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CHITIN SOLVENTS, COMPLEXES, AND PHYSICAL PROPERTIES

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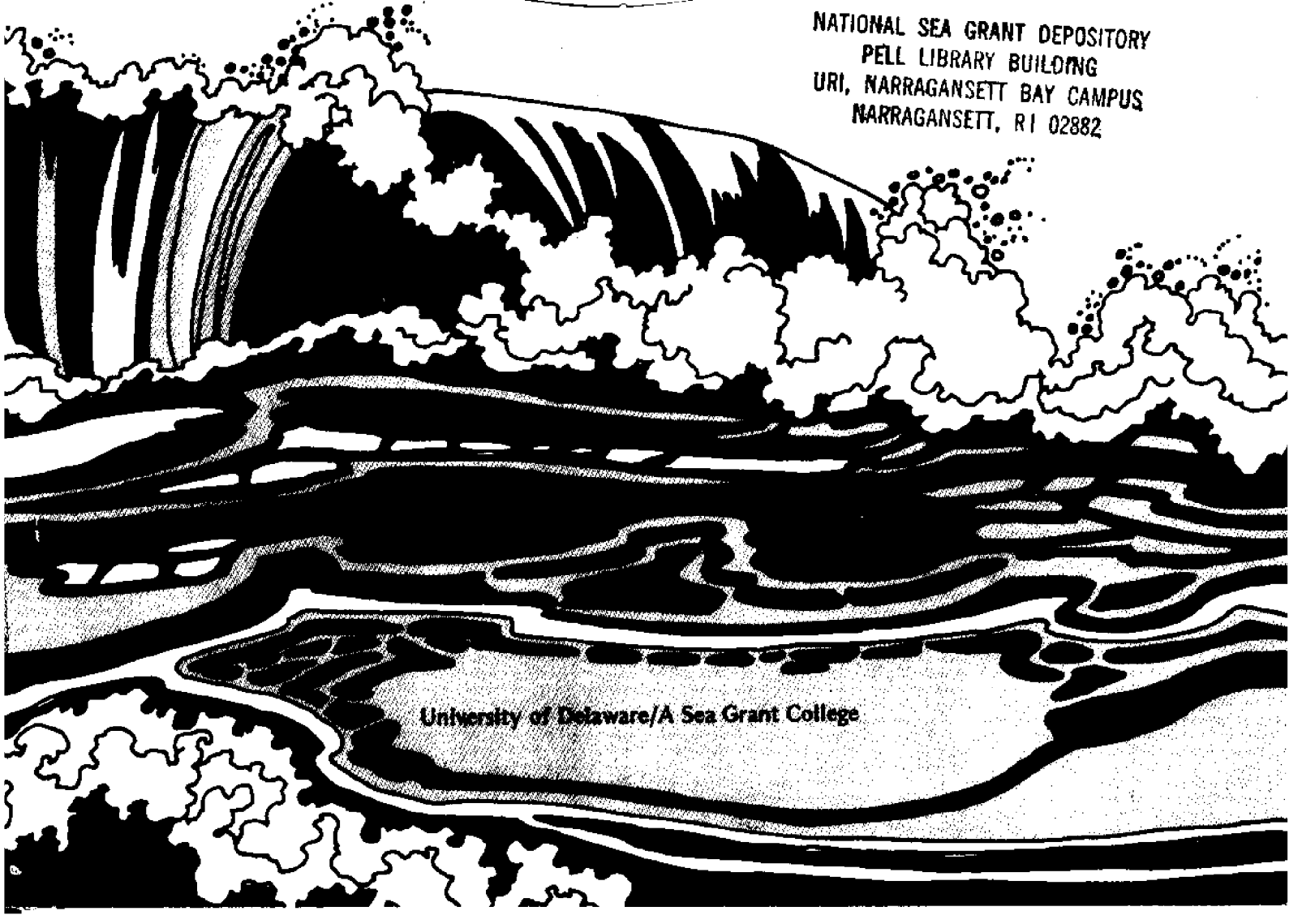

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CHITIN SOLVENTS, COMPLEXES, AND PHYSICAL PROPERTIES

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by

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CONTENTS

PREFACE

This report supplements the paper published by the authors in the *Proceedings of the First International Symposium on Chitin/Chitosan*, 1978, p. 182, entitled "Marine Chitin Properties and Solvents." Together, that report and this compilation cover most of the chitin research described in Mr. Rutherford's thesis, University of Delaware, 1976, as well as his subsequent investigations. The patents listed are assigned to the University of Delaware.

I. CHARACTERIZATION OF MARINE CHITINS USING A UNIQUE INERT SOLVENT*

Frank A. Rutherford, III

Chitin, a modified cellulose, has been examined as a potential marine resource; however, acid degradation that occurs during its isolation impairs the properties that make chitin valuable. In this study it was hypothesized that chitin prepared by milder processes should yield a product of superior quality. By using a chitin from a noncalcified source, *Limulus polyphemus*, the need for the acid treatment was eliminated; comparisons of properties were then made with chitins that had been isolated from other marine species through a process that included the acid treatment.

Characterizations were carried out in a newly discovered solvent for chitin, N,N-dimethylacetamide containing 5% LiCl. All chitins evaluated had sufficient molecular weight for fiber formation, but were partially deacetylated, which tended to reduce tensile strengths. Changes in the conformation of the glycoside linkages caused by the acids used in the isolations were observed by shifts in the optical activity. *Limulus* chitin had the highest solubility, molecular weight, and acetyl values of the chitins tested, together with the least change in native conformation. Renatured films from *Limulus* chitin had the greatest tensile strength, equal to that of naturally oriented chitin fibers.

*Abstract of thesis, University of Delaware, 1976.

II. FACTORS AFFECTING THE OPTICAL ROTATION OF CHITIN*

Frank A. Rutherford, III and Paul R. Austin

Summary

The discovery of an aprotic solvent for chitin, N,N-dimethylacetamide containing 5% LiCl, facilitated a systematic study of the effect of sample preparation on optical activity in comparison with that of native chitin. Experimental conditions such as acidity, alkalinity, temperature, and time of treatment alter the conformation and shift the optical rotation of chitin from its natural levo form to a dextrorotatory product. An important observation has been the *reversal* with the time of the optical rotation of chitin solutions, indicating at least partial recovery of the natural conformation of chitin in this environment. Anomerization of β-glycosidic linkages and transitional molecular forms, through random coil, helical structure, and molecular association, may all contribute to the optical rotation of the system. It seems likely that biological activity of chitin may also be affected by these variations.

Introduction

The abundance of chitin in seafood waste and the opportunity for promoting it as a new marine resource while alleviating a pollution problem have led to a burgeoning study of this unusual mucopolysaccharide. (See Neville, 1975; Muzzarelli, 1973 and 1977; and Muzzarelli and Pariser, 1978.) In view of the current use of crab meal in poultry feed and the potential nutritive value of crab meal and other chitinous products for calves and steers (Patton, et al., 1975) as well as crawfish (Meyers, 1974), and the efficacy of chitin in accelerating the healing of wounds (Prudden et al., 1970) it is timely to study its molecular conformation.

