of sample	cc. gelatin	cc. buffer	Time of gelation ' "	% of gelatin
1.	o.5o	o.5o	2 :15	5.o
2.	o.45	o.55	2 :5o	4.5
3.	o.4o	o.6o	3 :25	4.o
4.	o.35	o.65	5 :oo	3.5
5.	o.3o	o.7o	7 :4o	3.o
6.	o.25	o.75	9 :1o	2.5
7.	o.2o	o.8o	21 :oo	2.o

Solutions:-
Acetate buffer 0.2M pH. 4.7
+ Gelatin 10% in above buffer

Chart No. 1

RATE OF GELATION OF GELATIN SOLUTIONS WITH UREA AT pH. 4.7 *

No.of sample	cc. gelatin	cc. buffer	cc. urea	Mol.of urea	Time of gelation ' "
o.	o.5o	o.5o	o.oo	o.o	2 :15
1.	do.	o.45	o.o5	o.2	3 :oo
2.	do.	o.4o	o.1o	o.4	3 :55
3.	do.	o.35	o.15	o.6	5 :2o
4.	do.	o.3o	o.2o	o.8	7 :25
5.	do.	o.25	o.25	1.o	11 :oo
6.	do.	o.2o	o.3o	1.2	15 :45
7.	do.	o.15	o.35	1.4	22 :15
8.	do.	o.1o	o.4o	1.6	above 3o

Solutions:-
Acetate buffer. 0.2M pH. 4,7
+ Gelatin 10% in
+ above buffer.
Urea 4M in above buffer
All solutions are made to pH. 4.7 .

Chart No. 2

RATE OF GELATION OF GELATIN SOLUTIONS WITH METHYLUREA* at pH. 4.7

No.of sample	cc. gelatin	cc. buffer	cc.Meth-ylurea	Mol.of M-urea	Time of gelation ' "
o.	o.5o	o.5o	o.oo	o.o	2 :15
1.	do.	o.45	o.o5	o.2	2 :55
2.	do.	o.4o	o.1o	o.4	3 :4o
3.	do.	o.35	o.15	o.6	4 :55
4.	do.	o.3o	o.2o	o.8	6 :3o
5.	do.	o.25	o.25	1.o	8 :4o
6.	do.	o.2o	o.3o	1.2	11 :oo
7.	do.	o.15	o.35	1.4	13 :45
8.	do.	o.1o	o.4o	1.6	19 :oo

Solutions:-
Acetate buffer o.2M pH. 4.7 .
+ Gelatin 10% in above buffer.
+ Methylurea 4M in above buffer.

Chart No. 3

* Samples were immersed for ½ hour in a water bath at 37°C. then transferred into a water bath at 10°C. pH of solutions were determined between2o° & 25°C by a glass electrode(Beckman pH.meter)
+ pH. of solutions was corrected to 4.7 by the components of the buffer* (o.2M $NaH_3C_2O_2$ & o.2M $CH_3.COOH$)

RATE OF GELATION OF GELATIN WITH s-DIPHENYLGUANIDINE.HCl*
at pH. 4.7

No.of samp.	cc. gelat.	cc. buffer	cc. s-di-ph'guan.	Mol.of s-diph	time of gelation ' "
0.	o.5o	o.5o	o.oo	o.oo	2 : 15
1.	do	o.45	o.o5	o.o2	3 : oo
2.	do	o.4o	o.1o	o.o4	4 : 15
3.	do	o.35	o.15	o.o6	5 : 1o
4.	do	o.3o	o.2o	o.o8	6 : 4o
5.	do	o.25	o.25	o.1o	8.1515
6.	do	o.2o	o.3o	o.12	1o : 5o
7.	do	o.15	o.35	o.14	15 : 5o
8.	do	o.1o	o.4o	o.16	21 : oo

Solutions:-
Acetate buffer o.2M, pH. 4.7
+ Gelatin 1o% in above buffer
+ s-Diphenylguanidine.HClo.4M in above buffer

Chart No. 7

RATE OF FELATION OF GELATIN SOLUTUONS WITH ACETAMIDE
at pH. 4.7 *

No.of samp.	cc. gelat.	cc. buffer	cc. Acetamid	Mol.of acetam	Time of gelation ' "
o.	o.5o	o.5o	o.oo	o.oo	2 : 15
1.	do.	o.4o	o.1o	o.6	3 : 30
2.	do.	o.3o	o.2o	1.2	5 : 15
3.	do.	o.2o	o.3o	1.8	6 : 3o
4.	do.	o.1o	o.4o	2.4	9 : 15
5.	do.	o.oo	o.5o	3.o	1o : 2o

Solutions:-
Acetate buffer o.2M, pH. 4.7
+ Gelatin 1o% in above buffer
+ Acetamide 6M in above buffer

Chart No. 8

RATE OF GELATION OF GELATIN SOLUTIONS WITH POTASSIUM CHLORIDE at pH. 4.7 *

No.of Samp.	cc. gelat.	cc. buffer	cc. KCl.	Mol.of KCl.	Time of Gelation ' "
o.	o.5o	o.5o	o.oo	o.oo	2 : 15
1.	do.	o.45	o.o5	o.15	2 : 4o
2.	do.	o.4o	o.1o	o.3o	3 : o5
3.	do.	o.35	o.15	o.45	3 : 55
4.	do.	o.3o	o.2o	o.6o	4 : 45
5.	do.	o.25	o.25	o.75	5.:55
6.	do.	o.2o	o.3o	o.9o	6 : 5o
7.	do.	o.15	o.35	1.o5	8.: 15
8.	do.	o.1o	o.4o	1.2o	1o : 45
9.	do.	o.o5	o.45	1.35	14 : oo
1o.	do.	o.oo	o.5o	1.5o	19 : oo

Solutions:-
Acetate buffer o.2M, pH. 4.7
+ Gelatin 1o% in above buffer
+ KCl 3M in above buffer.

Chart No. 9

* Samples were immersed for ½ hour in water bath at 37°C then transferred into a water bath at 1o°C. pH. of solutions were determined between 2o° & 25°C by a glass electrode (Beckman pH. meter).

\+ pH. of solutions was corrected at 4.7 by the contents of the buffer (o.2M $NaH_3C_2O_2$ & o.2M $CH_3.COOH$)

GELATION OF GELATIN SOLUTIONS WITH CITRATE-PHOSPHATE BUFFER at pH. 4,7 *

No.of samp.	cc. gelatin	cc. buffer	% of gelatin	Time of gelation '	"
1.	o.5o	o.5o	5.o	2	oo
2.	o.45	o.55	4.5	2	1o
3.	o.4o	o.6o	4.o	2	3o
4.	o.35	o.65	3.5	3	2o
5.	o.3o	o.7o	3.o	4	4o
6.	o.25	o.75	2.5	7	3o
7.	o.2o	o.8o	2.o	17	oo

Solutions :-
Buffer, Citr.-Phosph. at pH.4.7
+ Gelatin, 10% in above buffer made to pH.4.7

Chart No. 10

GELATION OF GELATIN SOLUTIONS WITH GLUCOSE AT pH. 4.7 *

NO.of Samp.	cc. gelatin	cc. buffer	cc. glucose	Mol. of Glucose	Time of Gelation '	"
o.	o.3o	o.7o	o.oo	o.o	4	4o
1.	o.3o	o.6o	o.1o	o.2	4	40
2.	o.3o	o.4o	o.3o	o.6	4	37
3.	o.3o	o.2o	o.5o	1.o	4	32
4.	o.3o	o.oo	o.7o	1.4	4	25

Chart No 11

Solutions:-
Buffer, Citrate phosphate at pH. 4.7
+ Gelatin, 1o% in above buffer at pH. 4.7
+ Glucose 2M in above buffer at pH. 4.7

GELATION OF GELATIN WITH POTASSIUM SULFATE AT pH. 4.7 *

No.of Samp.	CC. Gelatin	cc. buffer	cc. K_2SO_4	Mol. of K_2SO_4	Time of Gelation '	"
0.	o.3o	o.7o	o.oo	o.oo	4	4o
1.	do.	o.6o	o.1o	o.o6	4	3o
2.	do.	o.4o	o.3o	o.18	4	o5
3.	do.	o.2o	o.5o	o.3o	3	25
4.	do.	o.oo	o.7o	o.42	2	5o

Chart No. 12

Solutions:-
Buffer, Citrate phosphater at pH. 4.7
+ Gelatin; in above buffer at pH. 4.7 ; 10%
+ K_2SO_4, 0.6M in above buffer at pH. 4.7

* Samples were immersed for ½ hour in water bath at 37° C. then transferred into a water bath at 10° C.
pH. of solutions were determined between (2o-25)° C. by a glass electrode (Beckman pH.meter.)
+ pH. of solutions were corrected to 4.7 by the contents of the buffer (o.2M Na_2HPO_4 ‡ o.1M Citric acid)

GELATION OF GELATIN WITH GLYCINE at pH. 4.7 *

Solutions:-

Buffer (0.2M Na_2HPO_4 + 0.1M Citric acid) pH. 4.7
Gelatin, 10% in above buffer at pH. 4.7
Glycine, 2M at pH. 4.7 in above buffer

No. of sample	cc. gelatin	cc. buffer	cc. glycine	Mol. of glycine	Time of Gelation
					' " "
0.	0.3o	0.7o	0.oo	o.o	4 : 4o
1.	do.	o.6o	o.1o	o.2	4 : 2o
2.	do.	o.4o	o.3o	o.6	3 : 35
3.	do.	o.2o	o.5o	1.o	2 : 55
4.	do.	o.oo	o.7o	1.4	2 : 3o
5.	do.				

Chart No. 13

GELATION OF GELATIN WITH ALANINE at pH. 4.7 *

Solutions :-

Buffer (0.2M Na_2HPO_4 0.1M Citric acid) pH. 4.7
Gelatin, 10% in above buffer at pH, 4.7
Alanine, 0.76M in above buffer, at pH. 4.7

No. of sample	cc. gelatin	cc. buffer	cc. alanine	Mol. of alanine	Time of Gelation
					' "
0.	0.3o	0.7o	o.oo	o.ooo	4 : 4o
1.	do.	o.6o	o.1o	o.o76	4 : 3o
2.	do.	o.4o	o.3o	o.228	4 : oo
3.	do.	o.2o	o.5o	o.38o	3 : 45
4.	do.	o.oo	o.7o	o.532	3 : 35

Chart No. 14

GELATION OF GELATIN AT pH. 3.45 IN PHOSPHATE-CITRATE BUFFER *

Solutions:-

Buffer (0.2M. Na_2HPO_4 +0.1M citric acid) at pH. 3.45
Gelatin 10% at pH. 3.45 in above buffer

No. of sample	cc. gelatin	cc. buffer	% of Gelatin	Time of gelation
				' "
1.	o.5o	o.5o	5.o	3 :00
2.	o.45	o.55	4.5	4 :10
3.	o.4o	o.6o	4.o	6 :oo
4.	o.35	o.65	3.5	8 :15
5.	o.3o	o.7o	3.o	12 :3o
6.	o.25	o.75	2.5	16 :4o
7.	o.2o	o.8o	2.o	32 :oo
8.	o.15	o.85	1.5	- - -

Chart No. 15

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* Samples were immersed for ½ hour in water bath at 37°C. then transferred into a water bath at 10°C.

pH. of solutions were corrected to 4.7 by the components of the buffer.

The pH. of the solutions was determined at (25-25)°C by a glass electrode (Beckman pH.meter.)

GELATION OF GELATIN WITH UREA at pH. 3.45 *
Solutions:-
Buffer,(o.2M Na_2HPO_4+o.1M Citric acid) at pH. 3.45
Gelatin 10% at pH. 3.45 in above buffer.
Urea 4M at pH. 3.45 in above buffer.

No. of sample	cc. gelatin	cc. buffer	cc. urea	Molarity of urea	Time of gelation
					' "
0.	0.5o	o.5o	o.oo	o.o	3 : oo
1.	do.	o.45	o.o5	o.2	4 : 15
2.	do.	o.4o	o.1o	o.4	6 : 45
3.	do.	o.35	o.15	o.6	1o : oo
4.	do.	o.3o	o.2o	o.8	15:25
5.	do.	o.25	o.25	1.o	24 : 15
6.	do.	o.2o	o.3o	1.2	- - - -

Chart No. 16

GELATION OF GELATIN WITH METHYLUREA at pH. 3.45 *
Solutions:-
Buffer, 0.2M Na_2HPO_4+o.1M Citric acid, at pH. 3.45
Gelatin 10% at pH. 3.45 in above buffer.
Methylurea(Murea) 3.81M at pH. 3.45 in above buffer.

No. of sample	cc. gelatin	cc. buffer	cc. Murea	Molarity of Murea	Time of gelation
					' "
0.	o.5o	o.5o	o.oo	o.oo	3 : oo
1.	do.	o.45	o.o5	o.19	5 : oo
2.	do.	o.4o	o.1o	o.38	6 : 45
3.	do.	o.35	o.15	o.57	9 : 3o
4.	do.	o.3o	o.2o	o.76	13 : 2o
5.	do.	o.25	o.25	o.95	19 : oo
6.	do.	o.2o	o.3o	1.14	24 : 3o
7.	do.	o.15	o.35	1.33	- - - -

Chart No. 17

GELATION OF GELATIN WITH ALLYLUREA at pH. 3.45 *
Solutions:-
Buffer, 0.2M Na_2HPO_4+0.1M Citric acid, at pH. 3.45
Gelatin 10% at pH. 3.45 in above buffer.
Allylurea 2M at pH. 3.45 in above buffer.(Aurea)

No. of sample	cc. gelatin	cc. buffer	cc. Aurea	Molarity of Aurea	Time of gelation
					' "
0.	o.5o	o.5o	o.oo	o.o	3 : oo
1.	do.	o.45	o.o5	o.1	4 : 3o
2.	do,	o.4o	o.1o	o.2	5 : 45
3.	do.	o.35	o.15	o.3	7 : 15
4.	do.	o.3o	o.2o	o.4	9 : 3o
5.	do.	o.25	o.25	o.5	13 : oo
6.	do.	o.2o	o.3o	o.6	15 : 35
7.	do.	o.15	o.35	o.7	21 : oo
8.	do.	o.1o	o.4o	o.8	29 : oo

Chart No. 18

* Samples were immersed for ½ hour in water bath at 37°C. then transferred into a water bath at 10°C.
pH. of the solutions were corrected by the components of the buffer to pH. 3.45
The pH. of the solutions was determined at (2o-25)°C. by a glass electrode (Beckman pH.meter).

GELATION OF GELATIN WITH THIOUREA at pH. 3.45 *
Solutions:-
Buffer, o.2M Na_2HPO_4 + o.1M Citric acid, at pH. 3.45
Gelatin 10% at pH. 3.45 in above buffer.
Thiourea 2M at pH. 3.45 in above buffer.

No. of sample	cc. gelatin	cc. buffer	cc. thiourea	Mol. of thiourea	Time of gelation
					' "
0.	0.50	0.50	0.00	0.0	3 : 00
1.	do.	0.45	0.05	0.1	5 : 25
2.	do.	0.40	0.10	0.2	8 : 40
3.	do	0.35	0.15	0.3	16 : 00
4.	do.	0.30	0.20	0.4	31 : 30
5.	do.	0.25	0.25	0.5	- - - -

Chart No. 19

GELATION OF GELATIN WITH GUANIDINE.HCl at pH. 3,45 *
Solutions:-
Buffer, o.2M Na_2HPO_4 + o.1M Citric acid, at at pH. 3.45
Gelatin 10% at pH. 3.45 in above buffer.
Guanidine.HCl 2M at pH. 3.45 in above buffer.

No. of sample	cc. gelatin	cc. buffer	cc. guan.HCl	Mol. of guan.HCl	Time of gelation
					' "
0.	0.50	0.50	0.00	0.0	3 : 00
1.	do.	0.45	0.05	0.1	4 : 50
2.	do.	0.40	0.10	0.2	6 : 50
3.	do.	0.35	0.15	0.3	9 : 25
4.	do.	0.30	0.20	0.4	12 : 55
5.	do.	0.25	0.25	0.5	25 : 10
6.	do.	0.20	0.30	0.6	- - - -

Chart No.20

GELATION OF GELATIN WITH s-DIPHENYLGUANIDINE.HCl at pH. 3.45*
Solutions:-
Buffer, o.2M Na_2HPO_4 + o.1M Citric acid, at pH. 3.45
Gelatin 10% at pH. 3.45 in above buffer.
s-Diphenylguanidine.HCl(sDGH)o.4M at pH. 3.45 in above buffer.

No. of sample	cc. gelatin	cc. buffer	cc. sDGH	Mol. of sDGH	Time of gelation
					' "
0.	o.5o	o.5o	o.oo	o.oo	3 : 00
1.	do.	o.45	o.o5	o.o2	4 : 45
2.	do.	o.4o	o.1o	o.o4	5 : 25
3.	do.	o.35	o.15	o.o6	6 : 3o
4.	do.	o.3o	o.2o	o.o8	8 : 3o
5.	do.	o.25	o.25	o.1o	1o : 3o
6.	do.	o.2o	o.3o	o.12	13 : 15
7.	do.	o.15	o.35	o.14	16 : 15

Chart No. 21

* Samples were immersed for ½ hour in water bath at 37°C. then transferred into a water bath at 10°C.
pH. of the solutions was determined at (2o-25)°C. by a glass electrode (Beckman pH.meter.),and corrected by the components of the buffer to pH. 3.45

GELATION OF GELATIN WITH ACETAMIDE at pH. 3.45 *

Solutions:-

Buffer, 0.2M Na_2HPO_4 + 0.1M Citric acid, at pH. 3.45

Gelatin 10% at pH. 3.45 in above buffer.

Acetamide 3.8M in above buffer, at pH. 3.45

No. of samples	cc. gelatin	cc. buffer	cc. acetamide	Mol. of acetamide	Time of gelation ' "
0.	0.50	0.50	0.00	0.00	3 : 00
1.	do.	0.40	0.10	0.38	5 : 10
2.	do.	0.30	0.20	0.76	7 : 20
3.	do.	0.20	0.30	1.14	10 : 30
4.	do.	0.10	0.40	1.52	14 : 15
5.	do.	0.00	0.50	1.90	17 : 30

Chart No. 22

GELATION OF GELATIN WITH THIOACETAMIDE AT pH. 3.45 *

Solutions:-

Buffer, 0.2M Na_2HPO_4 +0.1M Citric acid, at pH. 3.45

Gelatin 10% at pH. 3.45 in above buffer.

Thioacetamide (T-am.) 1.6M at pH. 3.45 in above buffer.