Chitin is predominately a homopolymer of N-acetyl-D-glucosamine (NAG) with multiple β-glycoside linkages, and is naturally levorotatory. Irvine (1969) developed a polarimetric method of identifying chitin. The hydrochloric-acid solutions containing chitin, which were initially levorotatory, slowly shifted to the dextro side upon hydrolysis of chitin to glucosamine. Falk et al., (1966) corroborated these findings. Because of the intractable nature of chitin, which is soluble only in strong mineral acids or concentrated salt solutions such as lithium thiocyanate (Clark and Smith, 1939), and the consequent difficulties in handling the polymer, its characterization by optical rotation was not pursued.

In connection with our chitin film and filament studies, dimethylacetamide containing 5% lithium chloride (DMAc-LiCl) was found to be an effective, nondegrading solvent system for chitin (Rutherford, 1976; Rutherford and Austin, 1977; and Austin, 1977). It was readily adapted to the determination of chitin properties by solution methods such as solubility itself, molecular weight, and optical activity. Thus, it has been possible to investigate the relation of optical rotation to chitin-isolation variables such as acid concentration for decalcification, alkali treatment for protein removal, time and temperature of reaction, and drying. One can interpret the accompanying changes in optical rotation in terms of the chiral nature of both individual molecular chains and their association in solution.

*Original manuscript this report.

Materials and Procedures

Excerpted pertinent data of chitin preparations, analyses, and physical measurements (Rutherford and Austin, 1978) and the new chiroptical information are summarized in Table 1. Shell from the horseshoe crab *(Limulus polyphemus)*, which is noncalcified, was deproteinated with 5% NaOH at 25°C and the chitin was separated. Blue-crab *(Callinectes sapidus)* chitin was obtained similarly, except that calcareous material was removed with 5% acetic acid followed by 2 N HCl at ambient temperature. The other chitins were commercial products: Japanese red crab *(Chionectes opilio)* from Eastman Kodak Company; Alaskan pink shrimp *(Pendalis borealis)* from Food, Chemical and Research Laboratories; and brown shrimp *(Penaeus aztecus)*, KY-10, from Hercules Incorporated.

Test samples were conditioned over silica gel and residual moisture (2.4% to 2.9%) was determined by vacuum drying at 100°C. Ash varied from 0.4% to 1.9%. Acetyl values, corrected for moisture and ash, were determined by hydrolysis of chitin with 50% NaOH, acidification with 85% H_3PO_4, dilution, distillation of the acetic acid-water mixture, and titration of aliquots of the distillate (Rutherford and Austin, 1978). Molecular weights were determined in DMAc-5% LiCl solutions using a Cannon-Fenske viscometer (Billmeyer, 1971).

Experiments were conducted to test separately and in combination the effects of acidity, alkalinity, and temperature on the conformation of chitin as indicated by its optical rotation. In each series, 0.5 g of brown-shrimp chitin (KY-10) ground to 20 mesh was treated under the specified condition for 6 hours. The samples were then rinsed with distilled water, dried, dissolved in 100 ml of DMAc-5% LiCl, filtered through felt, and centrifuged 30 minutes at 2600 rpm; the optical rotation was then determined (Table 2).

TABLE 1. Solution Properties of Chitins in DMAc-5% LiCl

Chitin	% Sol. Material	Acetyl * (%)	Mol. Wt. (x 10^6)	$[\alpha]_D^{25}$ (degrees)	
				Initial	In 2 wks.
Horseshoe crab	82	17.2	1.8	-56	-56
Blue crab	58	15.0	1.6	+33	-52
Red crab	76	16.6	1.3	+65	-22
Pink shrimp	62	13.8	0.4	+24	-54
Brown shrimp (KY-10)	92	20.7	0.8	-36	-36

*Theoretical value, 21.2%

For optical activity, the observed rotation, θ, of each chitin was measured with a Polyscience Polarimeter Model SR 6. The rotations (θ) were determined to the nearest 0.1 degree and converted to specific rotations by the equation

$$[\alpha]_D^{25} = \theta/lc$$

where c is concentration in g/ml and l is cell length in dm.

The concentration was determined by precipitation of chitin from an aliquot of solution with acetone, filtration, rinsing with acetone, drying, and removing the LiCl from the precipitate by several water washes. Concentrations were in the range of 3 to 4 gm/ml, depending on the percentage of the sample that was soluble (Table 1). Cell length was 2 dm.

Table 2. Effect of Chitin Isolation Variables on Optical Rotation*

Treatment	Temperature (°C)	$[\alpha]_D^{25}$ (degrees)
Aqueous NaOH (% solution)		
1%	Room	-60
3	Room	-63
3	60	+ 1
5	Room	-47
Aqueous HCl (% solution)		
1%	Room	-53
3	Room	-51
3	60	- 1
5	Room	-32
Dry heat†		
	25	-36
	40	-68
	50	-46
	60	-29
	70	-24
	80	-32
	90	-14
	100	-10

*6-hour treatment, each experiment; initial $[\alpha]_D^{25}$ for KY-10 was -36 degrees.

†Samples at 40°C and above gave cloudy solutions.

Results and Discussion

The wide range in properties of chitins with different sample histories is evident from Table 1. Variations in optical rotation on standing in DMAc-LiCl solutions, including reversion from dextro to the natural levo form, indicated marked conformation changes in the structure of the chitin.

In extending these studies, the separate contributions of chitin-processing variables were determined: acidity, alkalinity, and moderate heat. Table 2 shows that each treatment had a pronounced effect which increased with the severity of the conditions. Treatment of chitin at room temperature with 1% to 5% aqueous NaOH or HCl, or merely heating chitin for six hours initiated changes in rotation. In each case, 50° to 60°C appeared to be the threshold temperature for marked shifts from levo to dextro, perhaps through disruption of hydrogen bonds and subsequent changes in molecular conformation.

In a previous investigation (Falk et al., 1966), infrared absorption spectra of "chitan," a fully acetylated chitin, indicated a β-configuration at the anomeric center initially. They report also that after deuterium exchange in 8 to 10 N HCl at 40° to 80°C, the absorption band moved to a position indicative of an α-configuration. It appears from our studies that such conditions may indeed cause anomerization in chitin.

The conformation of chitin has been studied extensively (Neville, 1975; Rudall, 1973 and 1969; Ramakrishnan and Nageshwar, 1972; and Carlstrom, 1957) by means of X-ray diffraction analyses, infrared, electron micrography, and other techniques. The parameters of the orthorhombic unit cell and an antiparallel arrangement of three lateral adjacent chains have been established. The β-glycosidic oxygen connecting two hexosamine units is set at an angle and stabilized by intramolecular hydrogen bonding (Rudall and Kenchington, 1973) with side chains on alternate sides of consecutive residues. The polymer chains are helical in form with a two-fold screw axis. Adjacent chains are intermolecularly hydrogen bonded through the acetamido groups, and so form a laminar structure.

The variations and the reversal of optical rotation of some chitins on standing in the DMAc-LiCl solutions and the magnitude of the changes strongly suggests a shift from random coil to helical structure, followed by molecular association.

The structure of the chitin-DMAc-LiCl system that permits the facile random coil-helix shift is of interest. There may be an association between chitin and the solvent components by anaology with polyamides (Panar and Beste, 1976). Ciferri and Russo (1977) describe a definite coordination between Li^+ ion and carbonyl oxygen, and between Cl^- ion and the NH moiety in caprolactam-LiCl systems. A similar association in chitin-solvent systems seems likely.

Limitations of both the experimental data and their interpretation must be recognized. The treatments all involved heterogeneous systems in which reactions are slow and nonuniform, and only the soluble portions of the treated samples were involved in the solution tests. Nevertheless, one may postulate that the composite structure of chitin in solution may range from a highly ordered association of molecules (hydrogen bonded) with strong levo rotation to completely random coils that are

dextrorotatory. Intermediate between these are the mixed forms with some portion of the molecules anomerized or heat denatured irreversibly, their individual helical structure somewhat disrupted and their tendency to associate impaired; they exhibit modest levo or dextro rotations.

The random chains, intramolecularly hydrogen bonded in a ladder-like structure, have the dextro rotation of the many β-glycosidic NAG units. Rudall and Kenchington (1973) indicate that these chains themselves may form a helix that is right-handed. Association of these helical chains to a still more ordered structure, possibly in rope-like fashion, probably accounts for the strong levo rotation of natural chitin. A similar behavior of proteins is discussed by Morawetz (1965).

The behavior of the chitins described in Table 1 thus becomes more understandable. Horseshoe-crab and brown-shrimp chitins prepared under mild conditions show little change from their native levo rotation. The blue-crab and pink-shrimp chitins show marked destruction of chain association, but are not anomerized and recover their helical and associated structure in the DMAc-LiCl environment. Red-crab chitin appears intermediate; it may be partly anomerized or denatured so that recovery of its structure in solution is incomplete.

For the preparation of chitin, it appears that the mildest conditions of isolation and drying will lead to the least change in its native conformation and, presumably, to its optimum biological efficacy. Similarly, the best films and fibers have been prepared from undenatured chitins that permit the development of high molecular order in solution before extrusion or casting (Rutherford and Austin, 1978).

ACKNOWLEDGMENTS

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LITERATURE CITED

Austin, P. R. 1977. U.S. 4,059,457, Nov. 22.

Billmeyer, F. W. 1971. *Textbook of polymer science.* New York: Wiley-Interscience.

Carlstrom, D. 1957. *J. Biophys. Biochem. Cytol.*, 3:669.

Ciferri, A. and Russo, S. 1977. *Polymer preprints*, 1887, Am. Chem. Soc.

Falk, M.; Smith, D. G.; Maclachlan, J.; and McInnes, A. G. 1966. *Can. J. Chem.*, 44:2269.

Clark, G. L., and Smith, A. F. 1936. *J. Phys. Chem.*, 40:870.

Irvine, J. C. 1909. *Chem. Soc.*, 95:564.

Meyers, S. P. 1974. The crawfish industry. *Aquanotes*, 3:5, Louisiana State University.

Morawetz, H. 1965. *Macromolecules in solution.* High Polymer Series, 21. New York: Interscience.

Muzarelli, R. A.
1973. *Natural chelating polymers.* New York: Pergamon Press.
1977. *Chitin.* New York: Pergamon Press.

Muzzarelli, R. A., and Pariser, E. R., eds. 1978. *Proceedings of the First International Conference on Chitin/Chitosan*, MITSG 78-7.

Neville, A. C. 1975. *Biology of the arthropod cuticle.* New York: Springer-Verlag.

Panar, M., and Beste, L. F. 1976. *Polymer preprints*, 17:65, Am. Chem. Soc.

Patton, R. S., and Chandler, P. T. 1975. *J. Dairy Sci.*, 58:397.

Patton, R. S.; Chandler, P. T.; and Gonzalez, O. G. 1975. *J. Dairy Sci.*, 58:404.

Prudden, J. F.; Migel, P.; Hanson, P.; Friedrich, L.; and Balassa, L. 1970. *Am. J. Surgery*, 119:560.

Ramakrishnan, C., and Nageshwar, P. 1972. *Biochim. Biophys. Acta*, 261:123-135.

Rudall, K. M. 1969. *J. Polymer Sci.*, part C, no. 28, 83.

Rudall, K. M., and Kenchinton, W. 1973. *Biol. Rev.*, 48:597.

Rutherford, F. A. 1976. Characterization of marine chitins using a unique inert solvent. (Thesis, University of Delaware.)

Rutherford, F. A., and Austin, P. R. 1978. *Proceedings of the First International Conference on Chitin/Chitosan*, p. 182, MITSG 78-7.

III. CHITIN SOLUTION–U.S. PATENT 4,059,457

Paul R. Austin

United States Patent [19]

Austin

[11] **4,059,457**

[45] **Nov. 22, 1977**

[54] **CHITIN SOLUTION**

[75] Inventor: **Paul Rolland Austin**, Wilmington, Del.

[73] Assignee: **The University of Delaware**, Newark, Del.

[21] Appl. No.: **728,257**

[22] Filed: **Sept. 30, 1976**

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[63] Continuation-in-part of Ser. No. 659,280, Feb. 19, 1976.

[51] **Int. Cl.**[2] **C09J 3/04**

[52] **U.S. Cl.** **106/203**; 424/180; 536/20

[58] **Field of Search** ..... 106/203; 536/20; 260/32.8 N; 424/180

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,068,188	12/1962	Beste et al.	260/30.2
3,892,731	7/1975	Austin	536/20

Primary Examiner—Lorenzo B. Hayes
Assistant Examiner—Elizabeth A. Hatcher
Attorney, Agent, or Firm—John J. McDonnell

[57] **ABSTRACT**

Solutions of chitin in dimethylacetamide, N-methylpyrrolidone or mixtures of these in combination with a minor proportion of lithium chloride.

3 Claims, No Drawings

CHITIN SOLUTION

The Government of the United States has rights in this invention pursuant to Grant No. 04-3-158-30 with the Department of Commerce.

BACKGROUND OF THE INVENTION

This application is a continuation in part of U.S. application Ser. No. 659,280 filed Feb. 19, 1976, by Paul R. Austin.

This invention relates to new solvents for chitin and their use in the purification of chitin.

Chitin is an aminocellulose derivative that occurs widely in nature, for example, in the cell walls of fungi, bovine cartilage, cuttlefish bone and the hard shell of insects and crustaceans. The waste from shrimp, lobster and crab seafood industries contains 10–15% chitin and is a potentially important source of chitin. The isolation and purification of the chitin, associated in such waste with mineral components, protein and other ingredients, presents considerable difficulty and may cause hydrolytic degradation, denaturing or change in its natural conformation and optical activity.

The applications for chitin are not extensive, in part because it has been little investigated and in part because it is difficult to purify. The use of chitin for accelerating and promoting wound healing is described in U.S. Pat. No. 3,632,754, to L. L. Balassa, Jan. 4, 1972. In other literature, the difficulties of purification are mentioned frequently. Chitin is also employed in the manufacture of chitosan, a deacetylated chitin that is readily soluble in dilute acids and may find application in paper making and surface active agents, for example.

More specifically, chitin is a mucopolysaccharide, believed to be poly-N-acetyl-D-glucosamine, with an empirical formula of $(C_8H_{13}O_5N)_n$ in which n may be any number into the thousands range, but is commonly in the area of 100–10,000. Chitin is a generally intractable material, soluble only in strong mineral acids, lithium thiocyanate solutions, and other special concentrated salt solutions, most of which cause disintegration or rapid degradation with loss in molecular weight or hydrolysis of the acetyl groups or both. Chitin is insoluble in dilute acetic acid.