No. of sample	cc. gelatin	cc. buffer	cc. (T-am)	Mol. of (T-am.)	Time of gelation ' "
0.	0.50	0.50	0.00	0.00	3 : 00
1.	do.	0.40	0.10	0.16	6 : 30
2.	do.	0.30	0.20	0.32	12 : 00
3.	do.	0.20	0.30	0.48	25 : 00
4.	do.	0.10	0.40	0.64	- - -

Chart No. 23

GELATION OF GELATIN WITH POTASSIUM CHLORIDE at pH. 3.45 *

Solutions:-

Buffer, 0.1M Citric acid + 0.2M Na_2HPO4 , at pH. 3.45

Gelatin 10% at pH. 3.45 in above buffer.

Potassium chloride 3.8M at pH. 3.45 in above buffer.

No. of sample	cc. gelatin	cc. buffer	cc. KCl	Mol. of KCl	Time of gelation ' "
0.	0.50	0.50	0.00	0.00	3 : 00
1.	do.	0.45	0.05	0.19	3 : 35
2.	do.	0.40	0.10	0.38	4 : 10
3.	do.	0.35	0.15	0.57	5 : 25
4.	do.	0.30	0.20	0.76	6 : 40
5.	do	0.25	0.25	0.95	8 : 45
6.	do.	0.20	0.30	1.14	10 @ 00
7.	do.	0.15	0.35	1.33	14 : 15
8.	do.	0.10	0.40	1.52	18 : 40
9.	do.	0.05	0.45	1.71	24 : 45

Chart No. 24

* Samples were immersed in water bath at 37°C. for ½ hour then transferred into a water bath kept at 10°C.

pH. of the solutions was corrected by the components of the buffer to pH. 3.45

The pH. of the solutions was determined at (20-25)°C. by a glass electrode (Beckman pH. meter).

GELATION OF GELATIN IN CITRATE-PHOSPHATE BUFFER at pH.3.45 *

Solutions:-

Buffer, o.2M Na_2HPO_4 +o.1M Citric ACID, at pH.3.45

Gelatin 10% at pH. 3.45 in above buffer.

No.of Sample	cc. Gelatin	cc. buffer	% of Gelatin	time of Gelation ' "
1.	o.5o	o.5o	5.o	2 : 35
2.	o.45	o.55	4.5	3 : 15
3.	o.4o	o.6o	4.o	3 : 4o
4.	o.35	o.65	3.5	5 : 5o
5.	o.3o	o.7o	3.o	8 : 3o
6.	o.25	o.75	2.5	14 : oo
7.	o.2o	o.8o	2.o	23 : oo

Chart No. 25

GELATION OF GELATIN WITH GLYCINE at pH. 3.45 *

Solutions :-

Buffer, 0.2M Na_2HPO_4 +o.1M Citric acid, at pH. 3.45

Gelatin 10% at pH. 3.45 in above buffer.

Glycine 2M at pH. 3.45 in above buffer.

No. of sample	cc. gelatin	cc. buffer	cc. glycine	Mol.of glycine	Time of Gelation ' "
0.	o.5o	o.5o	o.oo	o.o	2 : 35
1.	do.	o.4o	o.1o	o.2	2 : 3o
2.	do.	o.3o	o.2o	o.4	2 : 2o
3.	do.	o.2o	o.3o	o.6	2 : o5
4.	do.	o.1o	o.4o	o.8	1 : 45
5.	do.	o.oo	o.5o	1.o	1 : 35
6.	o.3o	o.7o	o.oo	o.oo	9 :15
7.	do.	o.6o	o.1o	o.2o	8 :55
8.	do.	o.5o	o.2o	o.4o	8 : oo
9.	do.	o.4o	o.3o	o.6o	7 : 15
1o.	do.	o.3o	o.4o	o.8o	6 : 5o
11.	do.	o. 2o	o.5o	1.oo	6 : oo
12.	do.	o.1o	o.6o	1.2o	5 : 4o
	do.	o.oo	o.7o	1.4o	4 : 45

Chart No.26

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* Samples were immersed in a water bath at 37° C. for ½ hour then transferred into a water bath kept at 10°C.

pH. of solutions was corrected by the components of the buffer to pH. 3.45

The pH. of the solutions was determined at (2o-25)°C by a glass electrode (Beckman pH.meter).

GELATION OF GELATIN WITH ALANINE at pH. 3.45 *

Solutions:-

Buffer, o.1M Citric acid+o.2M Na_2HPO_4, at pH. 3.45

Gelatin 10% at pH. 3.45 in above buffer.

Alanine o.8M at pH. 3.45 inabove buffer.

	No. of sample	cc. gelatin	cc. buffer	cc. alanine	Mol. of alanine	time of gelation h : m
a.	0.	o.5o	o.5o	o.oo	o.oo	2 : 35
	1.	do.	o.4o	o.1o	o.o8	2 : 3o
	2.	do.	o.3o	o.2o	o.16	2 : 25
	3.	do.	o.2o	o.3o	o.24	2 : 35
	4.	do.	o.1o	o.4o	o.32	2 : 1o
	5.	do.	o.oo	o.5o	o.4o	2 : 1o
b.	X.	o.3o	o.7o	o.oo	o.oo	9 : 15
	6.	do.	o.6o	o.1o	o.o8	9 : 1o
	7.	do.	o.5o	o.2o	o.16	- --
	8.	do.	o.4o	o.3o	o.24	8 : 3o
	9.	do.	o.3o	o.4o	o.32	- --
	1o.	do.	o.2o	o;5o	o.4o	8 : 15
	11.	do.	o.1o	o.6o	o.48	- --
	12.	do.	o.oo	o.7o	o.56	7 : 45

Chart No.27

GELATION OF GELATIN WITH POTASSIUM SULFATE at pH. 3.45 *

Solutions:-

Buffer, o.1M Citric acid+o.2M Na_2HPO_4, at pH. 3.45

Gelatin 10% at pH. 3.45 in above buffer.

K_2SO_4 o.6M at pH. 3.45 in above buffer.

	No. of sample	cc. gelatin	cc. buffer	cc. K_2SO_4	Mol. of K_2SO_4	Time of gelation h : m
a.	o.	o.5o	o.5o	o.oo	o.oo	2 : 35
	1.	do.	o.4o	o.1o	o.o6	2 : 22
	2.	do.	o.3o	o.2o	o.12	2 : 1o
	3.	do;	o.2o	o.3o	o.18	1 : 55
	4.	do.	o.1o	o.4o	o.24	1 : 5o
	5.	do.	o.oo	o.5o	o.3o	1 : 45
b.	X.	o.3o	o.7o	o.oo	o.oo	9 : 15
	6.	o.3o	o.6o	o.1o	o.o6	7 : 2o
	7.	do.	o.5o	o.2o	o.12	6 : 35
	8.	do.	o.4o	o.3o	o.18	6 : 15
	9.	do.	o.3o	o.4o	o.24	5 : 4o
	1o.	do.	o.2o	o.5o	o.3o	5 : 15
	11.	do.	o.1o	o.6o	o.36	4 : 5o
	12.	do.	o.oo	o.7o	o.42	4 : 15

Chart No.28

* Samples were immersed in a water bath at 37° C. for ½ hour then transferred into a water bath kept at 1o° C.

pH. of solutions was corrected by the components of the buffer to pH. 3.45

The pH. of the solutions was determined at (2o-25)° C by a glass electrode (Beckman pH.meter).

GELATIO N OF GELATIN WITH GLUCOSE at pH. 3.45 *

Solutions:-

Buffer, 0.1M Citric acid+0.2M Na_2HPO_4, at pH. 3.45

Gelatin, 10%, at pH. 3.45, in above buffer.

Glucose, 2M, at pH. 3.45, in above buffer.

No. of sample	cc. gelatin	cc. glucose	cc. buffer	Mol. of glucose	Rate of gelation ' "
0.	o.3o	o.oo	o.7o	o.oo	9 : 15
1.	do.	o.1o	o.6o	o.2o	9 : 15
2.	do.	o.2o	o.5o	o.4o	9 : oo
3.	do.	o.3o	o.4o	o.6o	
4.	do.	o.4o	o.3o	o.8o	8 : 3o
5.	do.	o.5o	o.2o	1.oo	
6.	do.	o.6o	o.1o	1.2o	
7.	do.	o.7o	o.oo	1.4o	8 : 15

Chart No.29

GELATION OF GELATIN IN CITRATE PHOSPHATE BUFFER at pH. 8 *

No. of sample	cc. gelatin	cc. buffer	% of gelatin	Rate of gelation ' "
1.	o.5o	o.5o	5.o	2 : 3o
2.	o.45	o.55	4.5	2 : 55
3.	o.4o	o.6o	4.o	3 :5o
4.	o.35	o.65	3.5	6 : 2o
5.	o.3o	o.7o	3.o	8 : 45
6.	o.25	o.75	2.5	14 : oo
7.	o.2o	o.8o	2.o	28 : oo
8.	o.15	o.85	1.5	- - - -

Solutions:-
Buffer, o.1M Citric acid+o.2M Na_2HPO_4 at pH. 8
Gelatin 10% at pH. 8 in above buffer.

Chart No.30

GELATION OF GELatin AT pH.8 WITH UREA*

Solutions:- Buffer, o.1M Citric acid+o.2M Na_2HPO_4 at pH.8
Gelatin 10% at pH.8 in above buffer.
Urea 4M at pH.8 i n above buffer.

No. of samples	cc. gelatin	cc. buffer	cc. urea	Mol. of urea	Time of gelation ' "
0.	o.5o	o.5o	o.oo	o.o	2 : 3o
1.	do.	o.45	o.o5	o.2	3 : 15
2.	do.	o.4o	o.1o	o.4	4 : o5
3.	do.	o.35	o.15	o.6	6 : 4o
4.	do.	o.3o	o.2o	o.8	8 : 45
5.	do.	o.25	o.25	1.o	12 : 3o
6.	do.	o.2o	o.3o	1.2	2o : oo

Chart No.31

* Samples were immersed in a water bath at 37°C. for ½ hour then transferred into a water bath kept at 10°C.

The pH. of the solutions was corrected by the components of the buffer to the required one.

The pH. of the solutions was determined at (2o-25)°C. by a glass electrode (Beckman pH.meter.)

GELATION OF GELATIN WITH METHYLUREA at pH.8 *

No.of samp.	cc. gelat	cc. buff.	cc. urea	Mol. urea	Time of gelation ' "
0.	0.50	0.50	0.00	0.0	2 : 30
1.	do.	0.45	0.10	0.4	4 : 25
2.	do.	0.35	0.15	0.6	6 : 10
3.	do.	0.30	0.20	0.8	8.: 00
4.	do.	0.25	0.25	1.0	11 : 00
5.	do.	0.20	0.30	1.2	13 : 25
6.	do.	0.10	0.40	1.6	28 : 00
7.	do.	0.00	0.50	2.0	- - - -

Solutions:-
Buffer;0.1M citric acid +0.2M Na_2HPO_4 at pH8
Gelatin 10% at pH.8 in above buffer.
Methylurea 4M at pH.8 in above buffer.

Chart No. 32

GELATION OF GELATIN WI TH ALLYLUREA at pH.8 *

No.of samp.	cc. gelat	cc. buff.	cc.A urea	M.of Aurea	Time of gelation ' "
0.	0.50	0.50	0.00	0.0	2 : 30
1.	do.	0.45	0.05	0.1	3 : 10
2.	do.	0.40	0.10	0.2	3 : 50
3.	do.	0.35	0.15	0.3	5 :000
4.	do.	0.30	0.20	0.4	6 : 30
5.	do.	0.25	0.30	0.6	10 : 30
6.	do.	0.10	0.40	0.8	17 : 30
7.	do.	0.00	0.50	1.0	29 : 10

Solutions:-
Buffer,0.1M citric acid +0.2M Na_2HPO_4 at pH8
Gelatin 10% at pH.8 in above buffer.
Allylurea (Aurea) 2M at pH.8 in above buffer.

Chart No.33

GELATION OF GELATIN WITH THIOUREA at pH.8

No.of samp.	cc. gelat	cc. buff.	cc.T urea	M.of Turea	Time of gelation ' "
0.	0.50	0.50	0.00	0.0	2 : 30
1.	do.	0.45	0.05	0.1	3 : 15
2.	do.	0.40	0.10	0.2	5 : 10
3.	do.	0.35	0.15	0.3	7 : 40
4.	do.	0.30	0.20	0.4	13 : 00
5.	do.	0.25	0.25	0.5	24 : 50

Solutions:-
Buffer,0.1M citric acid +0.2M Na_2HPO_4 at pH.8
Gelatin 10% at pH.8 in above buffer.
Thiourea 2M at pH.8 in above buffer.

Turea for thiourea.

Chart No.34

* Samples were immersed in a water bath at 37°C. for ½ hour then transferred into a water bath kept at 10°C.
The pH. of the solutions was determined at (20-25)°C. by a glass electrode (Beckman pH.meter).
The pH. of the solutions was corrected by the components of the buffer to pH.8

GELATION OF GELATIN WITH GUANIDINE.HCl at pH.8 *

No.of samp.	cc. gelat	cc. buffer	cc. Guan	Mol.of Guan.	Time of gelation ' "
0.	0.50	0.50	0.00	0.0	2 : 30
1.	0.50	0.45	0.05	0.1	3 : 00
2.	0.50	0.40	0.10	0.2	4 : 00
3.	0.50	0.35	0.15	0.3	5 : 30
4.	0.50	0.30	0.20	0.4	7 : 30
5.	0.50	0.25	0.25	0.5	12 : 00
6.	0.50	0.20	0.30	0.6	18 : 50

Solutions:-
Buffer, o.1M citric acid+o.2M Na_2HPO_4 at pH.8
Gelatin 10% at pH.8 in above buffer.
Guanidine.HCl (Guan.) at pH.8 in above buffer.

Chart No. 35

GELATION OF GELATIN WITH ACETAMIDE at pH.8 *

No.of samp.	cc. gelat	cc. buff.	cc. acet.	Mol.of Acet.	Time of gelation ' "
0.	o.5o	o.5o	o.oo	o.0	2 : 30
1.	do.	o.4o	o.1o	o.4	3 : 25
2.	do.	o.3o	o.2o	o.8	4 : 40
3.	do.	o.2o	o.3o	1.2	6 : oo
4.	do.	o.1o	o.4o	1.6	7 : 25
5.	do.	o.oo	o.5o	2.o	9 : 3o

Solutions:-
Buffer, o.1M citric acid+o.2M Na_2HPO_4 at pH.8
Gelatin 10% at pH.8 in above buffer.
Acetamide, (Acet.) 4M at pH.8 in above buffer.

Chart No. 36

GELATION OF GELATIN WITH THIOACETAMIDE at pH.8 *

No.of samp.	cc. gelat	cc. buff.	cc. T¹acet.	Mol.of T¹acet.	Time of gelation ' "
0.	o.5o	o.5o	o.oo	o.0	2 : 30
1.	do.	o.45	o.o5	o.1	3 : 25
2.	do.	o.4o	o.1o	o.2	4 : 45
3.	do.	o.35	o.15	o.3	6 : 35
4.	do.	o.3o	o.2o	o.4	9 : 15
5.	do.	o.25	o.25	o.5	15 : oo
6.	do.	o.2o	o.3o	o.6	23 : oo
7.	do.	o.15	o.35	o.7	above 30

Solutions:-
Buffer, o.1M citric acid+o.2M Na_2HPO_4 at pH.8
Gelatin 10% at pH.8 in above buffer.
Thioacetamide, (T¹acet.) 2M at pH.8 in above buffer.

Chart No. 37

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* Samples were immersed in a water bath at 37°C. for ½ hour then transferred into a water bath kept at 10°C.
The pH.of the solutions was determined at (2o-25)C. by a glass electrode ( ) (Beckman pH.meter.)
The pH. of the solutions was corrected by the components of the buffer to pH.8 .

GELATION OF GELATIN WITH POTASSIUM CHLORIDE at pH.8*

No.of samp.	cc. @ cc. gelat	cc. buff.	cc. KCl	Mol.of KCl	Time of gelation ' "
0.	o.5o	o.5o	o.oo	o.oo	2 : 3o
1.	do.	o.4o	o.1o	o.35	3 : 4o
2.	do.	o.3o	o.2o	o.7o	5 : 3o
3.	do.	o.25	o.25	o.88	6 : 2o
4.	do.	o.2o	o.3o	1.o5	8 : oo
5.	do.	o.1o	o.4o	1.4o	11 : oo
6.	do.	o.oo	o.5o	1.75	16 : 15

Solutions:-
Buffer, o.1M citric acid + o.2M Na_2HPO_4 at pH.8
§ Gelatin 10% at pH.8 in above buffer.
§ KCl 3.5M at pH.8 in above buffer.

Chart No. 38

GELATION OF GELATIN WITH GLYCINE at pH.8 *

No.of samp.	cc. gelat	cc. buff.	cc. glyc	Mol.of glycine	Time of Gelation ' "
0.	o.5o	o.5o	o.oo	o.oo	2 : 3o
1.	o.5o	o.4o	o.1o	o.2o	2 : 15
2.	o.5o	o.3o	o.2o	o.4o	2 : 1o
3.	o.5o	o.2o	o.3o	o.6o	1 : 55
4.	o.5o	o.1o	o.4o	o.8o	1 : 5o
5.	o.5o	o.oo	o.5o	1.oo	1 : 3o

Solutions:- at pH.8
Buffer, o.1M citric acid + o.2M Na_2HPO_4
§ Gelatin 10% in above buffer.
§ Glycine, (glyc) 2M in above buffer.

Chart No. 39

GELATION OF GELATIN IN PHOSPHATE BUFFER at pH.11.5 *
Solutions:-
Buffer, o.1M NaOH + o.2M Na_2HPO_4, made to pH.11.5
Gelatin, 10% in above buffer made to pH.11.5

No. of sample	cc. gelatin	cc. buffer	% of gelatin	Time of Gelation ' "
1.	o.5o	o.5o	5.o	16
2.	o.45	o.55	4.5	22
3.	o.4o	o.6o	4.o	32
4.	o.35	o.65	3.5	--

Chart No. 40

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* Samples were immersed in a water bath at 37°C. for ½ hour then transferred into a water bath kept at 10° C.
The pH. of solutions was determined at (2o-25)°C. by a glass electrode (Beckman pH.meter).
§ The pH. of the solutions was corrected by the components of the buffer to 8

GELATION OF GELATIN WITH UREA at pH. 11.5 *

Solutions;

Buffer, o.1M NaOH+o.2M Na_2HPO_4 mixed to pH. 11.5
Gelatin,10%,in components of buffer made to pH. 11.5
Urea,4M,in components of buffer made to pH. 11.5

No. of sample	cc. gelatin	cc. buffer	cc. urea	Mol. of urea	Time of Gelation ' "
0.	o.5o	o.5o	o.oo	o.o	16
1.	o.5o	o.45	o.o5	o.2	23
2.	o.5o	o.4o	o.1o	o.4	53
3.	o.5o	o.35	o.15	o.6	--

Chart No. 41

GELATION OF GELATIN WITH THIOUREA at pH.11.5*%

Solutions:-

Buffer, o.1M NaOH + 0.2M Na_2HPO_4 , mixed to pH. 11.5
Gelatin,10%,in components of buffer made to pH. 11.5
Thiourea,2M,in components of buffer made to pH. 11.5

No. of sample	cc. gelatin	cc. buffer	cc. Thiourea	Mol.of Thoiurea	Time of Gelation '
0.	o.5o	o.5o	o.oo	o.o	16
1.	o.5o	o.45	o.o5	o.1	27
2.	o.5o	o.4o	o.1o	o.2	--

Chart No. 42

GELATION OF GELATIN WITH GUANIDINE.HCl at pH. 11.5 *

Solutions:-

Buffer, o.1M NaOH + o.2M Na_2HPO_4 , mixed to pH. 11.5
Gelatin,10%,in components of above buffer , pH. 11.5
Guanidine.HCl,2M,in " " " " " "
Guan. for guanidine.HCl

No. of sample	cc. gelatin	cc. Buffer	cc. Guan.	Mol.of Guan.	Time of Gelation '
0.	o.5o	o.5o	o.oo	o.o	16
1.	o.5o	o.45	o.o5	o.1	2o
2.	o.5o	o.4o	o.1o	o.2	47
3.	o.5o	o.35	o.15	o.3	--

Chart No. 43

* Samples were immersed in a water bath at 37°C. for ½ hour then transferred into a water bath kept at 10°C.

The pH. of the solutions was corrected by the components of the buffer to pH. 11.5

The pH. of the solutions was determined by a glass electrode (Beckman pH.meter)

GELATION OF GELATIN WITH POTASSIUM CHLORIDE at pH.11.5 *

Solutions:- at pH. 11.5

Buffer, 0.1M NaOH + 0.2M Na_2HPO_4

Gelatin, 10%, made with components of above buffer.

KCl, 3.5M, " " " " " " .

No. of sample	cc. gelatin	cc. buffer	cc. KCl	Mol. of KCl	Time of Gelation
					'
0.	0.5	0.50	0.00	0.00	16
1.	0.50	0.40	0.10	0.35	20
2.	0.50	0.30	0.20	0.70	32
3.	0.50	0.20	0.30	1.05	--

Chart No.44

GELATION OF GELATIN WITH Na-GLYCINATE at pH. 11.5 *

Solutions:- at pH, 11.5

Buffer, 0.1M NaOH + 0.2M Na_2HPO_4

Gelatin, 10%, made with components of above buffer.

Na-Glycinate, 2M, made with components of above buffer.

No. of sample	cc. gelatin	cc. buffer	cc. Na-glycin.	Mol. of Na-glycin.	Time of gelation
					' "
0.	0.50	0.50	0.00	0.0	16:00
1.	do.	0.40	0.10	0.2	11:30
2.	do.	0.30	0.20	0.4	9:00
3.	do.	0.20	0.30	0.6	9:00
4.	do.	0.10	0.40	0.8	11:00
5.	do.	0.00	0.50	1.0	11:00

Chart No.45

GELATION OF GELATIN IN CITRATE-PHOSPHATE BUFFER at pH. 3.45*

Solutions:- at pH. 3.45

Buffer, 0.1M Citric Acid + 0.2M Na_2HPO_4

Gelatin, 10%, made with components of above buffer.

No. of Samp.	cc. gelatin	cc. buffer	% conc. of gelatin	Time of Gelation
				' "
1.	0.9	0.1	9	1 : 10
2.	0.8	0.2	8	1 : 35
3.	0.7	0.3	7	2 : 00
4.	0.6	0.4	6	2 : 30
5.	0.5	0.5	5	3 : 20
6.	0.4	0.6	4	5 : 20

Chart No.46

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* Samples were immersed in a water bath at 37°C for ½ hour then transferred into a water bath, kept at 10°C.

The pH. of the solutions was determined by a glass electrode (Beckman pH. meter.)

GELATION OF GELATIN WITH POTASSIUM CHLORIDE*

Solutions:

Potassium chloride (24%) 3.22 M in dist. water.
Gelatin 10% in dist. water.
Hydrochloric acid 0.2 N
Potassium hydroxide 0.1 N

	No.of samp.	cc. gelatin	cc. KCl.	cc. HCl	cc. KOH	cc.dist. water	% of gelatin	mol.conc of KCl	time of gelation ' "	pH. of sample ' "
a.	A.	1.00	0.00	0.125	---	0.875	5.0	0.000	2 : 10	4.75
	1.	1.00	0.10	0.100	---	0.800	5.0	0.161	2 ; 30	
	2.	1.00	0.20	0.100	---	0.700	5.0	0.322	2 : 30	
	3.	1.00	0.30	0.150	---	0.550	5.0	0.483	3 : 40	
	4.	1.00	0.40	0.100	---	0.500	5.0	0.644	3 : 40	
	5.	1.00	0.50	0.100	---	0.400	5.0	0.805	4 : 40	
b.	b.	1.00	0.00	0.500	0.00	0.500	5.0	0.000	3 : 10	2.75
	6.	1.00	0.10	0.300	0.00	0.600	5.0	0.161	3 : 40	2.70
	7.	1.00	0.00	0.300	0.00	0.500	5.0	0.322	3 : 50	2.80
	8.	1.00	0.30	0.300	0.00	0.400	5.0	0.483	4 ; 20	2.80
	9.	1.00	0.40	0.300	0.00	0.300	5.0	0.644	4.: 50	2.80
	10.	1.00	0.50	0.400	0.00	0.100	5.0	0.805	5 : 40	2.60
c.	C.	1.00	0.00	----	0.40	0.600	5.0	0.00	3 : 30	11.70
	11.	1.00	0.10	----	0.40	0.50	5.0	0.161	3 : 40	11.65
	12.	1.00	0.20	----	0.40	0.40	5.0	0.322	3 : 20	11.55
	13.	1.00	0.30	-----	0.40	0.30	5.0	0.483	5 : 05	11.50
	14.	1.00	0.40	----	0.40	0.20	5.0	0.644	6 : 50	11.50
	15.	1.00	0.50	----	0.40	0.10	5.0	0.805	8 : 20	11.60

Chart No. 47

* Samples were immersed for ½ hour in a water bath at 37°C then transferred for gelation into a water bath at 10°C.
pH. of solutions was determined at room temperature (20-25)°C by a glass electrode, (Beckman pH. meter).

GELATION OF GELATIN WITH POTSSIUM NITRATE *

Solutions :-

Potassium Nitrate (20%) 1.98M.
Gelatin 10% in distilled water
Potassium hydroxide o.1N.
Nitric Acid o.2N.

	No.of Samp.	cc. gelatin	cc. KNO_3	cc. HNO_3	cc. KOH	cc.dist. water.	% of gelatin	mol. conc. of KNO_3	time of gelation I : II	pH. of sample
a	a.	1.00	o.oo	o.125	---	o.875	5.o	o.ooo	2 : 15	4.6
	1	do	o.1o	o.15o	o.o5	o.75	4.98	o.o99	2 : 25	4.75
	2	do	o.2o	o.o5o	---	o.75	5.o	o.198	3 : oo	4.75
	3.	do	o.3o	o.o5o	---	o.65	do	o.297	4 : 2o	4.75
	4.	do	o.4o	o.15o	o.o5	o.45	4.98	o.396	5 : 3o	4.7o
	5.	do	o.5o	o.o5o	---	o.45	5.o	o.495	7 : 4o	4.75
b	b.	1.00	o.oo	o.5o	---	o.5o	5.o	o.ooo	3 : 3o	2.7
	6.	do	o.1o	do	---	o.4o	do	o.o99	4 : 15	2.6
	7.	do	o.2o	do	---	o.3o	do	o.198	5 : 2o	2.6
	8.	do	o.3o	do	---	o.2o	do	o.297	6 : 5o	2.6
	9.	do	o.4o	do	---	o.1o	do	o.396	8 : 25	2.65
	1o.	do	o.5o	do	---	o.oo	do	o.495	11 : 5o	2.63
c	c.	1.00	o.oo	---	o.4o	o.6o	5.o	o.ooo	3 : 3o	11.7
	11.	do	o.1o	---	do	o.5o	do	o.o99	4.: 45	11.65
	12.	do	o.2o	---	do	o.4o	do	o.198	5 : 25	11.65
	13.	do	o.3o	---	do	o.3o	do	o.297	7 : 15	11.65

Chart No.48

* Samples were immersed for ½ hour in a water bath at 37°C then transferred for gelation into a water bath at 10°C.
pH. of solutions was determined at room temperature (20-25)°C by a glass electrode , (Beckman pH. meter).

GELATION OF GELATIN WITH POTASSIUM SULFATE*

Solutions: Potassium sulfate 0.591 N
Gelatin 10 % in distilled water.
Sulfuric acid 0.25 N
Potassium hydroxide 0.1 N

	No. of sample	cc. gelatin.	cc. pot. sulf.	cc. sulf. acid	cc. pot. hydr.	cc. dist. water.	% of gelatin	mol. conc. pot. sulf.	time of gelation	pH of samp.
a.	a.	0.60	0.00	0.03	-----	1.37	3.0	0.000	6' : 40"	4.7
	1.	0.60	0.20	0.03	-----	1.17	3.0	0.059	5 : 00	4.7
	2.	0.60	0.40	0.03	-------	0.97	3.0	0.118	4.0 00	4.7
	3.	0.60	0.60	0.03	-----	0.77	3.0	0.177	3 : 15	4.7
	4.	0.60	0.80	0.03	-----	0.57	3.0	0.236	2 : 30	4.7
	5.	0.60	1.00	0.03	-----	0.37	3.0	0.295	4 : 30	4.7
b.	b.	0.60	0.00	0.25	-----	1.15	3.0	0.000	13 : 00	2.75
	6.	0.60	0.20	0.27	-----	0.93	3.0	0.059	8 : 35	2.8
	7.	0.60	0.40	0.27	-----	0.73	3.0	0.118	7 : 05	2.8
	8.	0.60	0.60	0.27	-----	0.53	3.0	0.177	6 : 25	2.8
	9.	0.60	0.80	0.28	-----	0.32	3.0	0.236	5 : 50	2.8
	10.	0.60	1.00	0.31	-----	0.09	3.0	0.295	5 : 30	2.75
c.	c.	1.00	0.00	----	0.40	0.60	5.0	0.000	3 : 30	11.7
	11.	1.00	0.10	----	0.40	0.50	5.0	0.029	3 : 20	11.75
	12.	1.00	0.20	----	0.40	0.40	5.0	0.059	3 : 05	11.45
	13.	1.00	0.30	----	0.40	0.30	5.0	0.088	↑	11.7
	14.	1.00	0.40	----	0.40	0.20	5.0	0.118	|	11.7
	15.	1.00	0.50	----	0.40	0.10	5.0	0.148	2 : 45	11.8

Chart No.49

* Samples were immersed for ½ hour in a water bath at 37°C then transferred for gelation into a water bath at 10°C
pH. of samples was taken at room temperature (20-25°C) by a glass electrode, (Beckman pH. meter)

GELATION OF GELATIN WITH MAGNESIUM CHLORIDE*

Solutions:-

Magnesium chloride, $MgCl_2.6H_2O$, (40%) 1.97 Molar.
Gelatin 10% in distilled water.
Hydrochloric acid, HCl 0.2 M.

	No.of sample	cc. gelatin	cc. $MgCl_2$	cc. HCl	cc. H_2O	Total volume	% of gelatin	Mol.conc. of $MgCl_2$	Time of gelation ' "	pH.of sample
a.	a.	1.00	0.00	0.125	0.875	2.00	5.0	0.000	2 : 00	4.75
	1.	do.	0.10	0.120	0.78	do.	do.	0.098	2 : 30	4.65
	2.	do.	0.20	0.100	0.70	do.	do.	0.197	3 : 00	4.64
	3.	do.	0.30	0.075	0.625	do	do.	0.296	4 : 00	4.68
	4.	do.	0.40	0.050	0.55	do.	do.	0.394	5 : 20	4.73
	5.	do.	0.50	0.050	0.45	do.	do.	0.492	7 : 20	4.75
b.	b.	1.00	0.00	0.50	0.50	2.00	5.0	0.000	3 : 10	2.75
	6.	do	0.10	0.45	0.40	1.95	5.12	0.098	3 : 10	2.74
	7.	do	0.20	0.50	0.30	2.00	5.0	0.197	3 : 35	2.70
	8.	do.	0.30	0.475	0.225	do.	do.	0.296	4 : 40	2.75
	9.	do.	0.40	0.45	0.105	do.	do.	0.394	5 : 50	2.73
	10.	do.	0.50	0.45	0.05	do.	do.	0.492	7 : 45	2.76

Chart No.50

* Samples were immersed for ½ hour in a water bath at 37°C then transferred for gelation into a water bath at 10°C.
pH. of solution was determined at room temperature (20-25)°C by a glass electrode,(Beckman pH. meter)

GELATION OF GELATIN WITH MAGNESIUM SULFATE*

Solutions:-

Magnesium sulfate, $MgSO_4.7H_2O$, 1.83 M.
Gelatin 10% in distilled water
Sulfuric acid, H_2SO_4 , 0.25 N.

	No. of sample	cc. gelatin	cc. $MgSO_4$	cc. H_2SO_4	cc. H_2O	Total volume	% of gelatin	Mol. conc. of $MgSO_4$	Time of gelation	pH. of sample
									' "	
a.	a.	0.60	0.00	0.03	1.37	2.00	3.0	0.000	6 :40	4.7
	1.	do.	0.20	do.	1.17	do.	do.	0.183	4 :45	do.
	2.	do.	0.40	do.	0.97	do.	do.	0.366	4 :30	do.
	3.	do.	0.60	do.	0.77	do	do.	0.549	3 :15	do.
	4.	do.	0.80	do.	0.57	do.	do.	0.732	2 :40	do.
	5.	do.	1.00	do.	0.37	do.	do.	0.915	2 :15	do.
b.	b.	0.60	0.00	0.25	1.15	2.00	3.0	0.000	13 :00	2.75
	6.	do.	0.20	do.	0.95	do.	do.	0.183	6 :20	2.8
	7.	do.	0.40	do.	0.75	do.	do.	0.366	5 :00	do.
	8.	do.	0.60	do.7	0.55	do.	do.	0.549	4 :15	do.
	9.	do.	0.80	do.	0.35	do.	do.	0.732	cloudy precipit	do.

Chart No.51

* Samples were immersed for ½ hour im a water bath at 37°C. then transferred for gelation into a water bath at 10°C.
pH. of solutions was determined at room temperature(20-25)°C. by a glass electrode, (Beckman pH. meter.)

GELATION OF GELATIN WITH SODIUM SULFATE *

Solutions: Sodium sulfate 1.185 molar.
Gelatin 10% in distilled water.
Sulfuric acid 0.25 normal.
Sodium hydroxide 0.1 normal.

	No.of samp.	cc. gelatin	cc. sod.sulf.	cc.sulf acid	cc.sod. hydrox.	cc. water	% of gelat.	M.of sod.sulf	time of gelation	pH. of sample
a	a.	0.60	0.00	0.03	----	1.37	3.0	0.000	' "	4.7
	1.	0.60	0.20	0.03	----	1.17	3.0	0.118	6 : 40	4.7
	2.	0.60	0.40	0.03	----	0.97	3.0	0.237	6 : 30	4.7
	3.	0.60	0.60	0.03	----	0.77	3.0	0.356	5 : 15	4.7
	4.	0.60	0.80	0.03	----	0.57	3.0	0.474	4 : 00	4.7
	5.	0.60	1.00	0.03	----	0.37	3.0	0.592	2 : 45	4.7
									2 : 15	
b	b.	0.60	0.000	o.25	----	1.15	3.0	0.000	13 : 00	2.75
	6.	0.60	0.20	0.27	----	0.93	3.0	0.118	6 : 15	2.85
	7.	0.60	0.40	0.27	----	o.73	3.0	0.237	5 : 15	2.85
	8.	0.60	0.60	0.30	----	0.50	3.0	o.356	4 : 00	2.85
	9.	0.60	0.80	0.33	----	0.27	3.0	0.474	3(cloudy)	2.80
	10.	0.60	1.00						precipit.	2.8
c	c.	1.00	0.00.	----	0.40	0.60	5.0	0.000	3 : 35	11.68
	11.	1.00	0.10	----	0.40	0.50	5.0	0.059	3 : 30	11.58
	12.	1.00	0.20	----	0.40	0.40	5.0	0.118	3 : 10	11.46
	13.	1.00	0.30	----	o.40	0.3o	5.0	0.177	2 : 45	11.36
	14.	1.00	0.40	----	0.40	0.20	5.0	0.237	2 : 10	11.30
	15.	1.00	0.50	----	0.40	0.10	5.0	0.296	1 : 50	11.30

Chart No. 52

* Samples were immersed for ½ hour in a water bath at 37°C then transferred for gelation into a water bath at 10°C.
pH. of solutions was determined at room temperature (20-25)°C by a glass electrode, (Beckman pH. meter).

GELATION OF GELATIN WITH THIOUREA AT pH. 3.45 *
Solutions :- At pH. 3.45

No.of Samp.	cc. Gelat.	cc. buff.	cc.th. urea.	Mol.of th.urea	%of gelat.	time of gelation ' "
1.	o.9	o.o	o.1	o.2	9	2 :o5
2.	o.8	o.1	do.	do.	8	2 :5o
3.	o.7	o.2	do.	do.	7	4 :2o
4.	o.6	o.3	do.	do.	6	5 :5o
5.	o.5	o.4	do.	do.	5	9 :1o
6.	o.4	o.5	do.	do.	4	16 :3o

Chart No. 53

Buffer o.2M, Na_2HPO_4 + o.1M Citric Acid
Gelatin 10% made with components of above buffer.
(th.urea) thiourea, 2M in components of above buffer.

GELATION OF GELATIN WITH GUANIDINE.HCl at pH. 3.45 *
Solutions:- at pH. 3.45

No.of Samp.	cc. Gelat.	cc. buff.	cc. Guan.	Mol. of Guan.	% of Gelatine	Time of Gelation ' "
L.	o.9	o.o	o.1	o.2	9	1 : 5o
2.	o.8	o.1	o.1	do.	8.	2 : 3o
3.	o.7	o.2	o.1	do.	7	3 : oo
4.	o.6	o.3	o.1	do.	6	4 : 2o
5.	o.5	o.4	o.1	do.	5	6 : 25
6.	o.4	o.5	o.1	do.	4	1o : oo

Chart No.54

Buffer, o.2M, Na_2HPO_4 + o.1M Citric Acid.
Gelatin 10% made with components of above buffer.
(Guan.) Guanidine. HCl, 2M in components of above buffer.