More recently some new solvents for chitin are described by Paul R. Austin in U.S. Pat. No. 3,879,377, Apr. 22, 1975, and in U.S. Pat. No. 3,892,731, July 1, 1975. These solvents comprise a 1,2-chloroalcohol in admixture with an acidic solvent, e.g. sulfuric acid, and a chloracetic acid alone or in combination with other solvents, e.g. formic acid. These solvents provide useful means for purifying chitin and for regenerating the chitin in the form of films, fibers and the like. However, these solutions of chitin are not as stable as desired for storage for considerable lengths of time.

In the isolation and utilization of chitin it is desirable to set specifications for the chitin material, for example, molecular weight, viscosity or optical activity. All of these properties require a stable non-degrading solvent for their determination; the solvents of the prior art give transient values that are difficult to duplicate because of continuing chitin degradation.

It is an object of this invention to provide a new class of solvents for chitin.

It is a further object to provide a method for preparing viscosity-stable solutions of chitin that can be filtered, otherwise purified, processed, or their properties measured.

It is still another object to provide solutions of chitin from which the chitin can be regenerated in the form of films, fibers or other shaped objects.

It is a further object to provide chitin solutions that are free from hydrolytic degradation on storage for considerable periods of time, and that are able to convert certain dextrorotatory chitins to a natural levorotatory form.

SUMMARY OF THE INVENTION

It has now been found that chitin is dissolved by dimethylacetaminde, N-methylpyrrolidone or mixtures of these amides in conjunction with a minor amount, e.g. 2% up to the saturation point, of lithium chloride, at room temperature or on moderate heating, e.g. at 50° C. Solutions containing up to 15% chitin are readily obtained, depending to some extent on the molecular weight of the chitin. Solvency is limited by viscosity of the solution; the lower molecular weight chitins in general dissolve more readily and give lower viscosity systems. At higher concentrations of chitin, in the 10–15% range, an organisol system, plastic in character, is obtained. The chitin solutions can be purified by centrifuging, vacuum or pressure filtration, or other means as appropriate for the consistency of the solution and application involved.

For physical property determination and chitin characterization, dilute solutions of 1% or less of chitin are usually employed. For film and filament preparation by wet-casting and spinning technology, a solution containing 2–5% chitin is preferred and the shaped films or fibers are subsequently coagulated or renatured by treatment with excess ketone or alcohol non-solvent for chitin such as acetone, methylethyl ketone, methanol, ethanol, a propanol or a butanol, for example, washed with water and dried at ambient or elevated temperature. The resulting films and fibers are pliable and strong and can be cold drawn to orient them and to improve their strength.

At higher concentrations of chitin, in the range of 5–15%, the solutions become very viscous, approaching a plastic consistency. They are handled best by heavy duty mixers, pressure filtration and other organisol techniques. Films may be prepared by calendering or doctoring onto a moving belt, followed by solvent evaporation, water extraction and drying. Filaments may be extruded and renatured either by dry spinning with solvent evaporation or by a non-solvent precipitation as customary in wet spinning. Processing is completed as previously described.

As indicated above the dimethylacetaminde and N-methylpyrrolidone can be used alone or in mixtures of any proportions in combination with the lithium chloride. Proportions of lithium chloride ranging from 2–5% by weight of the tertiary amide solvent are preferred.

An important advantage of the chitin solutions of this invention is their improved stability to degradation on storage. They have a working life of at least one month at room temperature.

Natural chitin is known to be a beta glycoside with a levo (−) optical rotation; however, the method of isolation, which may involve strong acid, alkali or heat treatments often denatures it with a consequent shift in optical rotation to a dextro (+) value. Another advantage of the chitin solutions of this invention is their ability to convert the chitin therein from such denatured, dextro-

rotatory form to the natural levorotatory structure during storage. This reconversion of the molecular structure or conformation of chitin to the natural beta-glycoside form is believed to be of considerable importance in the use of chitin in wound healing acceleration and other physiological properties, and in its conversion to strong continuous films and filaments.

By way of explanation, but without limitation to the scope of the invention, it may be pointed out that the optical activity of chitin was investigated by J. C. Irvine long ago (J. Chem. Soc., 95, 564(1909)). He found that a chitin sample with a specific rotation, $[\alpha]_D^{25}$, of $-14°$ in hydrochloric acid, on standing slowly hydrolyzed at the glycoside linkages to glucosamine hydrochloride with a specific rotation, $[\alpha]_D^{25}$, of $+56°$. He suggested this test as a means of identifying chitin, but hydrolytic cleavage of the chitin molecular chain precluded its general acceptability. Thus the new solvent systems of the subject application at once provided a much improved tool for the characterization of chitin.

The asymmetry and hence the optical activity of polymer molecules may arise in several ways, but those most pertinent to the behavior of chitin appear to be the chirality of the carbon atoms, particularly those created by formation of the glycoside linkages, and the helical conformation of the polymer molecule as a whole (Morrison, R. T., Organic Chemistry, pp. 123, 1095, et. seq., Allyn and Bacon, Boston, 1975; Lehninger, A. L., Biochemistry, p. 113 et. seq., Worth Publishers, New York, 1970). Depending upon conditions of treatment or its environment, a polymer molecule may display these characteristics independently or together in an additive fashion. In each of these cases, respectively, optical activity may be changed by inversion of some of the chiral carbon atoms (Morrison, p. 462), or by unwinding of the helix to form a random coil (Lehninger, p. 113), for example as the result of acid treatments, or other denaturing agencies such as heat. The behavior of a series of chitins from various sources and preparational histories is illustrated in Table 1.

Optical Activity of Chitins

Chitin	Initial $[\alpha]_D^{25}$	$[\alpha]_D^{25}$ after 2 weeks
Horseshoe crab	−56°	−56°
Blue crab	+33	−52
Red crab	+23	−22
Pink shrimp	+75	−54
Brown shrimp	−36	−36

The horseshoe crab chitin was isolated under very mild conditions, without acid treatment as the horseshoe crab is not calcified, and the brown shrimp chitin sample was prepared with acid and alkali treatments close to neutrality; the other samples were isolated with more severe treatments of acid, alkali and heat.

Surprisingly, it was found that the solvent systems of this invention not only dissolved the chitin, but at the same time provided an environment for reversal of at least a portion of the optical activity. This is a quite unusual phenomenon, encountered only occasionally in polymer chemistry; most examples are in the highly polar protein and polyaminoacid field. No examples have been found in the cellulose area. Referring again to Table 1, column three, $[\alpha]_D^{25}$ after 2 weeks, it is seen that blue crab and pink shrimp chitins revert to their natural chitin levo rotation, while red crab chitin only partially reverts. Brown shrimp and the horseshoe crab chitins are unchanged. A possible explanation of this behavior is that the helical conformation of the chitin molecules can be reformed from random coils by the solvents of the subject application, whereas the inversion of the glycosidic linkages of the polymer chain cannot. Thus a partially inverted and random coil chitin sample such as the red crab sample can be converted to the helical form, but the chiral carbon atoms of the glycoside linkages are unaffected. Similarly the rotations of the horseshoe crab and brown shrimp chitins are unchanged by the subject solvent system environment. A further indication of this differential behavior is the observation that a sample of the alpha-ethylglycoside of N-acetylglucosamine was unchanged in optical rotation on standing 1 week in dimethylacetamide-5% LiCl; the $[\alpha]_D^{25}$ of the sample was $+135°$ in both cases.