Gelation OF GELATIN WITH POTASSIUM CHLORIDE at pH. 3.45 *
Solutions:- At pH. 3.45

No.of Samp.	cc. gelat.	cc. buff	cc. KCl.	Mol.of KCl	% of KCl	Time of Gelation ' "
1.	o.8	o.o	o.2	o.8	8	2 : 4o
2.	o.7	o.1	do.	do.	7	3 : 55
3.	o.6	o.2	do.	do.	6	4 : 15
4.	o.5	o.3	do.	do.	5	6 : oo
5.	o.4	o.4	do.	do.	4	1o : 2o

Chart No.55

Buffer, o.2M, Na_2HPO_4 + o.1M Citric Acid
Gelation 10% made with components of above buffer.
KCl 4M in components of above buffer.

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* Samples were immersed in a water bath at 37°C for ½ hour then transferred into a water bath, kept at 1o°C.

The pH. of the solutions was determined by a glass electrode (Beckman pH. meter).

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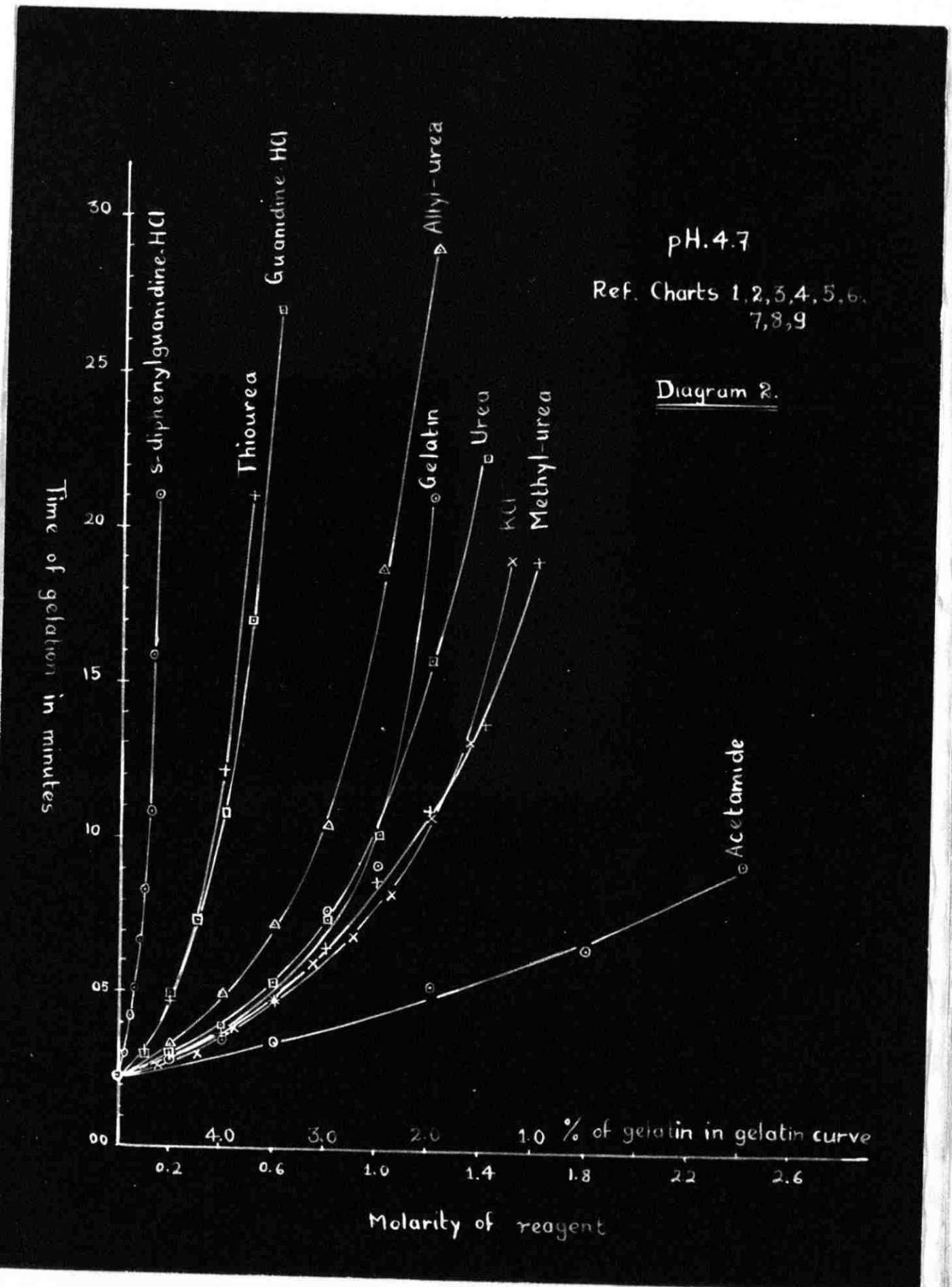
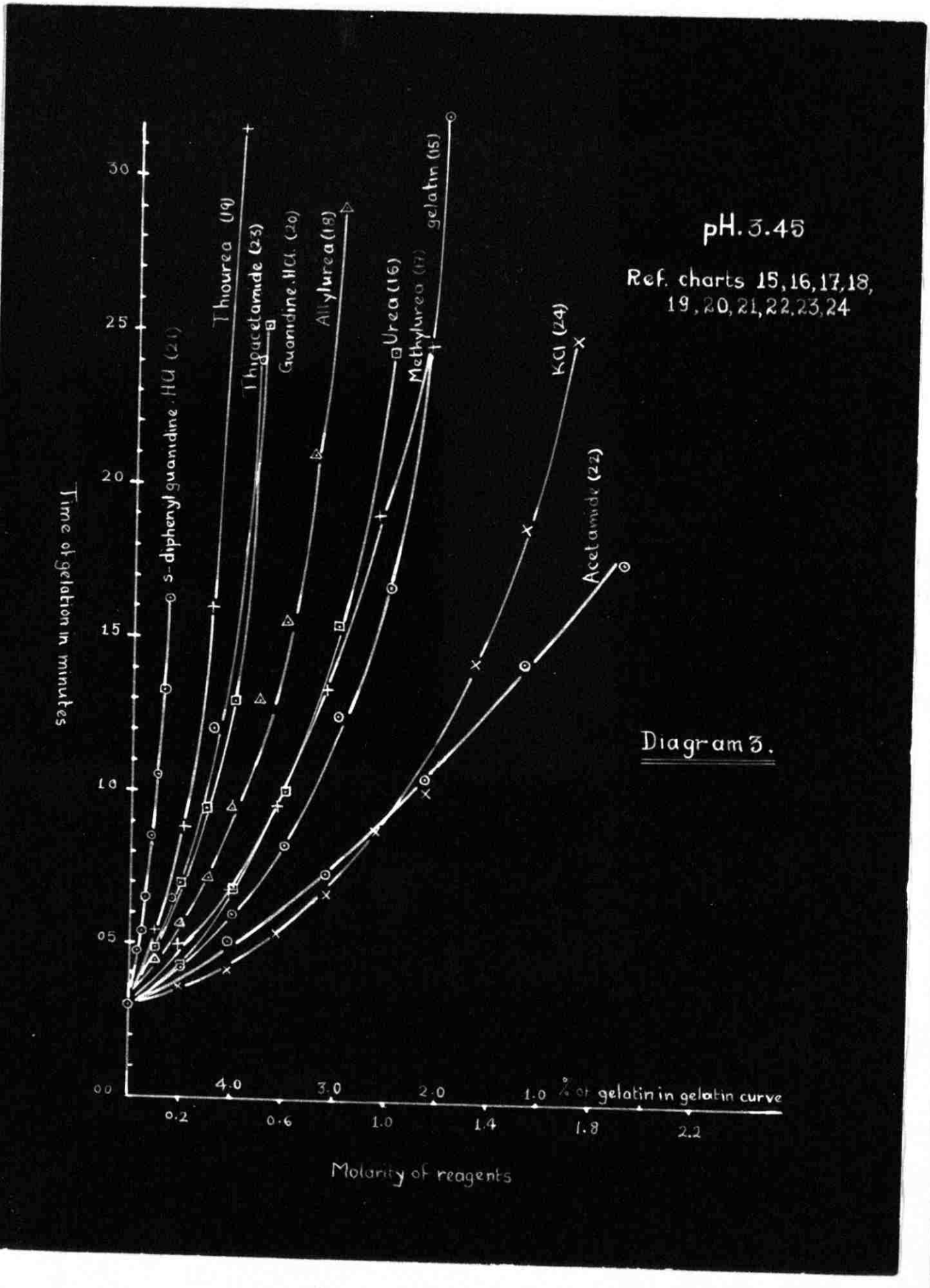
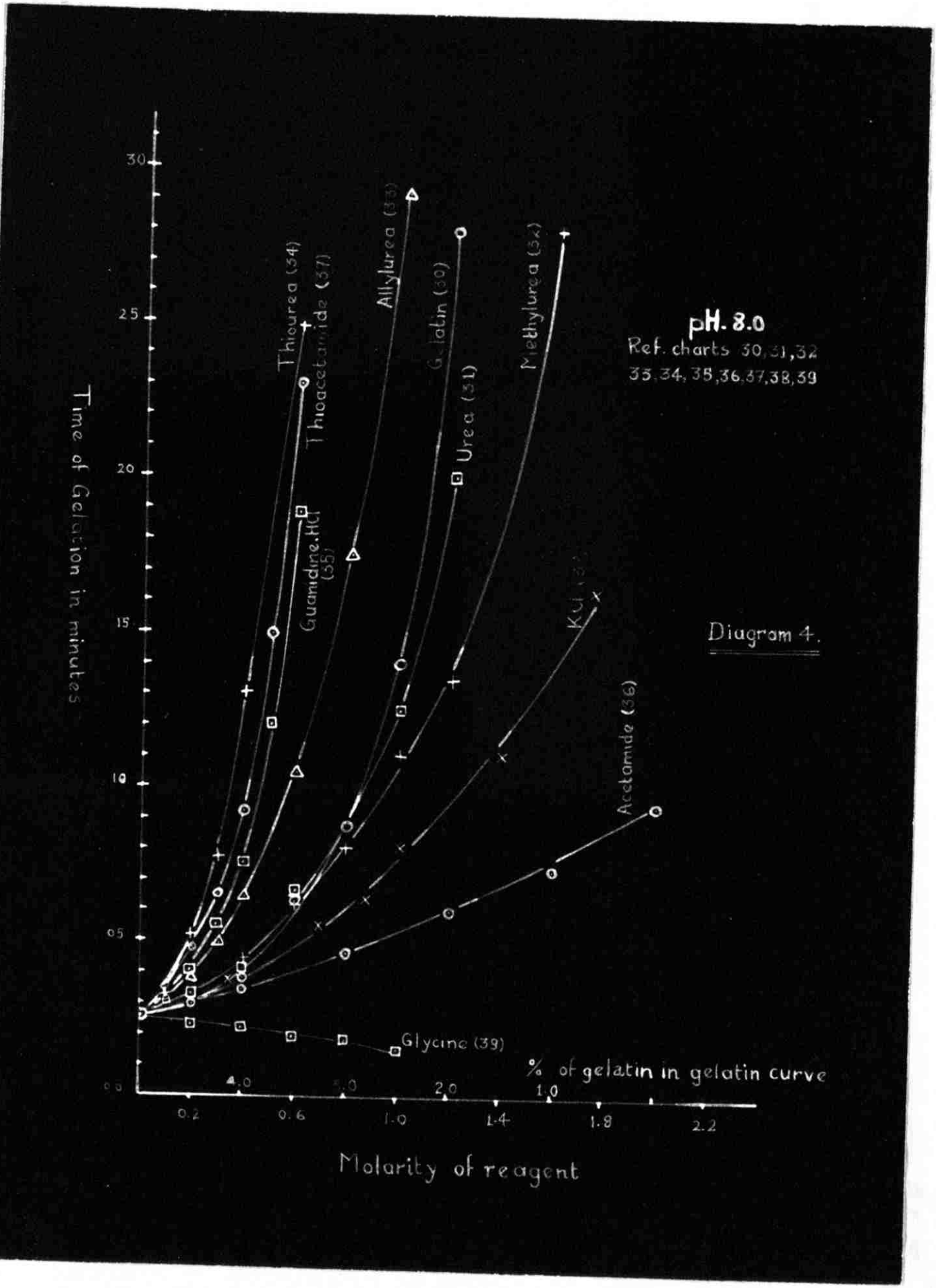
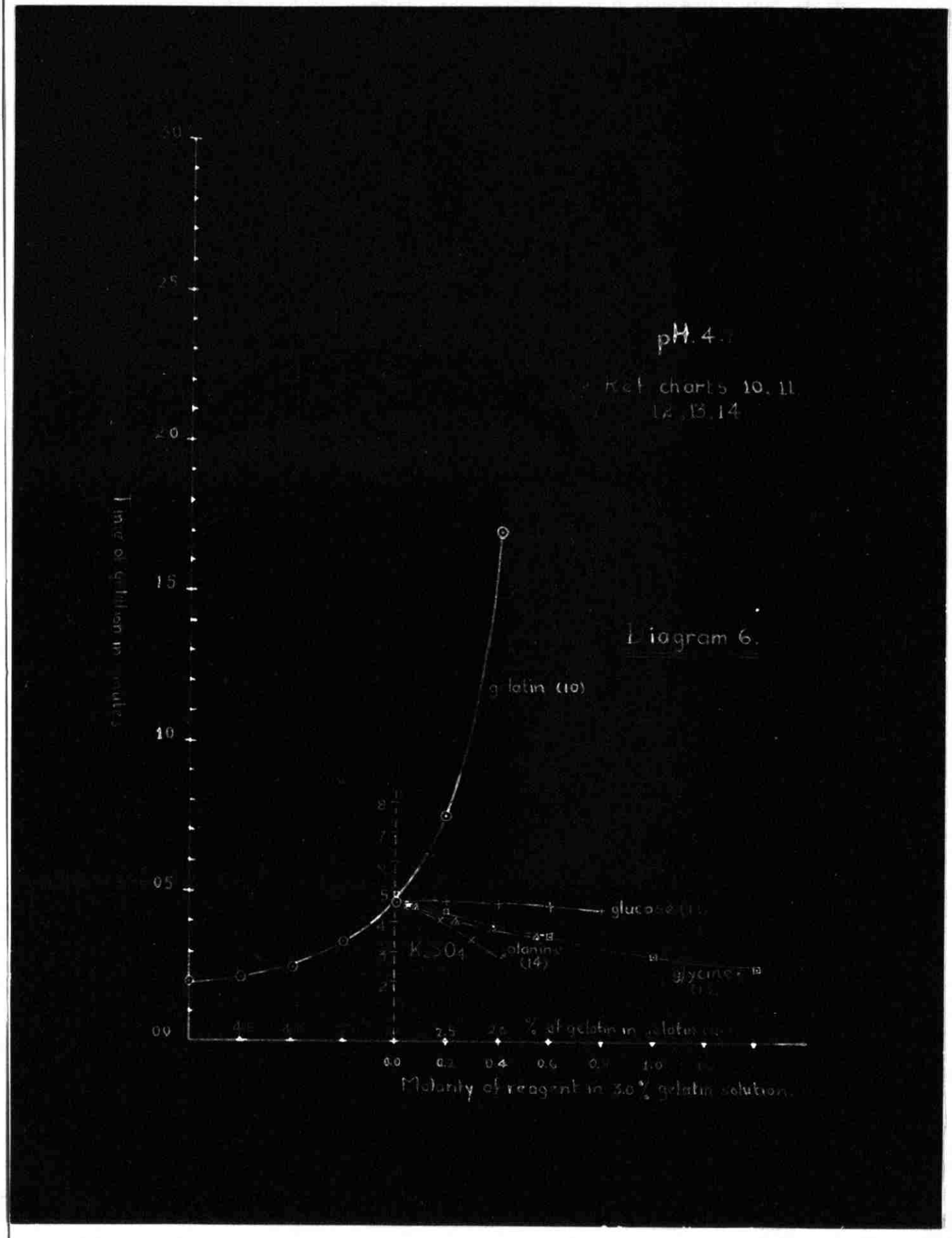
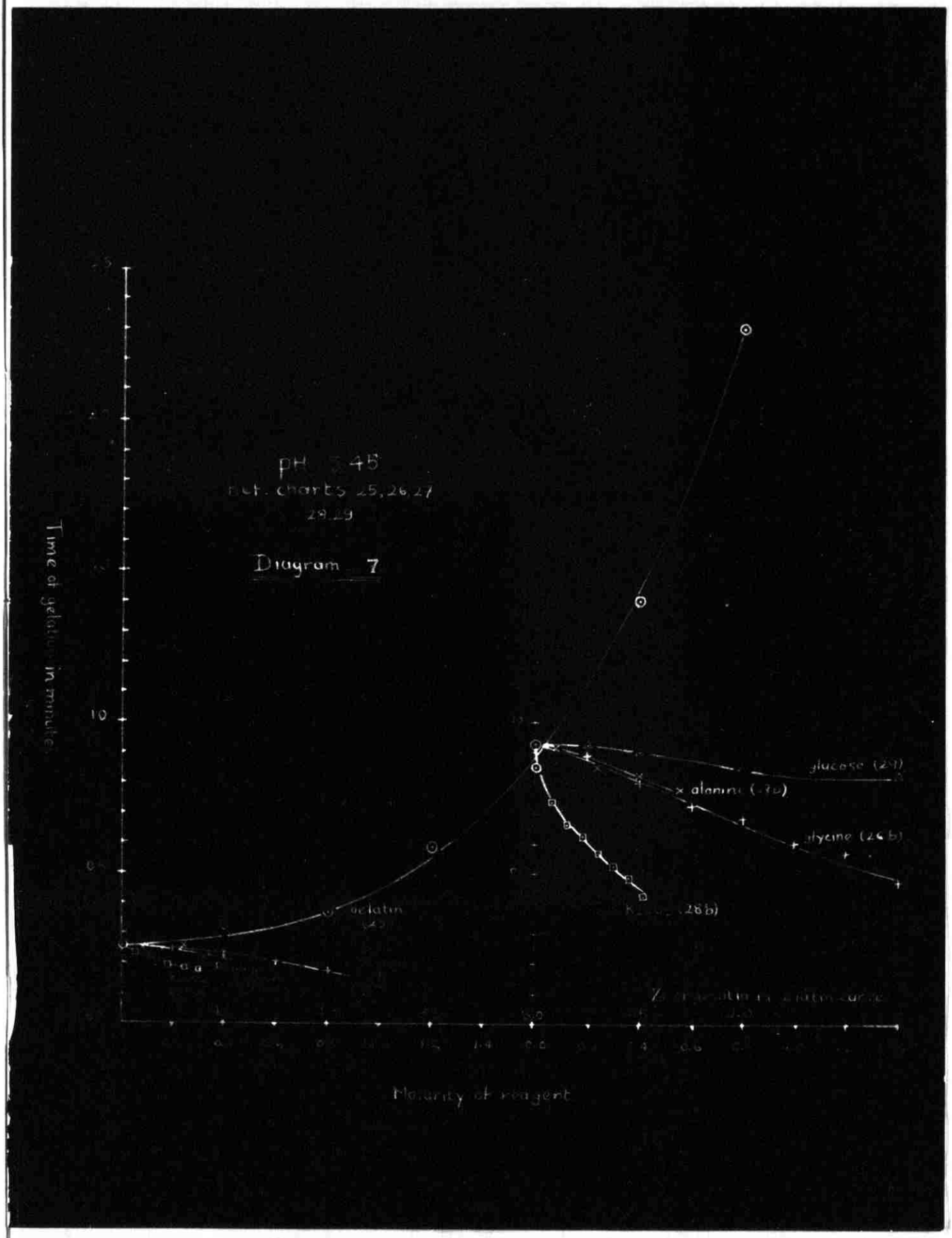

pH.4.7
Ref. Charts 1,2,3,4,5,6,
7,8,9
Diagram 2.
Time of gelation in minutes
30
25
20
15
10
05
00
s-diphenylguanidine.HCl
Thiourea
Guanidine.HCl
Allyl-urea
Gelatin
Urea
KCl
Methyl-urea
Acetamide
4.0
3.0
2.0
1.0
% of gelatin in gelatin curve
0.2
0.6
1.0
1.4
1.8
2.2
2.6
Molarity of reagent

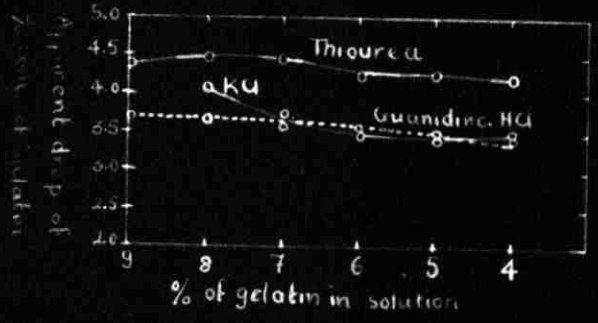

pH. 3.45
Ref. charts 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Diagram 3.
Time of gelation in minutes
Molarity of reagents
% of gelatin in gelatin curve
s-diphenylguanidine. HCl (21)
Thiourea (19)
Thiguetamide (23)
Guanidine. HCl (20)
Allylurea (18)
Urea (16)
Methylurea (17)
gelatin (15)
KCl (24)
Acetamide (22)
00
05
10
15
20
25
30
0.2
0.6
1.0
1.4
1.8
2.2
4.0
3.0
2.0
1.0


pH. 8.0
Ref. charts 30, 31, 32
33, 34, 35, 36, 37, 38, 39
Diagram 4.
Time of Gelation in minutes
Molarity of reagent
% of gelatin in gelatin curve
Thiourea (34)
Thioacetamide (37)
Allylurea (33)
Gelatin (30)
Urea (31)
Methylurea (32)
Guanidine.HCl (35)
Acetamide (36)
Glycine (39)
30
25
20
15
10
05
4.0
2.0
1.0
0.2
0.6
1.0
1.4
1.8
2.2

pH. 11.5
Ref. charts 40, 41, 43, 43, 44, 45.
Diagram 5.
Guanidine HCl (43)
Urea (43)
Thio-urea (43)
Gelatin (43)
KCl (44)
Na glycurate (43)
Molarity of reagent

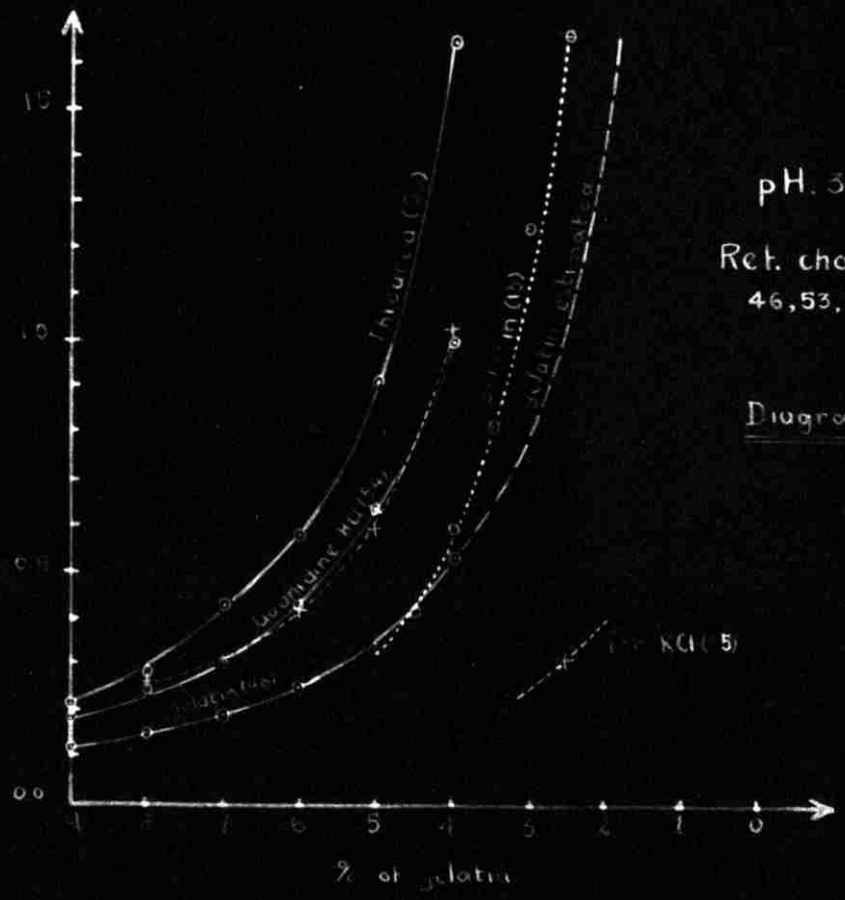

pH. 4.
Ref charts 10. 11
12. 13. 14
Diagram 6.
gelatin (10)
glucose
alanine (14)
glycine
K2SO4
% of gelatin in gelatin
Molarity of reagent in 3.0 % gelatin solution.


pH 3.45
Ref. charts 25, 26, 27
29, 29
Diagram 7
Time of gelation in minutes
10
glucose (29)
alanine (28)
glycine (26b)
gelatin
(26)
Molarity of reagent



Ref. Diag. 8

Diagram 9.



pH. 3.45

Ref. charts 13, 46, 53, 54, 55.

Diagram 8.

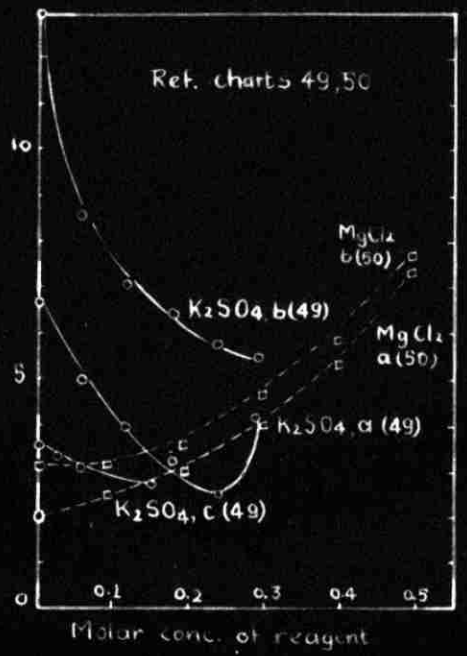


Diagram 11

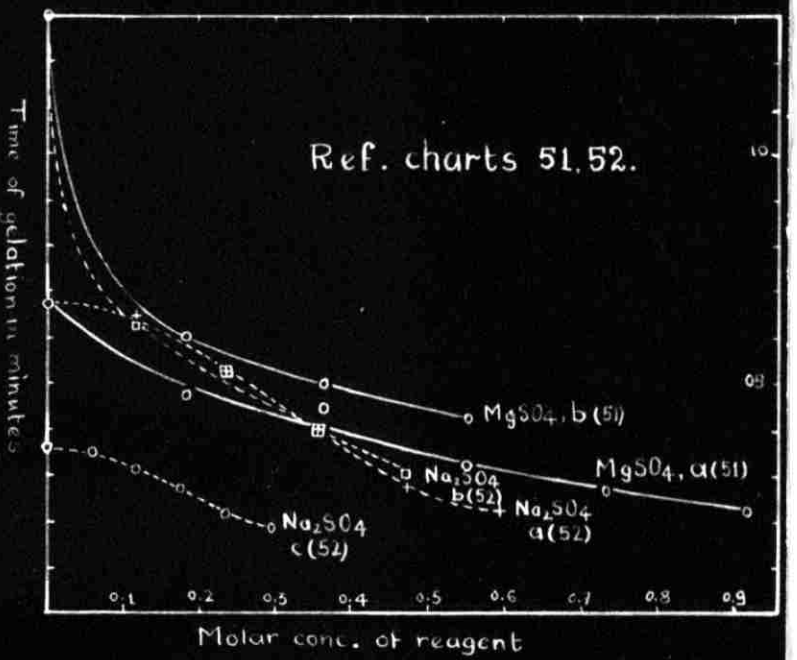


Diagram 12

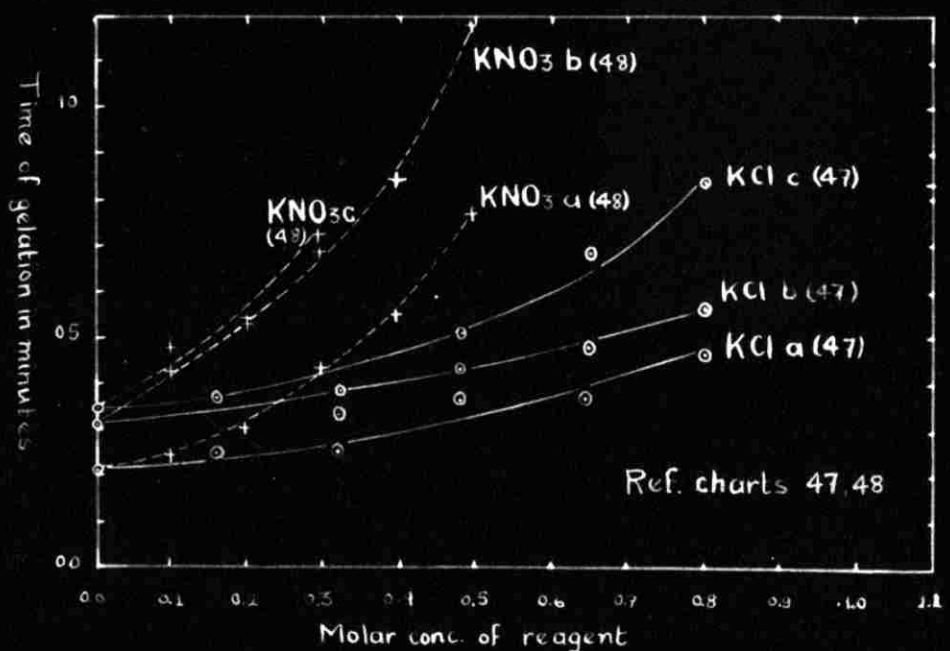


Diagram 10

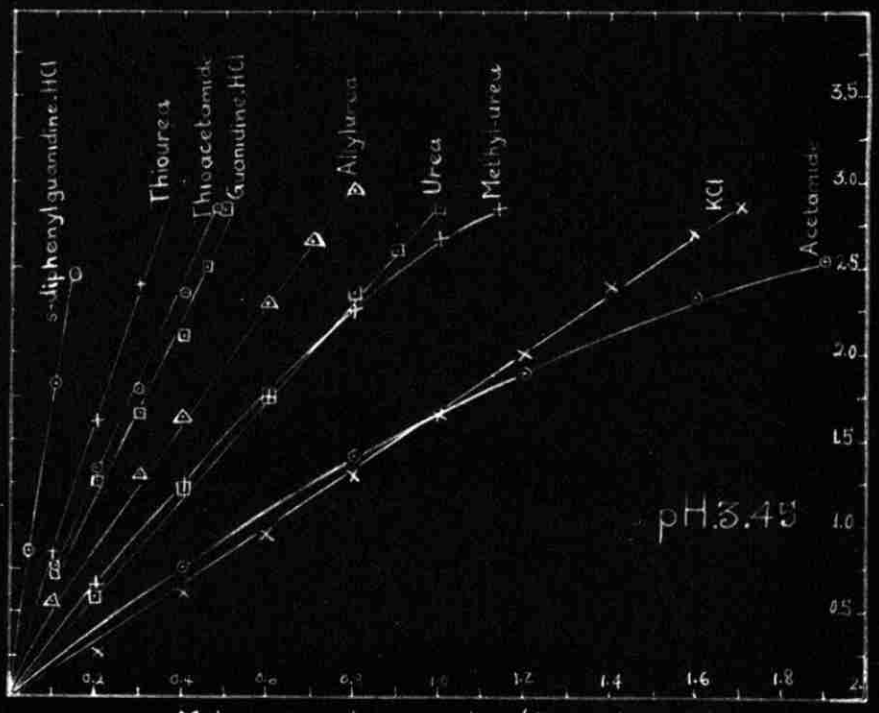


Diagram 14

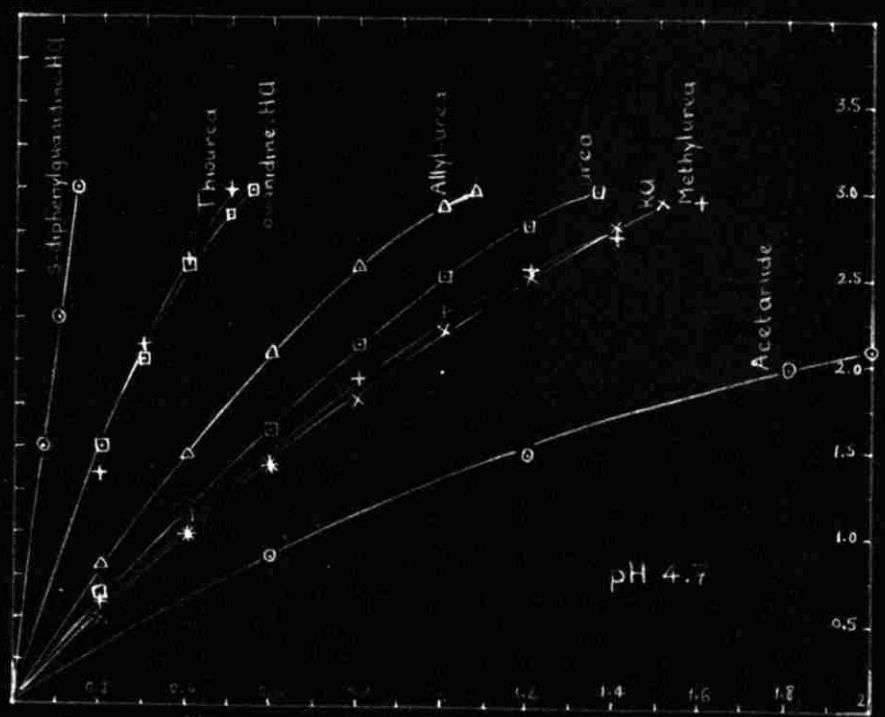


diagram 15

Diagram 15.

Diagram 16.

RATE OF GELATION OF GELATIN SOLUTIONS AT pH.4.7*

No.of sample	cc. gelatin	cc. buffer	Time of gelation ' "	% of gelatin
1.	0.5o	0.5o	2 :15	5.0
2.	0.45	0.55	2 :5o	4.5
3.	0.4o	0.6o	3 :25	4.0
4.	0.35	0.65	5 :oo	3.5
5.	0.3o	0.7o	7 :4o	3.o
6.	0.25	0.75	9 :1o	2.5
7.	0.2o	0.8o	21 :oo	2.o

Solutions:-
Acetate buffer 0.2M pH. 4.7
‡ Gelatin 10% in above buffer

Chart No. 1

RATE OF GELATION OF GELATIN SOLUTIONS WITH UREA AT pH. 4.7 *

No.of sample	cc. gelatin	cc. buffer	cc. urea	Mol.of urea	Time of gelation ' "
o.	o.5o	o.5o	o.oo	o.o	2 :15
1.	do.	o.45	o.o5	o.2	3 :oo
2.	do.	o.4o	o.1o	o.4	3 :55
3.	do.	o.35	o.15	o.6	5 :2o
4.	do.	o.3o	o.2o	o.8	7 :25
5.	do.	o.25	o.25	1.o	11 :oo
6.	do.	o.2o	o.3o	1.2	15 :45
7.	do.	o.15	o.35	1.4	22 :15
8.	do.	o.1o	o.4o	1.6	above 3o

Solutions:-
Acetate buffer. 0.2M pH. 4,7
+ Gelatin 10% in
+ above buffer.
Urea 4M in above buffer
All solutions are made to pH. 4.7 .

Chart No. 2.

RATE OF GELATION OF GELATIN SOLUTIONS WITH METHYLUREA*
at pH. 4.7

No.of sample	cc. gelatin	cc. buffer	cc.Methylurea	Mol.of M-urea	Time of gelation ' "
o.	o.5o	o.5o	o.oo	o.o	2 :15
1.	do.	o.45	o.o5	o.2	2 :55
2.	do.	o.4o	o.1o	o.4	3 :4o
3.	do.	o.35	o.15	o.6	4 :55
4.	do.	o.3o	o.2o	o.8	6 :3o
5.	do.	o.25	o.25	1.o	8 :4o
6.	do.	o12o	o.3o	1.2	11 :oo
7.	do.	o.15	o.35	1.4	13 :45
8.	do.	o.1o	o.4o	1.6	19 :oo

Solutions:-
Acetate buffer o.2M pH. 4.7 .
+ Gelatin 10% in above buffer.
+ Methylurea 4M in above buffer.

Chart No. 3

* Samples were immersed for ½ hour in a water bath at 37 C. then transferred into a water bath at 10 C. pH of solutions were determined between2o & 25 C by a glass electrode(Beckman pH.meter)
+ pH. of solutions were corrected to 4.7 by the components of the buffer (o.2M $NaH_3C_2O_2$ & o.2M $CH_3.COOH$)

RATE OF GELATION OF GELATION SOLUTIONS WITH ALLYLUREA *
at pH. 4.7

No. of Samp.	cc. gelat.	cc. buffer	cc. allylurea	Mol. of A-urea	Time of Gelation ' "
o.	o.5o	o.5o	o.oo	o.o	2 :15
1.	do.	o.45	o.o5	o.2	3 :2o
2.	do.	o.4o	o.1o	o.4	5 :oo
3.	do.	o.35	o.15	o.6	7 :15
4.	do.	o.3o	o.2o	o.8	1o :3o
5.	do.	o.25	o.25	1.o	18 :4o
6.	do.	o.2o	o.3o	1.2	29 :oo

Solutions:-
acetate buffer o.2M, pH. 4.7
+ Gelatin 10% in above buffer
+ Allylurea 4M in above buffer.