Chitin from various sources can be used with the solvents of this invention. Thus, chitin from red, blue, rock, king and Dungeness crabs, from lobsters, shrimp, and other crustaceans, and from the cell walls of fungi and the hard shells of insects are all operable.

The following examples illustrate in further detail the solvents of this invention and their use in the preparation of chitin films and fibers.

EXAMPLE 1

To a solution of one part of lithium chloride in 20 parts of dimethylacetamide is added 0.6 parts of chitin (red crab) and the mixture stirred at room temperature for 1 hour. Then 20 parts of N-methylpyrrolidone is added and the mixture stirred for two hours. The resulting solution is filtered through felt and the filtrate spread on a glass plate at a thickness of 1 mm. The wet film is dried for one hour in a current of air at room temperature, and then placed in excess acetone for 16 hours to extract solvent from the film. The film is extracted for another 24 hours with fresh acetone, and finally rinsed again with fresh acetone and dried in air at room temperature for 30 minutes. The film is cut into small strips and these are cold drawn to produce a 66% increase in length. The drawn film is strong and pliable.

EXAMPLE 2

To a solution of 1 part of lithium chloride in 20 parts of dimethylacetamide is added 0.6 parts of chitin (blue crab) and the mixture stirred at room temperature for 1 hour; then 10 parts of dimethylacetamide is added and the mixture stirred for 2 hours. The resulting solution is filtered through felt and the filtrate spread on a glass plate at a thickness of 1 mm. The chitin is regenerated into a film as in Example 1. The film is cut into small strips (2 mm × 2.5 cm) and these are cold drawn 83%. The tensile strength of the drawn film is 16.1 Kg/mm².

The chitin films cast from solution in dimethylacetamide and dimethylacetamide/N-methylpyrrolidone systems show syneresis, i.e. solvent exudes from the film as it stands for a short time, e.g. for an hour. This has the effect of concentrating the chitin in the resident film and facilitating its renaturing by immersion in acetone.

EXAMPLE 3

To a solution of 2.5 parts LiCl in 50 parts N-methyl-2-pyrrolidone is added 0.25 parts chitin (Dungeness crab) and the mixture is stirred at room temperature for 1.5 hours. The resulting solution is filtered through felt and the filtrate spread on a glass plate at a thickness of 1 mm. The chitin is regenerated into a film as in the previous examples. The film is cold drawable.

EXAMPLE 4

Five parts of finely divided chitin and 50 parts of dimethylacetamide containing 5% of lithium chloride is rapidly mixed at room temperature to thoroughly wet all of the chitin. At first the chitin appears to absorb the solvent to form discrete gel particles which upon standing and intermittent stirring convert to a very viscous mass. This mixture is worked periodically with a stirrer to promote gel particle attrition and develop a more coherent gelatinous system. After standing 5 days, the gelatinous material is doctored onto a platen and pressed to a coherent film. The solvent is removed by evaporation, followed by a water wash and drying. The resultant film is pliable and tough. The film can be cold drawn with typical necking down. Upon breaking at the extension limit, the fracture line shows fibrillation, as does polyethylene and other polymers capable of being cold drawn.

EXAMPLE 5

To 100 parts dimethylacetamide containing 5% lithium chloride 3.5 parts of chitin (red crab) is added and the mixture stirred for 18 hours. The resulting solution is filtered through felt and allowed to stand for 24 hours. Some of the solution is then spread on a glass plate to a thickness of 1 mm and regenerated as in example 1. The quality of film produced is tested by cold drawing and it is found to be capable of being cold drawn 40%. The remaining solution is stored for 48 days. It is noted that viscosity of the solution at the end of 48 days is the same as the fresh solution. Some of this solution that had been stored 48 days is then spread on a glass plate to a thickness of 1 mm and regenerated in the same manner as the film made from the fresh solution. The quality of this film is tested by cold drawing and it is found to be capable of being cold drawn 75%, thus indicating that storage in the solution does not degrade the chitin.

The following Examples, carried out with samples of chitin different from those described in Table 1, illustrate the conversion of the dextrorotatory form of chitin obtained by acid purification of chitin to the natural levorotatory form on storage of dimethylacetamide-lithium chloride solutions of the chitins at room temperature for several days.

EXAMPLE 6

To a solution of 100 parts of dimethylacetamide-5% lithium chloride one part of red crab chitin is added and the mixture stirred for one and a half hours. The solution is filtered through wool felt and then centrifuged for one hour at 2600 RPM. The optical activity of the sample is followed with time. Immediately following the centrifugation the optical activity, $[\alpha]_D^{25}$, of the sample is $+65°$. After 6 days the optical activity changes to 0°, and after 21 days in solution the optical activity stabilizes at $-22°$.

EXAMPLE 7

To a solution of 40 parts of dimethylacetamide-5% lithium chloride one part of pink shrimp chitin is added and the mixture stirred for one and a half hours. The solution is filtered through felt and then centrifuged for one hour at 2600 RPM. The optical activity of the chitin solution is observed over time. The initial optical activity, $[\alpha]_D^{25}$, is $+24°$ and after 21 days stabilizes at $-54°$.

While particular examples of the present invention have been shown and described it is apparent that changes and modification may be made therein without departing from the invention in its broader aspects. The aim of the appended claims, therefore, is to cover all such changes and modifications as fall within the true spirit and scope of the invention.

I claim:

1. A solution of chitin in dimethylacetamide or N-methylpyrrolidone or mixtures of these amides in conjunction with a minor amount of lithium chloride.

2. The solution of claim 1 wherein the lithium chloride is present in the solution in an amount of at least 2% of the solvent.

3. The solution of claim 1 which has been held until the chitin which in solution originally, if it had dextro optical rotation, is converted to the levo rotatory form.

* * * * *

IV. SOLVENTS FOR AND PURIFICATION OF CHITIN–U.S. PATENT 4,062,921

Paul R. Austin

United States Patent [19]

Austin

[11] **4,062,921**

[45] **Dec. 13, 1977**

[54] **SOLVENTS FOR AND PURIFICATION OF CHITIN**

[75] Inventor: **Paul R. Austin, Wilmington, Del.**

[73] Assignee: **University of Delaware, Newark, Del.**

[21] Appl. No.: **659,280**

[22] Filed: **Feb. 19, 1976**

[51] **Int. Cl.**[2] **C07H 5/06**

[52] **U.S. Cl.** ..... **264/233**; 106/203; 260/32.8 N; 264/186; 264/207; 264/217; 264/299

[58] **Field of Search** ..... 264/186, 194, 207, 217, 264/218, 233, 299; 260/32.8 N; 106/203

[56] **References Cited**

U.S. PATENT DOCUMENTS

2,040,880	5/1936	Rigby	536/20
2,689,244	9/1954	Jones	536/20
3,068,188	12/1962	Beste et al.	260/30.2
3,703,570	11/1972	Busch et al.	264/41
3,879,377	4/1975	Austin	536/20
3,892,731	7/1975	Austin	536/20
3,989,535	11/1976	Capozza	106/203

OTHER PUBLICATIONS

Solubility & Reactivity of Marine Polymers in Dimethylformamide-N_2O_4, Allan et al., 1/1971, Chemistry and Industry, p. 127.

Polyamides–Solubility–Kirk–Othmer, Encyclopedia of Chemical Technology, vol. 16, pp. 6, 7.