Chart No.4

RATE OF GELATION OF GELATION SOLUTIONS WITH THIOUREA *
at pH. 4.7

No.of Samp.	cc. gelat.	cc. buffer	cc. Thio-urea.	Mol. of T-urea	Time of Gelation ' "
o.	o.5o	o.5o	o.oo	o.o	2 :15
1.	do.	o.45	o.o5	o.1	3 :1o
2.	do.	o.4o	o.1o	o.2	4.45
3.	do.	o.35	o.15	o.3	7 :3o
4.	do.	o.3o	o.2o	o.4	12 :15
5.	do.	o.25	o.25	o.5	21 :oo,
6.	do.	o.2o	o.3o	o.6	above 3o

Solutions:-
acetate buffer o.2M, pH. 4.7
+ Gelatin 1o% in above buffer
+ Thiourea 2M in above buffer.

Chart No.5

RATE OF GELATION OF GELATION SOLUTIONS WITH GUANIDINE HYDROCHLORIDE * at pH. 4.7

No. of Samp.	cc. Gelat.	cc. buffer	cc. guanidine HCl	Mol. of Guani.	Time of Gelation ' "
o.	o.5o	o.5o	o.oo	o.o	2 :15
1.	do.	o.45	o.o5	o.1	3 :oo
2.	do.	o.4o	o.1o	o.2	5 :oo
3.	do.	o.35	o.15	o.3	7 :2o
4.	do.	o.3o	o.2o	o.4	10 :5o
5.	do.	o.25	o.25	o.5	17 :oo
6.	do.	o.2o	o.3o	o.6	27 :3o
7.	do.	o.15	o.35	o.7	47 :oo

Solutions :-
acetate buffer o.2M, pH. 4.7
+ Gelatin 1o% in above buffer
+ Guanidine.HCl 2M in above buffer.

Chart No.6

* Samples were immersed for ½ hour in a water bath at 37 C then transferred into a water bath at 1o C. pH. of solutions were determined between 2o & 25 C by a glass electrode (Beckman pH. meter).

\+ pH. of solutions were corrected at 4.7 by the contents of the buffer (o.2M $NaH_3C_2O_2$ & o.2M $CH_3.COOH$)

RATE OF GELATION OF GELATIN WITH s-DIPHENYLGUANIDINE.HCl*
at pH. 4.7

No.of samp.	cc. gelat.	cc. buffer	cc.s-diphenylguan.	Mol.of s-diph	time of gelation ' "
0.	0.5o	0.5o	0.00	0.00	2 : 15
1.	do	0.45	0.05	0.02	3 : 00
2.	do	0.4o	0.1o	0.04	4 : 15
3.	do	0.35	0.15	0.06	5 : 1o
4.	do	0.3o	0.2o	0.08	6 : 4o
5.	do	0.25	0.25	0.1o	8.1515
6.	do	0.2o	0.3o	0.12	1o : 5o
7.	do	0.15	0.35	0.14	15 : 5o
8.	do	0.1o	0.4o	0.16	21 : oo

Solutions:-
Acetate buffer o.2M, pH. 4.7
+ Gelatin 1o% in above buffer
+ s-Diphenylguanidine.HCl o.4M in above buffer

Chart No. 7

RATE OF FELATION OF GELATIN SOLUTUONS WITH ACETAMIDE
at pH. 4.7 *

No.of samp.	cc. gelat.	cc. buffer	cc. Acetamid	Mol.of acetam	Time of gelation ' "
o.	c.5o	o.5o	o.oo	o.oo	2 : 15
1.	do.	o.4o	o.1o	o.6	3 : 30
2.	do.	o.3o	o.2o	1.2	5 : 15
3.	do.	o.2o	o.3o	1.8	6 : 3o
4.	do.	o.1o	o.4o	2.4	9 : 15
5.	do.	o.oo	o.5o	3.o	1o : 2o

Solutions:-
Acetate buffer o.2M, pH. 4.7
+ Gelatin 1o% in above buffer
+ Acetamide 6M in above buffer

Chart No. 8

RATE OF GELATION OF GELATIN SOLUTIONS WITH POTASSIUM CHLORIDE at pH. 4.7 *

No.of Samp.	cc. gelat.	cc. buffer	cc. KCl.	Mol.of KCl.	Time of Gelation ' "
o.	o.5o	o.5o	o.oo	o.oo	2 : 15
1.	do.	o.45	o.o5	o.15	2 : 4o
2.	do.	o.4o	o.1o	o.3o	3 : o5
3.	do.	o.35	o.15	o.45	3 : 55
4.	do.	o.3o	o.2o	o.6o	4 : 45
5.	do.	o.25	o.25	o.75	5.:55
6.	do.	o.2o	o.3o	o.9o	6 : 5o
7.	do.	o.15	o.35	1.o5	8.: 15
8.	do.	o.1o	o.4o	1.2o	1o : 45
9.	do.	o.o5	o.45	1.35	14 : oo
1o.	do.	o.oo	o.5o	1.5o	19 : oo

Solutions:-
Acetate buffer o.2M, pH. 4.7
+ Gelatin 1o% in above buffer
+ KCl 3M in above buffer.

Chart No. 9

* Samples were immersed for ½ hour in water bath at 37 C then transferred into a water bath at 1o C. pH. of solutions were determined between 2o & 25 C by a glass electrode (Beckman pH. meter).

\+ pH. of solutions were corrected at 4.7 by the contents of the buffer (o.2M $NaH_3C_2O_2$ & o.2M $CH_3.COOH$)

GELATION OF GELATIN SOLUTIONS WITH CITRATE-PHOSPHATE BUFFER at pH. 4,7 *

No.of samp.	cc. gelatin	cc. buffer	% of gelatin	Time of gelation '	"
1.	0.50	0.50	5.0	2 :	00
2.	0.45	0.55	4.5	2 :	10
3.	0.40	0.60	4.0	2 :	30
4.	0.35	0.65	3.5	3 :	20
5.	0.30	0.70	3.0	4 :	40
6.	0.25	0.75	2.5	7 :	30
7.	0.20	0.80	2.0	17 :	00

Solutions :-
Buffer, Citr.-Phosph. at pH.4.7
+ Gelatin, 10% in above buffer made to pH.4.7

Chart No. 10

GELATION OF GELATIN SOLUTIONS WITH GLUCOSE AT pH. 4.7 *

NO.of Samp.	cc. gelatin	cc. buffer	cc. glucose	Mol. of Glucose	Time of Gelation '	"
0.	0.30	0.70	0.00	0.0	4 :	40
1.	0.30	0.60	0.10	0.2	4 :	40
2.	0.30	0.40	0.30	0.6	4 :	37
3.	0.30	0.20	0.50	1.0	4 :	32
4.	0.30	0.00	0.70	1.4	4 :	25

Chart No.11

Solutions:-
Buffer, Citrate phosphate at pH. 4.7
+ Gelatin, 10% in above buffer at pH. 4.7
+ Glucose 2M in above buffer at pH. 4.7

GELATION OF GELATIN WITH POTASSIUM SULFATE AT pH. 4.7 *

No.of Samp.	CC. Gelatin	cc. buffer	cc. K_2SO_4	Mol. of K_2SO_4	Time of Gelation '	"
0.	0.30	0.70	0.00	0.00	4 :	40
1.	do.	0.60	0.10	0.06	4 :	30
2.	do.	0.40	0.30	0.18	4 :	05
3.	do.	0.20	0.50	0.30	3 :	25
4.	do.	0.00	0.70	0.42	2 :	50

Chart No.12

Solutions:-
Buffer, Citrate phosphater at pH. 4.7
+ Gelatin; in above buffer at pH. 4.7 ; 10%
+ K_2SO_4, 0.6M in above buffer at pH. 4.7

---

* Samples were immersed for ½ hour in water bath at 37 C.then transferred into a water bath at 10 C.

pH. of solutions were determined between (20-25 C. by a glass electrode (Beckman pH.meter.)

+ pH. of solutions were corrected to 4.7 by the contents of the buffer (0.2M Na_2HPO_4 ‡ 0.1M Citric acid)

GELATION OF GELATIN WITH GLYCINE at pH. 4.7 *

Solutions:-

Buffer (0.2M Na_2HPO_4 + 0.1M Citric acid) pH. 4.7
Gelatin, 10% in above buffer at pH. 4.7
Glycine, 2M at pH. 4.7 in above buffer

No. of sample	cc. gelatin	cc. buffer	cc. glycine	Mol.of glycine	Time of Gelation ' "
0.	o.3o	o.7o	o.oo	o.o	4 : 4o
1.	do.	o.6o	o.1o	o.2	4 : 2o
2.	do.	o.4o	o.3o	o.6	3 : 35
3.	do.	o.2o	o.5o	1.o	2 : 55
4.	do.	o.oo	o.7o	1.4	2 : 3o
5.	do.				

Chart No.13

GELATION OF GELATIN WITH ALANINE at pH. 4.7 *

Soltions :-

Buffer (0.2M Na_2HPO_4 0.1M Citric acid) pH. 4.7
Gelatin, 10% in above buffer at pH, 4.7
Alanine, 0.76M in above buffer, at pH. 4.7

No. of sample	cc. gelatin	cc. buffer	cc. alanine	Mol. of alanine	Time of Gelation ' "
0.	o.3o	o.7o	o.oo	o.ooo	4 : 4o
1.	do.	o.6o	o.1o	o.o76	4 : 3o
2.	do.	o.4o	o.3o	o.228	4 : oo
3.	do.	o.2o	o.5o	o.38o	3 : 45
4.	do.	o.oo	o.7o	o.532	3 : 35

Chart No.14

GELATION OF GELATIN AT pH. 3.45 IN PHOSPHATE-CITRATE BUFFER *

Solutions:-

Buffer (0.2M. Na_2HPO_4+0.1M citric acid) at pH. 3.45
Gelatin 10% at pH. 3.45 in above buffer

No. of sample	cc. gelatin	cc. buffer	% of Gelatin	Time of gelation ' "
1.	o.5o	o.5o	5.o	3 :00
2.	o.45	o.55	4.5	4 :10
3.	o.4o	o.6o	4.o	6 :oo
4.	o.35	o.65	3.5	8 :15
5.	o.3o	o.7o	3.o	12 :3o
6.	o.25	o.75	2.5	16 :4o
7.	o.2o	o.8o	2.o	32 :oo
8.	o.15	o.85	1.5	- - -

Chart No.15

---

* Samples were immersed for ½ hour in water bath at 37 C. then transferred into a water bath at 10 C.

pH. of solutions were corrected to 4.7 by the components of the buffer.

The pH. of the solutions was determined at (25-25)C by a glass electrode (Beckman pH.meter.)

GELATION OF GELATIN WITH UREA at pH. 3.45 *

Solutions:-

Buffer,(o.2M Na_2HPO_4+o.1M Citric acid) at pH. 3.45
Gelatin 10% at pH. 3.45 in above buffer.
Urea 4M at pH. 3.45 in above buffer.

No. of sample	cc. gelatin	cc. buffer	cc. urea	Molarity of urea	Time of gelation
					' "
0.	0.5o	o.5o	o.oo	o.o	3 : 00
1.	do.	o.45	o.o5	o.2	4 : 15
2.	do.	o.4o	o.1o	o.4	6 : 45
3.	do.	o.35	o.15	o.6	1o : oo
4.	do.	o.3o	o.2o	o.8	15:25
5.	do.	o.25	o.25	1.o	24 : 15
6.	do.	o.2o	o.3o	1.2	- - - -

Chart No. 16

GELATION OF GELATIN WITH METHYLUREA at pH. 3.45 *

Solutions:-

Buffer, 0.2M Na_2HPO_4+o.1M Citric acid, at pH. 3.45
Gelatin 10% at pH. 3.45 in above buffer.
Methylurea(Murea) 3.81M at pH. 3.45 in above buffer.

No. of sample	cc. gelatin	cc. buffer	cc. Murea	Molarity of Murea	Time of gelation
					' "
0.	o.5o	o.5o	o.oo	o.oo	3 : 00
1.	do.	o.45	o.o5	o.19	5 : oo
2.	do.	o.4o	o.1o	o.38	6 : 45
3.	do.	o.35	o.15	o.57	9 : 3o
4.	do.	o.3o	o.2o	o.76	13 : 2o
5.	do.	o.25	o.25	o.95	19 : oo
6.	do.	o.2o	o.3o	1.14	24 : 3o
7.	do.	o.15	o.35	1.33	- - - -

Chart No. 17

GELATION OF GELATIN WITH ALLYLUREA at pH. 3.45 *

Solutions:-

Buffer, 0.2M Na_2HPO_4+0.1M Citric acid, at pH. 3.45
Gelatin 10% at pH. 3.45 in above buffer.
Allylurea 2M at pH. 3.45 in above buffer.(Aurea)

No. of sample	cc. gelatin	cc. buffer	cc. Aurea	Molarity of Aurea	Time of gelation
					' "
0.	o.5o	o.5o	o.oo	o.o	3 : 00
1.	do.	o.45	o.o5	o.1	4 : 3o
2.	do,	o.4o	o.1o	o.2	5 : 45
3.	do.	o.35	o.15	o.3	7 : 15
4.	do.	o.3o	o.2o	o.4	9 : 3o
5.	do.	o.25	o.25	o.5	13 : oo
6.	do.	o.2o	o.3o	o.6	15 : 35
7.	do.	o.15	o.35	o.7	21 : oo
8.	do.	o.1o	o.4o	o.8	29 : oo

Chart No. 18

* Samples were immersed for ½ hour in water bath at 37 C. then transferred into a water bath at 10 C.

pH. of the solutions were corrected by the components of the buffer to pH. 3.45

The pH. of the solutions was determined at (2o-25)C. by a glass electrode (Beckman pH.meter).

GELATION OF GELATIN WITH THIOUREA at pH. 3.45 *

Solutions:-

Buffer, o.2M Na_2HPO_4 + o.1M Citric acid, at pH. 3.45
Gelatin 10% at pH. 3.45 in above buffer.
Thiourea 2M at pH. 3.45 in above buffer.

No. of sample	cc. gelatin	cc. buffer	cc. thiourea	Mol. of thiourea	Time of gelation
					' "
0.	0.5o	o.5o	o.oo	o.o	3 : 0o
1.	do.	o.45	o.o5	o.1	5 : 25
2.	do.	o.4o	o.1o	o.2	8 : 4o
3.	do	o.35	o.15	o.3	16 : oo
4.	do.	o.3o	o.2o	o.4	31 : 3o
5.	do.	o.25	o.25	o.5	- - - -

Chart No.19

GELATION OF GELATIN WITH GUANIDINE.HCl at pH. 3,45 *

Solutions:-

Buffer, o.2M Na_2HPO_4 + o.1M Citric acid, at at pH. 3.45
Gelatin 10% at pH. 3.45 in above buffer.
Guanidine.HCl 2M at pH. 3.45 in above buffer.

No. of sample	cc. gelatin	cc. buffer	cc. guan.HCl	Mol. of guan.HCl	Time of gelation
					' "
0.	o.5o	o.5o	o.oo	o.o	3 : 0o
1.	do.	o.45	o.o5	o.1	4 : 5o
2.	do.	o.4o	o.1o	o.2	6 : 5o
3.	do.	o.35	o.15	o.3	9 : 25
4.	do.	o.3o	o.2o	o.4	12 : 55
5.	do.	o.25	o.25	o.5	25 : 1o
6.	do.	o.2o	o.3o	o.6	- - - -

Chart No.20

GELATION OF GELATIN WITH s-DIPHENYLGUANIDINE.HCl at pH. 3.45*

Solutions:-

Buffer, o.2M Na_2HPO_4 + o.1M Citric acid, at pH. 3.45
Gelatin 10% at pH. 3.45 in above buffer.
s-Diphenylguanidine.HCl(sDGH)o.4M at pH. 3.45 in above buffer.

No. of sample	cc. gelatin	cc. buffer	cc. sDGH	Mol. of sDGH	Time of gelation
					' "
0.	o.5o	o.5o	o.oo	o.oo	3 : 0o
1.	do.	o.45	o.o5	o.o2	4 : 45
2.	do.	o.4o	o.1o	o.o4	5 : 25
3.	do.	o.35	o.15	o.o6	6 : 3o
4.	do.	o.3o	o.2o	o.o8	8 : 3o
5.	do.	o.25	o.25	o.1o	1o : 3o
6.	do.	o.2o	o.3o	o.12	13 : 15
7.	do.	o.15	o.35	o.14	16 : 15

Chart No.21

* Samples were immersed for ½ hour in water bath at 37 C. then transferred into a water bath at 10 C.

pH. of the solutions was determined at (2o-25) C. by a glass electrode (Beckman pH.meter.),and corrected by the components of the buffer to pH. 3.45

GELATION OF GELATIN WITH ACETAMIDE at pH. 3.45 *

Solutions:-

Buffer, 0.2M Na_2HPO_4 + 0.1M Citric acid, at pH. 3.45

Gelatin 10% at pH. 3.45 in above buffer.

Acetamide 3.8M in above buffer, at pH. 3.45

No. of samples	cc. gelatin	cc. buffer	cc. acetamide	Mol. of acetamide	Time of gelation ' "
0.	0.50	0.50	0.00	0.00	3 : 00
1.	do.	0.40	0.10	0.38	5 : 10
2.	do.	0.30	0.20	0.76	7 : 20
3.	do.	0.20	0.30	1.14	10 : 30
4.	do.	0.10	0.40	1.52	14 : 15
5.	do.	0.00	0.50	1.90	17 : 30

Chart No. 22

GELATION OF GELATIN WITH THIOACETAMIDE AT pH. 3.45 *

Solutions:-

Buffer, 0.2M Na_2HPO_4 +0.1M Citric acid, at pH. 3.45

Gelatin 10% at pH. 3.45 in above buffer.

Thioacetamide (T-am.) 1.6M at pH. 3.45 in above buffer.

No. of sample	cc. gelatin	cc. buffer	cc. (T-am)	Mol. of (T-am.)	Time of gelation ' "
0.	0.50	0.50	0.00	0.00	3 : 00
1.	do.	0.40	0.10	0.16	6 : 30
2.	do.	0.30	0.20	0.32	12 : 00
3.	do.	0.20	0.30	0.48	25 : 00
4.	do.	0.10	0.40	0.64	- - -

Chart No. 23

GELATION OF GELATIN WITH POTASSIUM CHLORIDE at pH. 3.45 *

Solutions:-

Buffer, 0.1M Citric acid + 0.2M Na_2HPO4 , at pH. 3.45

Gelatin 10% at pH. 3.45 in above buffer.

Potassium chloride 3.8M at pH. 3.45 in above buffer.

No. of sample	cc. gelatin	cc. buffer	cc. KCl	Mol. of KCl	Time of gelation ' "
0.	0.50	0.50	0.00	0.00	3 : 00
1.	do.	0.45	0.05	0.19	3 : 35
2.	do.	0.40	0.10	0.38	4 : 10
3.	do.	0.35	0.15	0.57	5 : 25
4.	do.	0.30	0.20	0.76	6 : 40
5.	do	0.25	0.25	0.95	8 : 45
6.	do.	0.20	0.30	1.14	10 : 00
7.	do.	0.15	0.35	1.33	14 : 15
8.	do.	0.10	0.40	1.52	18 : 40
9.	do.	0.05	0.45	1.71	24 : 45

Chart No. 24

* Samples were immersed in water bath at 37 C. for ½ hour then transferred into a water bath kept at 10 C.

pH. of the solutions was corrected by the components of the buffer to pH. 3.45

The pH. of the solutions was determined at (20-25) C. by a glass electrode (Beckman pH. meter).

GELATION OF GELATIN IN CITRATE+PHOSPHATE BUFFER at pH.3.45 *

Solutions:-

Buffer, o.2M Na_2HPO_4+o.1M Citric ACID, at pH.3.45

Gelatin 10% at pH. 3.45 in above buffer.

No.of Sample	cc. Gelatin	cc. buffer	% of Gelatin	time of Gelation ' "
1.	o.5o	o.5o	5.o	2 : 35
2.	o.45	o.55	4.5	3 : 15
3.	o.4o	o.6o	4.o	3 : 4o
4.	o.35	o.65	3.5	5 : 5o
5.	o.3o	o.7o	3.o	8 : 3o
6.	o.25	o.75	2.5	14 : oo
7.	o.2o	o.8o	2.o	23 : oo

Chart No.25

GELATION OF GELATIN WITH GLYCINE at pH. 3.45 *

Solutions :-

Buffer, 0.2M Na_2HPO_4+o.1M Citric acid, at pH. 3.45

Gelatin 10% at pH. 3.45 in above buffer.

Glycine 2M at pH. 3.45 in above buffer.

	No. of sample	cc. gelatin	cc. buffer	cc. glycine	Mol.of glycine	Time of Gelation ' "
a.	0.	o.5o	o.5o	o.oo	o.o	2 : 35
	1.	do.	o.4o	o.1o	o.2	2 : 3o
	2.	do.	o.3o	o.2o	o.4	2 : 2o
	3.	do.	o.2o	o.3o	o.6	2 : o5
	4.	do.	o.1o	o.4o	o.8	1 : 45
	5.	do.	o.oo	o.5o	1.o	1 : 35
b.	6.	o.3o	o.7o	o.oo	o.oo	9 : 15
	7.	do.	o.6o	o.1o	o.2o	8 : 55
	8.	do.	o.5o	o.2o	o.4o	8 : oo
	9.	do.	o.4o	o.3o	o.6o	7 : 15
	1o.	do.	o.3o	o.4o	o.8o	6 : 5o
	11.	do.	o. 2o	o.5o	1.oo	6 : oo
	12.	do.	o.1o	o.6o	1.2o	5 : 4o
		do.	o.oo	o.7o	1.4o	4 : 45

Chart No. 26

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* Samples were immersed in a water bath at 37 C. for ½ hour then transferred into a water bath kept at 10 C.

pH. of solutions was corrected by the components of the buffer to pH. 3.45

The pH. of the solutions was determined at (2o-25)C by a glass electrode (Beckman pH.meter).

GELATION OF GELATIN WITH ALANINE at pH. 3.45 *

Solutions:-

Buffer, o.1M Citric acid+o.2M Na_2HPO_4, at pH. 3.45

Gelatin 10% at pH. 3.45 in above buffer.

Alanine o.8M at pH. 3.45 inabove buffer.

	No. of sample	cc. gelatin	cc. buffer	cc. alanine	Mol. of alanine	time of gelation '	"
a.	0.	0.5o	o.5o	o.oo	o.oo	2	35
	1.	do.	o.4o	o.1o	o.o8	2	3o
	2.	do.	o.3o	o.2o	o.16	2	25
	3.	do.	o.2o	o.3o	o.24	2	35
	4.	do.	o.1o	o.4o	o.32	2	1o
	5.	do.	o.oo	o.5o	o.4o	2	1o
b.	X.	o.3o	o.7o	o.oo	o.oo	9	15
	6.	do.	o.6o	o.1o	o.o8	9	1o
	7.	do.	o.5o	o.2o	o.16	-	--
	8.	do.	o.4o	o.3o	o.24	8	3o
	9.	do.	o.3o	o.4o	o.32	-	--
	1o.	do.	o.2o	o;5o	o.4o	8	15
	11.	do.	o.1o	o.6o	o.48	-	--
	12.	do.	o.oo	o.7o	o.56	7	45

Chart No. 27

GELATION OF GELATIN WITH POTASSIUM SULFATE at pH. 3.45 *

Solutions:-

Buffer, o.1M Citric acid+o.2M Na_2HPO_4, at pH. 3.45

Gelatin 10% at pH. 3.45 in above buffer.

K_2SO_4 o.6M at pH. 3.45 in above buffer.

	No. of sample	cc. gelatin	cc. buffer	cc. K_2SO_4	Mol. of K_2SO_4	Time of gelation '	"
a.	o.	o.5o	o.5o	o.oo	o.oo	2	35
	1.	do.	o.4o	o.1o	o.o8	2	22
	2.	do.	o.3o	o.2o	o.16	2	1o
	3.	do;	o.2o	o.3o	o.18	1	55
	4.	do.	o.1o	o.4o	o.24	1	5o
	5.	do.	o.oo	o.5o	o.3o	1	45
b.	X.	o.3o	o.7o	o.oo	o.oo	9	15
	6.	o.3o	o.6o	o.1o	o.o6	7	2o
	7.	do.	o.5o	o.2o	o.12	6	35
	8.	do.	o.4o	o.3o	o.18	6	15
	9.	do.	o.3o	o.4o	o.24	5	4o
	1o.	do.	o.2o	o.5o	o.3o	5	15
	11.	do.	o.1o	o.6o	o.36	4	5o
	12.	do.	o.oo	o.7o	o.42	4	15

Chart No.28

* Samples were immersed in a water bath at 37 C. for ½ hour then transferred into a water bath kept at 1o C.

pH. of solutions was corrected by the components of the buffer to pH. 3.45

The pH. of the solutions was determined at (2o-25)C by a glass electrode (Beckman pH.meter).

GELATIO N OF GELATIN WITH GLUCOSE at pH. 3.45 *

Solutions:-

Buffer, 0.1M Citric acid+0.2M Na_2HPO_4, at pH. 3.45

Gelatin, 10%, at pH. 3.45, in above buffer.

Glucose, 2M, at pH. 3,45, in above buffer.

No. of sample	cc. gelatin	cc. glucose	cc. buffer	Mol. of glucose	Rate of gelation ' "
0.	o.3o	o.oo	o.7o	o.oo	9 : 15
1.	do.	o.1o	o.6o	o.2o	9 : 15
2.	do.	o.2o	o.5o	o.4o	9 : oo
3.	do.	o.3o	o.4o	o.6o	
4.	do.	o.4o	o.3o	o.8o	8 : 3o
5.	do.	o.5o	o.2o	1.oo	
6.	do.	o.6o	o.1o	1.2o	
7.	do.	o.7o	o.oo	1.4o	8 : 15

Chart No.29

GELATION OF GELATIN IN CITRATE PHOSPHATE BUFFER at pH. 8 *

No. of sample	cc. gelatin	cc. buffer	% of gelatin	Rate of gelation ' "
1.	o.5o	o.5o	5.o	2 : 3o
2.	o.45	o.55	4.5	2 : 55
3.	o.4o	o.6o	4.o	3 :5o
4.	o.35	o.65	3.5	6 : 2o
5.	o.3o	o.7o	3.o	8 : 45
6.	o.25	o.75	2.5	14 : oo
7.	o.2o	o.8o	2.o	28 : oo
8.	o.15	o.85	1.5	- - - -

Solutions:-

Buffer, o.1M Citric acid+o.2M Na_2HPO_4 at pH. 8

Gelatin 10% at pH. 8 in above buffer.

Chart No.30

GELATION OF GELatin AT pH.8 WITH UREA*

Solutions:- Buffer, o.1M Citric acid+o.2M Na_2HPO_4 at pH.8

Gelatin 10% at pH.8 in above buffer.

Urea 4M at pH.8 i n above buffer.

No. of sample	cc. gelatin	cc. buffer	cc. urea	Mol. of urea	Time of gelation ' "
0.	o.5o	o.5o	o.oo	o.o	2 : 3o
1.	do.	o.45	o.o5	o.2	3 : 15
2.	do.	o.4o	o.1o	o.4	4 : o5
3.	do.	o.35	o.15	o.6	6 : 4o
4.	do.	o.3o	o.2o	o.8	8 : 45
5.	do.	o.25	o.25	1.o	12 : 3o
6.	do.	o.2o	o.3o	1.2	2o : oo

Chart No.31

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* Samples were immersed in a water bath at 37 C. for ½ hour then transferred into a water bath kept at 10 C.

The pH. of the solutions was corrected by the components of the buffer to the required one.

The pH. of the solutions was determined at (2o-25)C. by a glass electrode (Beckman pH.meter.)

GELATION OF GELATIN WITH METHYLUREA at pH.8 *

No. of samp.	cc. gelat	cc. buff.	cc. urea	Mol. urea	Time of gelation ' "
0.	o.5o	o.5o	o.oo	o.o	2 : 3o
1.	do.	o.45	o.1o	o.4	4 : 25
2.	do.	o.35	o.15	o.6	6 : 1o
3.	do.	o.3o	o.2o	o.8	8.: oo
4.	do.	o.25	o.25	1.o	11 : oo
5.	do.	o.2o	o.3o	1.2	13 : 25
6.	do.	o.1o	o.4o	1.6	28 : oo
7.	do.	o.oo	o.5o	2.o	- - - -

Solutions:-
Buffer;o.1M citric acid +o.2M Na_2HPO_4 at pH8
Gelatin 10% at pH.8 in above buffer.
Methylurea 4M at pH.8 in above buffer.

Chart No.32

GELATION OF GELATIN WI TH ALLYLUREA at pH.8 *

No. of samp.	cc. gelat	cc. buff.	cc. A urea	M. of Aurea	Time of gelation ' "
0.	o.5o	o.5o	o.oo	o.o	2 : 3o
1.	do.	o.45	o.o5	o.1	3 : 1o
2.	do.	o.4o	o.1o	o.2	3 : 5o
3.	do.	o.35	o.15	o.3	5 :ooo
4.	do.	o.3o	o.2o	o.4	6 : 3o
5.	do.	o.2o	o.3o	o.6	1o : 3o
6.	do.	o.1o	o.4o	o.8	17 : 3o
7.	do.	o.oo	o.5o	1.o	29 : 1o

Solutions:-
Buffer,0.1M citric acid +0.2M Na_2HPO_4 at pH8
Gelatin 10% at pH.8 in above buffer.
Allylurea (Aurea) 2M at pH.8 in above buffer.

Chart No.33

GELATION OF GELATIN WITH THIOUREA at pH.8

No. of samp.	cc. gelat	cc. buff.	cc. T urea	M. of Turea	Time of gelation ' "
o.	o.5o	o.5o	o.oo	o.o	2 : 3o
1.	do.	o.45	o.o5	o.1	3 : 15
2.	do.	o.4o	o.1o	o.2	5 : 1o
3.	do.	o.35	o.15	o.3	7 : 4o
4.	do.	o.3o	o.2o	o.4o	13 : oo
5.	do.	o.25	o.25	o.5	24 : 5o

Solutions:-
Buffer,0.1M citric acid +0.2M Na_2HPO_4 at pH.8
Gelatin 10% at pH.8 in above buffer.
Thiourea 2M at pH.8 in above buffer.
Turea for thiourea.

Chart No. 34

* Samples were immersed in a water bath at 37 C. for ½ hour then transferred into a water bath kept at 10 C.
The pH. of the so;utions was determined at (2o-25)C. by a glass electrode (Beckman pH.meter).
The pH. of the solutions was corrected by the components of the buffer to pH.8

GELATION OF GELATIN WITH GUANIDINE.HCl at pH.8 *

No.of samp.	cc. gelat	cc. buffer	cc. Guan	Mol.of Guan.	Time of gelation '	"
0.	0.50	0.50	0.00	0.0	2	30
1.	0.50	0.45	0.05	0.1	3	00
2.	0.50	0.40	0.10	0.2	4	00
3.	0.50	0.35	0.15	0.3	6	30
4.	0.50	0.30	0.20	0.4	7	30
5.	0.50	0.25	0.25	0.5	12	00
6.	0.50	0.20	0.30	0.6	18	50

Solutions:-
Buffer, o.1M citric acid + o.2M Na_2HPO_4 at pH.8
Gelatin 10% at pH.8 in above buffer.
Guanidine.HCl (Guan.) at pH.8 in above buffer.
Chart No. 35

GELATION OF GELATIN WITH ACETAMIDE at pH.8 *

No.of samp.	cc. gelat	cc. buff.	cc. acet.	Mol.of Acet.	Time of gelation '	"
0.	0.50	0.50	0.00	0.0	2	30
1.	do.	0.40	0.10	0.4	3	25
2.	do.	0.30	0.20	0.8	4	40
3.	do.	0.20	0.30	1.2	6	00
4.	do.	0.10	0.40	1.6	7	25
5.	do.	0.00	0.50	2.0	9	30

Solutions:-
Buffer, o.1M citric acid + o.2M Na_2HPO_4 at pH.8
Gelatin 10% at pH.8 in above buffer.
Acetamide, (Acet.) 4M at pH.8 in above buffer.
Chart No. 36

GELATION OF GELATIN WITH THIOACETAMIDE at pH.8 *

No.of samp.	cc. gelat	cc. buff.	cc. T¹ acet.	Mol.of T¹acet.	Time of gelation '	"
0.	0.50	0.50	0.00	0.0	2	30
1.	do.	0.45	0.05	0.1	3	25
2.	do.	0.40	0.10	0.2	4	45
3.	do.	0.35	0.15	0.3	6	35
4.	do.	0.30	0.20	0.4	9	15
5.	do.	0.25	0.25	0.5	15	00
6.	do.	0.20	0.30	0.6	23	00
7.	do.	0.15	0.35	0.7	above	30

Solutions:-
Buffer, o.1M citric acid + o.2M Na_2HPO_4 at pH.8
Gelatin 10% at pH.8 in above buffer.
Thioacetamide, (T¹acet.) 2M at pH.8 in above buffer.
Chart No. 37.

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* Samples were immersed in a water bath at 37 C. for ½ hour then transferred into a water bath kept at 10 C.
The pH. of the solutions was determined at (2o-25)C. by a glass electrode ( ) (Beckman pH.meter.)
The pH. of the solutions was corrected by the components of the buffer to pH.8 .

GELATION OF GELATIN WITH POTASSIUM CHLORIDE at pH.8*

No.of samp.	cc. § gelat	cc. buff.	cc. KCl	Mol.of KCl	Time of gelation ' "
0.	o.5o	o.5o	o.oo	o.oo	2 : 3o
1.	do.	o.4o	o.1o	o.35	3 : 4o
2.	do.	o.3o	o.2o	o.7o	5 : 3o
3.	do.	o.25	o.25	o.88	6 : 2o
4.	do.	o.2o	o.3o	1.o5	8 : oo
5.	do.	o.1o	o.4o	1.4o	11 : oo
6.	do.	o.oo	o.5o	1.75	16 : 15

Solutions:-
Buffer, o.1M citric acid + o.2M Na_2HPO_4 at pH.8
§ Gelatin 10% at pH.8 in above buffer.
§ KCl 3.5M at pH.8 in above buffer.
Chart No.38

GELATION OF GELATIN WITH GLYCINE at pH.8 *

No.of samp.	cc. gelat	cc. buff.	cc. glyc	Mol.of glycine	Time of Gelation ' "
0.	o.5o	o.5o	o.oo	o.oo	2 : 3o
1.	o.5o	o.4o	o.1o	o.2o	2 : 15
2.	o.5o	o.3o	o.2o	o.4o	2 : 1o
3.	o.5o	o.2o	o.3o	o.6o	1 : 55
4.	o.5o	o.1o	o.4o	o.8o	1 : 5o
6665.	o.5o	o.oo	o.5o	1.oo	1 : 3o

Solutions:- at pH.8
Buffer, o.1M citric acid + o.2M Na_2HPO_4
§ Gelatin 10% in above buffer.
§ Glycine, (glyc) 2M in above buffer.
Chart No.39

GELATION OF GELATIN IN PHOSPHATE BUFFER at pH.11.5 *
Solutions:-
Buffer, o.1M NaOH + o.2M Na_2HPO_4, made to pH.11.5
66 Gelatin, 10% in above buffer made to pH.11.5

No. of sample	cc. gelatin	cc. buffer	% of gelatin	Time of gelat Gelation ' "
1.	o.5o	o.5o	5.o	16
2.	o.45	o.55	4.5	22
3.	o.4o	o.6o	4.o	32
4.	o.35	o.65	3.5	--

Chart No.40

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* Samples were immersed in a water bath at 37 C. for ½ hour then transferred into a water bath kept at 10 C.
The pH. of solutions was determined at (2o-25) C. by a glass electrode (Beckman pH.meter).
§ The pH. of the solutions was corrected by the components of the buffer to 8

GELATION OF GELATIN WITH UREA at pH. 11.5 *
Solutions;
Buffer, o.1M NaOH+o.2M Na_2HPO_4 mixed to pH. 11.5
Gelatin 10% in components of buffer made to pH. 11.5
Urea 4M in components of buffer made to pH. 11.5

No. of sample	cc. gelatin	cc. buffer	cc. urea	Mol. of urea	Time of Gelation ' "
0.	o.5o	o.5o	o.oo	o.o	16
1.	o.5o	o.45	o.o5	.o.2	23
2.	o.5o	o.4o	o.1o	o.4	53
3.	o.5o	o.35	o.15	o.6	--

Chart No.41

GELATION OF GELATIN WITH THIOUREA at pH.11.5*
Solutions:-
Buffer, o.1M NaOH + 0.2M Na_2HPO_4 , mixed to pH. 11.5
Gelatin 10% in components of buffer made to pH. 11.5
Thiourea 2M in components of buffer made to pH. 11.5

No. of sample	cc. gelatin	cc. buffer	cc. Thiourea	Mol.of Thoiurea	Time of Gelation '
0.	o.5o	o.5o	o.oo	o.8	16
1.	o.5o	o.45	o.o5	o.1	27
2.	o.5o	o.4o	o.1o	o.2	--

Chart No.42

GELATION OF GELATIN WITH GUANIDINE.HCl at pH. 11.5 *
Solutions:-
Buffer, o.1M NaOH + o.2M Na_2HPO_4 , mixed to pH. 11.5
Gelatin 10% in components of above buffer , pH. 11.5
Guanidine.HCl 2M in " " " " "
Guan. for guanidine.HCl

No.mof sample	cc. gelatin	cc. Buffer	cc. Guan.	Mol.of Guan.	Time of Gelation '
0.	o.5o	o.5o	o.oo	o.o	16
1.	o.5o	o.45	o.o5	o.1	2o
2.	o.5o	o.4o	o.1o	o.2	47
3.	o.5o	o.35	o.15	o.3	--

Chart No.43

* Samples were immersed in a water bath at 37 C. for ½ hour then transferred into a water bath kept at 10 C.