Primary Examiner—Jeffery R. Thurlow

Attorney, Agent or Firm—John J. McDonnell

[57] **ABSTRACT**

New solvents for chitin comprising dimethylacetamide, N-methylpyrrolidone or mixtures of these in combination with a minor proportion of lithium chloride, and their use in the purification of chitin for regeneration in the form of films and fibers are described.

2 Claims, No Drawings

SOLVENTS FOR AND PURIFICATION OF CHITIN

The Government of the United States has rights in this invention pursuant to Grant No. 04-3-158-30 with the Department of Commerce.

BACKGROUND OF THE INVENTION

This invention relates to new solvents for chitin and their use in the purification of chitin.

Chitin is an aminocellulose derivative that occurs widely in nature, for example, in the cell walls of fungi, bovine cartilage, cuttlefish bone and the hard shell of insects and crustaceans. The waste from shrimp, lobster and crab seafood industries contains 10–15% chitin and is a potentially important source of chitin, although the isolation and purification of the chitin, associated therein with mineral components, protein and other ingredients, presents considerable difficulty.

The applications for chitin are not extensive, in part because it has been little investigated and in part because it is difficult to purify. The use of chitin for accelerating and promoting wound healing is described in U.S. Pat. No. 3,632,754, to L. L. Balassa, Jan. 4, 1972. In other literature, the difficulties of purification are mentioned frequently. Chitin is also employed in the manufacture of chitosan, a deacetylated chitin that is readily soluble in dilute acids and may find application in paper making and surface active agents, for example.

More specifically, chitin is a mucopolysaccharide, believed to be poly-N-acetyl-D-glucosamine, with an empirical formula of $(C_8H_{13}O_5N)_n$ in which n may be any number into the thousands range, but is commonly in the area of 100–10,000. Chitin is a generally intractable material, soluble only in strong mineral acids, lithium thiocyanate solutions, and other special concentrated salt solutions, most of which cause disintegration or rapid degradation with loss in molecular weight or hydrolysis of the acetyl groups or both.

More recently some new solvents for chitin are described by Paul R. Austin in U.S. Pat. No. 3,879,377, Apr. 22, 1975, and in U.S. Pat. No. 3,892,731, July 1, 1975. These solvents comprise a 1,2-chloroalcohol in admixture with an acidic solvent, e.g. sulfuric acid, and a chloracetic acid alone or in combination with other solvents, e.g. formic acid. These solvents provide useful means for purifying chitin and for regenerating the chitin in the form of films, fibers and the like. However these solutions of chitin are not as stable as desired for storage for considerable lengths of time.

In the isolation and utilization of chitin it is desirable to set specifications for the chitin material, for example, molecular weight, viscosity or optical activity. All of these properties require a stable non-degrading solvent for their determination; the solvents of the prior art give transient values that are difficult to duplicate because of continuing chitin degradation.

It is an object of this invention to provide a new class of solvents for chitin.

It is a further object to provide a method for preparing solutions of chitin that can be filtered, otherwise purified, processed, or their properties measured.

It is still another object to provide solutions of chitin from which the chitin can be regenerated in the form of films, fibers or other shaped objects.

It is a still further object to provide chitin solutions that are stable on storage for considerable periods of time.

SUMMARY OF THE INVENTION

It has now been found that chitin is dissolved by dimethylacetamide, N-methylpyrrolidone or mixtures of these amides in conjunction with a minor amount, e.g. 2% up to the saturation point, of lithium chloride, at room temperature or on moderate heating, e.g. at 50° C. Solutions containing up to 15% chitin are readily obtained, depending to some extent on the molecular weight of the chitin. Solvency is limited by viscosity of the solutions; the lower molecular weight chitins in general dissolve more readily and give lower viscosity systems. At higher concentrations of chitin, in the 10–15% range, an organisol system, plastic in character, is obtained. The chitin solutions can be purified by centrifuging, vacuum or pressure filtration, or other means as appropriate for the consistency of the solution and application involved.

For physical property determination and chitin characterization, dilute solutions of 1% or less of chitin are usually employed. For film and filament preparation by wet-casting and spinning technology, a solution 2–5% chitin is preferred and the shaped films or fibers are subsequently coagulated or renatured by treatment with excess acetone, for example, washed with water and dried at ambient or elevated temperature. The resulting films and fibers are pliable and strong and can be cold drawn to orient them and to improve their strength.

At higher concentrations of chitin, in the range of 5–15%, the solutions become very viscous, approaching a plastic consistency. They are handled best by heavy duty mixers, pressure filtration and other organisol techniques. Films may be prepared by calendering or doctoring onto a moving belt, followed by solvent evaporation, water extraction and drying. Filaments may be extruded and renatured either by dry spinning with solvent evaporation or by a non-solvent precipitation as customary in wet spinning. Processing is completed as previously described.

As indicated above the dimethylacetamide and N-methylpyrrolidone can be used alone or in mixtures of any proportions in combination with the lithium chloride. Proportions of lithium chloride ranging from 2–5% by weight of the tertiary amide solvent are preferred.

An important advantage of the chitin solutions of this invention is their improved stability on storage. They have a working life of at least one month at room temperature.

Chitin from various sources can be used with the solvents of this invention. Thus, chitin from red, blue, rock, king and Dungeness crabs, from lobsters, shrimp, and other crustaceans, and from the cell walls of fungi and the hard shells of insects are all operable.

The following examples illustrate in further detail the solvents of this invention and their use in the preparation of chitin films and fibers.

EXAMPLE 1

To a solution of one part of lithium chloride in 20 parts of dimethylacetamide is added 0.6 parts of chitin (red crab) and the mixture stirred at room temperature for one hour. Then 20 parts of N-methylpyrrolidone is added and the mixture stirred for 2 hours. The resulting solution is filtered through felt and the filtrate spread on a glass plate at a thickness of 1 mm. The wet film is dried for one hour in a current of air at room temperature, and then placed in excess acetone for 16 hours to extract solvent from the film. The film is extracted for

another 24 hours with fresh acetone, and finally rinsed again with fresh acetone and dried in air at room temperature for 30 minutes. The film is cut into small strips and are cold drawn to produce a 66% increase in length. The drawn film is strong and pliable.

EXAMPLE 2

To a solution of 1 part of lithium chloride in 20 parts of dimethylacetamide is added 0.6 parts of chitin (blue crab) and the mixture stirred at room temperature for one hour; then 10 parts of dimethylacetamide is added and the mixture stirred for 2 hours. The resulting solution is filtered through felt and the filtrate spread on a glass plate at a thickness of 1 mm. The chitin is regenerated into a film as in Example 1. The film was cut in small strips (2 mm × 2.5 cm) and were cold drawn 83%. The tensile strength of the drawn film is 16.1 Kg/mm^2.

The chitin films cast from solution in dimethylacetamide and dimethylacetamide/N-methylpyrrolidone systems show syneresis, i.e. solvent exudes from the film as it stands for a short time, e.g. for an hour. This has the effect of concentrating the chitin in the resident film and facilitating its renaturing by immersion in acetone.

EXAMPLE 3

To a solution of 2.5 parts LiCl in 50 parts N-methyl-2-pyrrolidone is added 0.25 parts chitin (Dungeness crab) and the mixture is stirred at room temperature for 1.5 hours. The resulting solution is filtered through felt and the filtrate spread on a glass plate at a thickness of 1 mm. The chitin is regenerated into a film as in the previous examples. The film was cold drawable.