The pH. of the solutions was corrected by the components of the buffer to pH. 11.5

The pH. of the solutions was determined by a glas electrode (Beckman pH.meter)

GELATION OF GELATIN WITH POTASSIUM CHLORIDE at pH.11.5 *

Solutions:- at pH. 11.5

Buffer, 0.1M NaOH + 0.2M Na_2HPO_4

Gelatin 10% made with components of above buffer.

KCl 3.5M " " " " " "

No. of sample	cc. gelatin	cc. buffer	cc. KCl	Mol.of KCl	Time of Gelation
					'
0.	0.50	0.50	0.00	0.00	16
1.	0.50	0.40	0.10	0.35	20
2.	0.50	0.30	0.20	0.70	32
3.	0.50	0.20	0.30	1.05	--

Chart No.44

GELATION OF GELATIN WITH Na-GLYCINATEatat pH. 11.5 *

Solutions:- at pH, 11.5

Buffer, 0.1M NaOH + 0.2M Na_2HPO_4

Gelatin 10% made with components of above buffer.

Na-Glycinate 2M made with components of above buffer.

No.of sample	cc. gelatin	cc. buffer	cc. Na-glycin.	Mol.of Na-glycin.	Time of gelation
					' "
0.	0.50	0.50	0.00	0.0	16:00
1.	do.	0.40	0.10	0.2	11:30
2.	do,	0.30	0.20	0.4	9:00
3.	do.	0.20	0.30	0.6	9:00
4.	do.	0.10	0.40	0.8	11:00
5.	do.	0.00	0.50	1.0	11:00

Chart No. 45

GELATION OF GELATIN IN CITRATE-PHOSPHATE BUFFER at pH. 3.45*

Solutions:- at pH. 3.45

Buffer, 0.1M Citric Acid + 0.2M Na_2HPO_4

Gelatin 10% made with components of above buffer.

No. of Samp.	cc. gelatin	cc. buffer	% cons.of gelatin	Time of Gelation
				' "
1.	0.9	0.1	9	1 : 10
2.	0.8	0.2	8	1 : 35
3.	0.7	0.3	7	2 : 00
4.	0.6	0.4	6	2 : 30
5.	0.5	0.5	5	3 : 20
6.	0.4	0.6	4	5 : 20

Chart No. 46

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* Samples were immersed in a water bath at 37 C for ½ hour then transferred into a water bath, kept at 10 C.

The pH. of the solutions was determined by a glass electrode (Beckman pH. meter.)

Chart No. 47

GELATION OF GELATIN WITH POTASSIUM CHLORIDE*

Solutions:

Potassium chloride (24%) 3.22 M in dist. water.
Gelatin 10% in dist. water.
Hydrochloric acid 0.2 N
Potassium hydroxide 0,1 N

	No.of samp.	cc. gelatin	cc. KCl.	cc. HCl	cc. KOH	cc.dist. water	% of gelatin	mol.conc of KCl	time of gelation ' "	pH. of sample ' "
a)	A.	1.00	0.00	0.125	---	0.875	5.0	0.000	2 : 10	4.75
	1.	1.00	0.10	0.100	---	0.800	5.0	0.161	2 ; 30	
	2.	1.00	0.20	0.100	---	0.700	5.0	0.322	2 : 30	
	3.	1.00	0.30	0.150	---	0.550	5.0	0.483	3 : 40	
	4.	1.00	0.40	0.100	---	0.500	5.0	0.644	3 : 40	
	5.	1.00	0.50	0.100	---	0.400	5.0	0.805	4 : 40	
b)	B.	1.00	0.00	0.500	0.00	0.500	5.0	0.000	3 : 10	2.75
	6.	1.00	0.10	0.300	0.00	0.600	5.0	0.161	3 : 40	2.70
	7.	1.00	0.00	0.300	0.00	0.500	5.0	0.322	3 : 50	2.80
	8.	1.00	0.30	0.300	0.00	0.400	5.0	0.483	4 ; 20	2.80
	9.	1.00	0.40	0.300	0.00	0.300	5.0	0.644	4.: 50	2.80
	10.	1.00	0.50	0.400	0.00	0.100	5.0	0.805	5 : 40	2.60
c)	C.	1.00	0.00	----	0.40	0.600	5.0	0.00	3 : 30	11.70
	11.	1.00	0.10	----	0.40	0.50	5.0	0.161	3 : 40	11.65
	12.	1.00	0.20	----	0.40	0.40	5.0	0.322	3 : 20	11.55
	13.	1.00	0.30	-----	0.40	0.30	5.0	0.483	5 : 05	11.50
	14.	1.00	0.40	----	0.40	0.20	5.0	0.644	6 : 50	11.50
	15.	1.00	0.50	----	0.40	0.10	5.0	0.805	8 : 20	11.60

* Samples were immersed for ½ hour in a water bath at 37 C then transferred for gelation into a water bath at 10 C.
pH. of solutions were determined at room temperature (20-25) C by a glass electrode, (Beckman pH. meter).

GELATION OF GELATIN WITH POTSSIUM NITRATE *

Chart No. 48

Solutions :-

Potassium Nitrate (20%) 1.98M.
Gelatin 10% in distilled water
Potassium hydroxide 0.1N.
Nitric Acid 0.2N.

	No.of Samp.	cc. @gelatin	cc. KNO_3	CC. HNO_3	cc. KOH	cc.dist. water.	% of @gelatin	mol. conc. of KNO_3	time of gelation	pH. of sample
a.	a.	1.00	0.00	0.125	---	0.875	5.0	0.000	2 : 15	4.6
	1	do	0.10	0.150	0.05	0.75	4.98	0.099	2 : 25	4.75
	2	do	0.20	0.050	---	0.75	5.0	0.198	3 : 00	4.75
	3.	do	0.30	0.050	---	0.65	do	0.297	4 : 20	4.75
	4.	do	0.40	0.150	0.05	0.45	4.98	0.396	5 : 30	4.70
	5.	do	0.50	0.050	---	0.45	5.0	0.495	7 : 40	4.75
b.	b.	1.00	0.00	0.50	---	0.50	5.0	0.000	3 : 30	2.7
	6.	do	0.10	do	---	0.40	do	0.099	4 : 15	2.6
	7.	do	0.20	do	---	0.30	do	0.198	5 : 20	2.6
	8.	do	0.30	do	---	0.20	do	0.297	6 : 50	2.6
	9.	do	0.40	do	---	0.10	do	0.396	8 : 25	2.65
	10.	do	0.50	do	---	0.00	do	0.495	11 : 50	2.63
c.	c.	1.00	0.00	---	0.40	0.60	5.0	0.000	3 : 30	11.7
	11.	do	0.10	---	do	0.50	do	0.099	4 : 45	11.65
	12.	do	0.20	---	do	0.40	do	0.198	5 : 25	11.65
	13.	do	0.30	---	do	0.30	do	0.297	7 : 15	11.65

* Samples were immersed for ½ hour in a water bath at 37 C then transferred for gelation into a water bath at 10 C.
pH. of solutions were determined at room temperature (20-25) C by a glass electrode , (Beckman pH. meter).

GELATION OF GELATIN WITH POTASSIUM SULFATE*

Chart No. 49

Solutions: Potassium sulfate 0.591 N
Gelatin 10 % in distilled water.
Sulfuric acid 0.25 N
Potassium hydroxide 0.1 N

	No. of sample	cc. gelatin.	cc. pot. sulf.	cc. sulf. acid	cc. pot. hydr.	cc. dist. water.	% of gelatin	mol. conc. pot. sulf.	time of gelation	pH of samp.
a	a.	0.60	0.00	0.03	-----	1.37	3.0	0.000	6' ; 40"	4.7
	1.	0.60	0.20	0.03	-----	1.17	3.0	0.059	5 : 00	4.7
	2.	0.60	0.40	0.03	-------	0.97	3.0	0.118	4.: 00	4.7
	3.	0.60	0.60	0.03	-----	0.77	3.0	0.177	3 : 15	4.7
	4.	0.60	0.80	0.03	-----	0.57	3.0	0.236	2 : 30	4.7
	5.	0.60	1.00	0.03	-----	0.37	3.0	0.295	4 : 30	4.7
b	b.	0.60	0.00	0.25	-----	1.15	3.0	0.000	13 : 00	2.75
	6.	0.60	0.20	0.27	-----	0.93	3.0	0.059	8 : 35	2.8
	7.	0.60	0.40	0.27	-----	0.73	3.0	0.118	7 : 05	2.8
	8.	0.60	0.60	0.27	-----	0.53	3.0	0.177	6 : 25	2.8
	9.	0.60	0.80	0.28	-----	0.32	3.0	0.236	5 : 50	2.8
	10.	0.60	1.00	0.31	-----	0.09	3.0	0.295	5 : 30	2.75
c	c.	1.00	0.00	----	0.40	0.60	5.0	0.000	3 : 30	11.7
	11.	1.00	0.10	----	0.40	0.50	5.0	0.029	3 : 20	11.75
	12.	1.00	0.20	----	0.40	0.40	5.0	0.059	3 : 05	11.45
	13.	1.00	0.30	----	0.40	0.30	5.0	0.088	^	11.7
	14.	1.00	0.40	----	0.40	0.20	5.0	0.118	|	11.7
	15.	1.00	0.50	----	0.40	0.10	5.0	0.148	2 : 45	11.8

* Samples were immersed for ½ hour in a water bath at 37 C ~~for~~ then transferred for gelation into a water bath at 10 C
pH. of samples were taken at room temperature (20-25 C) by a glass electrode, (Beckman pH. meter)

GELATION OF GELATIN WITH MAGNESIUM CHLORIDE*

Chart No. 50

Solutions:-

Magnesium chloride, $MgCl_2.6H_2O$, (40%) 1.97 Molar.
Gelatin 10% in distilled water.
Hydrochloric acid, HCl 0.2 M.

	No. of sample	cc. gelatin	cc. $MgCl_2$	cc. HCl	cc. H_2O	Total volume	% of gelatin	Mol. conc. of $MgCl_2$	Time of gelation	pH. of sample
a.	A.	1.00	0.00	0.125	0.875	2.00	5.0	0.000	2 : 00	4.75
	1.	do.	0.10	0.120	0.78	do.	do.	0.098	2 : 30	4.65
	2.	do.	0.20	0.100	0.70	do.	do.	0.197	3 : 00	4.64
	3.	do.	0.30	0.075	0.625	do	do.	0.296	4 : 00	4.68
	4.	do.	0.40	0.050	0.55	do.	do.	0.394	5 : 20	4.73
	5.	do.	0.50	0.050	0.45	do.	do.	0.492	7 : 20	4.75
b.	B.	1.00	0.00	0.50	0.50	2.00	5.0	0.000	3 : 10	2.75
	6.	do	0.10	0.45	0.40	1.95	5.12	0.098	3 : 10	2.74
	7.	do	0.20	0.50	0.30	2.00	5.0	0.197	3 : 35	2.70
	8.	do.	0.30	0.475	0.225	do.	do.	0.296	4 : 40	2.75
	9.	do.	0.40	0.45	0.105	do.	do.	0.394	5 : 50	2.73
	10.	do.	0.50	0.45	0.05	do.	do.	0.492	7 : 45	2.76

* Samples were immersed for ½ hour in a water bath at 37 C then transferred for gelation into a water bath at 10 C.
pH. of solution were determined at room temperature (20-25) C by a glass electrode, (Beckman pH. meter)

GELATION OF GELATIN WITH MAGNE IUM SULFATE*

Chart No.51

Solutions:-

Magnesium sulfate, $MgSO_4.7H_2O$, 1.83 M.
Gelatin 10% in distilled water
Sulfuric acid, H_2SO_4 , 0.25 N.

	No.of sample	cc. gelatin	cc. $MgSO_4$	cc. H_2SO_4	cc. H_2O	Total volume	% of gelatin	Mol.conc. of $MgSO_4$	Time of gelation	pH.of sample
									h m	
a	a.	o.6o	o.oo	o.o3	1.37	2.oo	3.o	o.ooo	6 :4o	4.7
	1.	do.	o.2o	do.	1.17	do.	do.	o.183	4 :45	do.
	2.	do.	o.4o	do.	o.97	do.	do.	o.366	4 :3o	do.
	3.	do.	o.6o	do.	o.77	do	do.	o.549	3 :15	do.
	4.	do.	o.8o	do.	o.57	do.	do.	o.732	2 :4o	do.
	5.	do.	1.oo	do.	o.37	do.	do.	o.915	2 :15	do.
b	b.	o.6o	o.oo	o.25	1.15	2.oo	3.o	o.ooo	13 :oo	2.75
	6.	do.	o.2o	do.	o.95	do.	do.	o.183	6 :2o	2.8
	7.	do.	o.4o	do.	o.75	do.	do.	o.366	5 :oo	do.
	8.	do.	o.6o	do.7	o.55	do.	do.	o.549	4 :15	do.
	9.	do.	o.8o	do.	o.35	do.	do.	o.732	cloudy precipit.	do.

* Samples were immersed for ½ hour im a water bath at 37 C. then transferred for gelation into a water bath at 10 C.
pH. of solutions were determined at room temperature(2o-25) C. by a glass electrode, (Beckman pH. meter.)

GELATION OF GELATIN WITH SODIUM SULFATE *

Chart No. 52

Solutions: Sodium sulfate 1.185 molar.
Gelatin 10% in distilled water.
Sulfuric acid 0.25 normal.
Sodium hydroxide 0.1 normal.

	No.of samp.	cc. gelatin	cc. sod.sulf.	cc.sulf acid	cc.sod. hydrox.	cc. water	% of gelat.	M.of sod.sulf	time of gelation	pH. of sample
a	a.	0.60	0.00	0.03	----	1.37	3.0	0.000	' " 6 : 40	4.7
	1.	0.60	0.20	0.03	----	1.17	3.0	0.118	6 : 30	4.7
	2.	0.60	0.40	0.03	----	0.97	3.0	0.237	5 : 15	4.7
	3.	0.60	0.60	0.03	----	0.77	3.0	0.356	4 : 00	4.7
	4.	0.60	0.80	0.03	----	0.57	3.0	0.474	2 : 45	4.7
	5.	0.60	1.00	0.03	----	0.37	3.0	0.592	2 : 15	4.7
b	b.	0.60	0.000	0.25	----	1.15	3.0	0.000	13 : 00	2.75
	6.	0.60	0.20	0.27	----	0.93	3.0	0.118	6 : 15	2.85
	7.	0.60	0.40	0.27	----	0.73	3.0	0.237	5 : 15	2.85
	8.	0.60	0.60	0.30	----	0.50	3.0	0.356	4 : 00	2.85
	9.	0.60	0.80	0.33	----	0.27	3.0	0.474	3(cloudy precipit	2.80 2.8
	10.	0.60	1.00							
c	c.	1.00	0.00.	----	0.40	0.60	5.0	0.000	3 : 35	11.68
	11.	1.00	0.10	----	0.40	0.50	5.0	0.059	3 : 30	11.58
	12.	1.00	0.20	----	0.40	0.40	5.0	0.118	3 : 10	11.46
	13.	1.00	0.30	----	0.40	0.30	5.0	0.177	2 : 45	11.36
	14.	1.00	0.40	----	0.40	0.20	5.0	0.237	2 : 10	11.30
	15.	1.00	0.50	----	0.40	0.10	5.0	0.296	1 : 50	11.30

* Samples were immersed for ½ hour in a water bath at 37 C then transferred for gelation into a water bath at 10 C.
pH. of solutions were determined at room temperature (20-25)C by a glass electrode, (Beckman pH. meter).

GELATION OF GELATIN WITH THIOUREA AT pH. 3.45 *
Solutions :- At pH. 3.45

No.of Samp.	cc. Gelat.	cc. buff.	cc. th. urea.	Mol. of th. urea	% of gelat	time of gelation ' "
1.	o.9	o.o	o.1	o.2	9	2 :o5
2.	o.8	o.1	do.	do.	8	2 :5o
3.	o.7	o.2	do.	do.	7	4 :2o
4.	o.6	o.3	do.	do.	6	5 :5o
5.	o.5	o.4	do.	do.	5	9 :1o
6.	o.4	o.5	do.	do.	4	16 :3o

Chart No. 53

Buffer o.2M, Na_2HPO_4 + o.1M Citric Acid
Gelatin 10% made with components of above buffer.
(th.urea) thiourea 2M in components of above buffer.

GELATION OF GELATIN WITH GUANIDINE.HCl at pH. 3,45 *
Solutions:- at pH. 3.45

No.of Samp.	cc. Gelat	cc. buff	cc. Guan.	Mol. of Guan.	% of Gelatine	Time of Gelation ' "
L.	o.9	o.o	o.1	o.2	9	1 : 5o
2.	o,8	o.1	o.1	do.	8.	2 : 3o
3.	o.7	o,2	o.1	do.	7	3 : oo
4.	o.6	o.3	o.1	do.	6	4 : 2o
5.	o.5	o.4	o.1	do.	5	6 : 25
6.	o.4	o.5	o.1	do.	4	1o : oo

Chart No. 54

Buffer, o.2M, Na_2HPO_4 + o.1M Citric Acid.
Gelatin 10% made with components of above buffer.
(Guan.) Guanidine. HCl 2M in components of above buffer.

Gelation OF GELATIN WITH POTASSIUM CHLORIDE at pH. 3.45 *
Solutions:- At pH. 3.45

No.of Samp.	cc. gelat	cc. buff	cc. KCl.	Mol. of KCl	% of KCl	Time of Gelation ' "
1.	o.8	o.o	o.2	o.8	8	2 : 4o
2.	o.7	o.1	do.	do.	7	3 : 05
3.	o.6	o.2	do.	do.	6	4 : 15
4.	o.5	o.3	do.	do.	5	6 : oo
5.	o.4	o.4	do.	do.	4	1o : 2o

Chart No. 55

Buffer, o.2M, Na_2HPO_4 + o.1M Citric Acid
Gelation 10% made with components of above buffer.
KCl 4M in components of above buffer.

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* Samples were immersed in a water bath at 37 C for ½ hour then transferred into a water bath, kept at 1o C.

The pH. of the solutions was determined by a glass electrode (Beckman pH. meter).

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