EXAMPLE 4

Five parts of finely divided chitin and 50 parts of dimethylacetamide containing 5% of lithium chloride is rapidly mixed at room temperature to thoroughly wet all of the chitin. At first the chitin appears to absorb the solvent to form discrete gel particles which upon standing and intermittent stirring convert to a very viscous mass. This mixture is worked periodically with a stirrer to promote gel particle attrition and develop a more coherent gelatinous system. After standing five days, the gelatinous material is doctored onto a platten and pressed to a coherent film. The solvent is removed by evaporation, followed by a water wash and drying. The resultant film is pliable and tough. The film can be cold drawn with typical necking down. Upon breaking at the extension limit, the fracture line shows fibrillation, as does polyethylene and other polymers capable of being cold drawn.

EXAMPLE 5

To 100 parts dimethylacetamide containing 5% lithium chloride 3.5 parts of chitin (red crab) was added and the mixture stirred for 18 hours. The resulting solution was filtered through felt and allowed to stand for 24 hours. Some of the solution was then spread on a glass plate to a thickness of 1 mm and regenerated as in example 1. The quality of film produced was tested by cold drawing and it was found to be capable of being cold drawn 40%. The remaining solution was stored for 48 days. It was noted that viscosity of the solution at the end of 48 days was the same as the fresh solution. Some of this solution that had been stored 48 days was then spread on a glass plate to a thickness of 1 mm and regenerated in the same manner as the film made from the fresh solution. The quality of this film was tested by cold drawing and it was found to be capable of being cold drawn 75%, thus indicating that storage in the solution does not degrade the chitin.

While particular examples of the present invention have been shown and described it is apparent that changes and modification may be made therein without departing from the invention in its broader aspects. The aim of the appended claims, therefore, is to cover all such changes and modifications as fall within the true spirit and scope of the invention.

I claim:

1. A process for forming films and fibers from chitin that is insoluble in dilute acids which comprises dissolving of said chitin in a solvent consisting of at least 2% lithium chloride in dimethylacetamide or N-methylpyrrolidone or mixtures thereof, casting the solution in the form of a film or fiber, removing the solvent by evaporation or by immersion in a non-solvent followed by washing in water and then drying said film or fiber.

2. The process of claim 1 wherein the non-solvent is acetone.

* * * * *

V. CHITIN COMPLEXES WITH ALCOHOLS AND CARBONYL COMPOUNDS—U.S. PATENT 4,063,016

Paul R. Austin

United States Patent [19]

Austin

[11] **4,063,016**

[45] **Dec. 13, 1977**

[54] **CHITIN COMPLEXES WITH ALCOHOLS AND CARBONYL COMPOUNDS**

[75] Inventor: **Paul R. Austin**, Wilmington, Del.

[73] Assignee: **University of Delaware**, Newark, Del.

[21] Appl. No.: **640,583**

[22] Filed: **Dec. 15, 1975**

[51] **Int. Cl.²** **C08B 37/08**

[52] **U.S. Cl.** **536/20; 424/180**

[58] **Field of Search** ..... 260/211 R; 536/20

[56] **References Cited**

U.S. PATENT DOCUMENTS

1,929,786	10/1933	Meyer	260/211 R
2,072,771	3/1937	Rigby	260/211 R
2,163,104	6/1939	Salzberg	260/211 R
2,669,529	2/1954	Rust	260/211 R
2,670,329	2/1954	Jones	260/211 R
2,783,148	2/1957	Gyorgy et al.	260/211 R
2,842,049	7/1958	Delangre	260/211 R
3,847,897	11/1974	Dunn et al.	260/211 R

OTHER PUBLICATIONS

Seichertova, O. et al., "Chem. Abst." vol. 79, p. 112580(b), 1973.

Primary Examiner—Johnnie R. Brown

[57] **ABSTRACT**

New compositions of matter comprising complexes of chitin with lower molecular weight alcohols, aldehydes and ketones have been discovered. These complexes are useful in the solution and purification of chitin and in the preparation of modified chitins for fibers, films and plastics, and for pharmaceutical purposes.

7 Claims, 5 Drawing Figures

CHITIN COMPLEXES WITH ALCOHOLS AND CARBONYL COMPOUNDS

The Government has rights in this invention pursuant to Grant No. 04-3-158-30 awarded by the U.S. Department of Commerce.

This invention relates to new addition compounds or complexes of chitin and their method of manufacture.

PRIOR ART

Chitin is a cellulose-like material that occurs widely in nature, for example, in the cell walls of fungi and the hard shells of insects and crustaceans. More specifically, chitin is a mucopolysaccharide, poly-N-acetyl-D-glucosamine, of relatively high molecular weight. However, in the natural state it occurs only in small flakes or as short fibrous material, and is not capable of forming useful shaped articles without solution and reprecipitation or renaturing. Methods of dissolving chitin in certain solvents are described in the literature. For example, Clarke and Smith, J. Phys. Chem., 40, 863 (1936), used aqueous acids or lithium salts for solution and regeneration of chitin. These authors observed the formation of addition compounds of chitin with lithium thiocyanate and with sodium hydroxide under certain conditions. However, the formation of addition compounds, or complexes, of chitin with organic compounds has not hitherto been described.

OBJECTS OF THE INVENTION

It is an object of this invention to provide novel complexes of chitin with low molecular weight alcohols, aldehydes and ketones. It is a further object to provide a process for preparing these now chitin complexes.

DRESCRIPTION OF THE INVENTION

The compositions of this invention are complexes of chitin with alcohols, aldehydes and ketones preferably having up to 10 carbon atoms. A preferred group of the complexes are those having a mole ratio of the N-acetylglucosamine unit of chitin to oxygen-containing complexing agent ranging from 1:6 to 5:1. . Especially preferred compositions of this invention are the complexes of chitin with alcohols, aldehydes and ketones having up to five carbon atoms. The alcohols, aldehydes and ketones can have one or more halogen substituents, e.g., chlorine.

The chitin complexes of this invention can be prepared by direct contact of chitin with an excess of lower molecular weight alcohol, aldehyde or ketone, i.e., at least five parts by weight of the complexing agent to one part of chitin. However, it is preferred to use a water- or solvent-swelled chitin with the complexing agent. It is also preferred to use a finely comminuted chitin to increase the rate of interaction between the chitin and the alcohol or carbonyl compound.

A mass action effect is observed in this process. That is, one complexing agent in a chitin complex can be displaced with another if a substantial excess of the second complexing agent is allowed to interact with the previously formed complex.

With low boiling complexing agents such as methanol and acetone the chitin complex has a relatively high vapor pressure of the alcohol or carbonyl component under ambient conditions which results in decomposition of the complex with drying of the chitin, unless the complex is stored in a closed container in the presence

of a slight excess of the complexing alcohol or carbonyl compound.

In other cases, where molecular association forces are stronger, when chemical reaction may be involved, or when higher boiling complexing agents are employed, even an excess of a second complexing agent may not displace a favored complexing agent.

The chitin complexes of this invention have a number of unusual properties, which make them useful in a variety of applications. For example, they have a voluminous physical form which makes them easily handled and filtered in the preparation of highly purified chitin for pharmaceutical and other purposes. Furthermore, they are more readily dissolved in chitin solvents than natural chitin, and hence are useful in preparing chitin solutions for the fabrication of chitin in the form of films, fibers, or other shaped articles; they also may contribute a plasticizing effect that enhances the cold drawing of chitin fibers and films. The complexes, in addition, facilitate renaturing of chitin to fibrillar material useful for wound-healing acceleration, e.g., in the manufacture of fibrous mats for absorbable, internal reinforcement of herniated areas. Chitin complexes with higher molecular weight aldehydes and ketones have greater stability and exhibit the toughness and formability required for the manufacture of plastic products.

The products and processes of this invention are illustrated in greater detail in the following examples in which quantities of ingredients are expressed in parts by weight or percent by weight, unless otherwise specified.

The drawings are graphs supporting the fact that complexes are formed as fully explained in the examples.

EXAMPLE I

One part of water-swelled chitin is placed in 10 parts of methanol and comminuted to permit thorough penetration and interaction of the methanol. After standing for two hours the chitin alcoholate slurry is filtered off and redispersed in 10 parts of fresh methanol. After standing an additional hour the chitin alcoholate is filtered off and spread on a tray to dry; the product is broken up and turned during the drying period to present fresh surface facilitate drying. This operation is carried out at room temperature in an area of good air flow.

To demonstrate chitin complex formation, weight loss of the drying complex is determined versus time, in the expectation that superficial solvent would be lost more rapidly than complexed solvent, as is the case with such known materials as calcium sulfate and its hydrates. The intercept of the two rate curves approximates the composition of the complex. The data follow:

Time, min.	Parts by wt.	
0	3.3	
7	2.7	
13	2.3	
28	1.3	
57	0.8	
67	0.7	(dry)

It will be noted from the table and especially from FIG. 1, which expresses these data in graphical form, that there is an abrupt change in solvent evaporation

rate after the fourth period, with an intercept corresponding to a complex of 0.7 part of chitin monomer and 0.36 part of methanol (0.0034 and 0.011 moles, respectively) or a ratio of 3.2 moles of methanol per N-acetylglucosamine residue.

The chitin-methanol complex is a pasty, somewhat cheesy material that is stable at room temperature if kept in a closed vessel in the presence of a slight excess of methanol. However, in the open, it will lose methanol gradually as indicated above.

EXAMPLE II

One part of water-swelled chitin is placed in 10 parts of methanol and comminuted to permit thorough penetation and interaction of the methanol. After standing for two hours the chitin alcoholate is filtered off and redispersed in 10 parts of acetone. The dispersion, standing for 15 minutes, and filtration is repeated two more times to assure complete displacement of the methanol and formation of the acetonate. The acetonate is somewhat more rubbery and cheesy than the alcoholate and filters very rapidly.

After the last filtration, the acetone-wet chitin acetonate is dried in a tray at room temperature with good air movement around it. The weight loss of a sample is measured at time intervals until the sample is thoroughly dry. The data follow:

Time, min.	Parts by wt.	Time, min.	Parts by wt.
9	0.076		
10	.064	20	.039
11	.054	25	.035
12	0.48	30	.033
13	.045	35	.031
14	.043	40	.030
15	.042	60	.029
16	.041	120	.028
18	.040	180	.025 (dry)

In this case, inspection of the data does not give a ready indication of the difference in rate of solvent loss of simple evaporation versus complex breakdown and diffusion of solvent vapor, but by plotting time versus weight loss on a simple graph, FIG. 2, it is clearly seen that two different rates are involved. The intersection of these curves corresponds to a product comprising 0.025 part of chitin and 0.019 part of acetone (0.000123 mole of chitin monomer and 0.00033 mole of acetone) or a mole ratio of acetone to N-acetylglucosame of 2.7:1. This chitin acetonate complex is slowly soluble in formic acid at room temperature whereas the original chitin is not soluble in formic acid.

Another method of characterizing the chitin complexes of this invention involves the use of differential thermal analysis, which measures the heat energy change occurring in a substance as a function of temperature. The curves obtained, called thermograms, show any chemical change that is accompanied by a heat energy change. Differential thermal analysis has been widely used in studies of the chemistry of inorganic and organic compounds, including studies of hydrates of various compounds. The thermogram obtained on the chitin-acetone complex of this Example is given in FIG. 3. In this determination the temperature of the chitin-acetonate and the inert control material is increased at a constant rate of 20° C per minute. The resulting thermogram shows a sharp endothermic heat change beginning at 75° C. The thermograms obtained with the ketone complexes of this Example and those of Examples V and VI show the strong association of the ketones to the chitin.

EXAMPLE III

One part of an amorphous, precipitated chitin and 0.7 part of camphor are dissolved in 17 parts of formic acid. The homogeneous solution is spread on glass and the formic acid allowed to evaporate. A hard, flaky, tough camphor complex results that appears to be stable in air. In contrast to the original powdery chitin, the product is difficult to grind; it is tough rather than friable. Upon grinding, some fibrillar material is formed along with a nearly white powder. There is no indication of the separation of camphor itself.

EXAMPLE IV

Two parts of red crab chitin are dissolved in 87 parts of a solvent mixture comprised of 40 percent trichloroacetic acid, 40 percent chloral hydrate and 20 percent methylene chloride. Solution is accelerated by warming slightly, below 40° C, stirring for 30 minutes. A very thick, viscous solution is obtained that is filtered through wool felt.

A portion of the filtered chitin solution is spread upon glass to a thickness of about one-sixteenth inch. It is immersed in acetone to coagulate it and is washed in four successive acetone washes, each lasting 15 minutes. It is then neutralized and washed with a solution of potassium hydroxide in 2-propanol and finally with water to a pH of 7. The film is tough, clear and ductile, and has a sp. gr. of 1.46 – 1.47. Analysis shows the presence of 33.28 percent chlorine, indicating a ratio of about one mole of unhydrated chloral to one mole of N-acetylglucosamine in the complex (30.4 percent chlorine calculates for 1:1 complex.)

In this example, the strong affinity of chloral for chitin is illustrated, as the successive acetone and alkaline 2-propanol washes do not form new complexes, although they probably remove superficial chloral. However, when the complex is extracted for 12 hours with methylene chloride, with continuous removal of the extract and treatment with fresh solvent (Soxhlet), the complex contains 9.45 percent chlorine, indicating a ratio of about 5 N-acetylglucosamine units to one of unhydrated chloral.

EXAMPLE V

One part of water-swelled chitin is washed with two successive portions of acetone, and dried superficially in air at room temperature to remove excess acetone, but not enough to decompose the complex. The resulting chitin-acetone complex is immersed in five parts of methyl isobutyl ketone and comminuted for 15 minutes to permit thorough penetration and interaction of the methyl isobutyl ketone. The chitin ketonate is then filtered off and redispersed in three more five-part portions of methyl isobutyl ketone with filtration after each successive soaking. This treatment produces complete replacement of the acetone in the acetonate with formation of the chitin methyl isobutyl ketonate. The ketonate is dried superfically in air at room temperature with mechanical working of the product with a spatula.

The differential thermal analysis thermogram of the ketonate of this Example is shown in FIG. 4. With the chitin complex and the inert control material heated at a constant rate of increase of 20° C per minute an endothermic heat change begins at about 104° C and becomes a sharp change at about 114° C.

EXAMPLE VI

One part of water-swelled chitin is immersed in five parts of methyl ethyl ketone and comminuted for 15 minutes to permit thorough penetration of the methyl ethyl ketone into the porous chitin. The swollen chitin complex is then filtered off and redispersed in three more five-part portions of methyl ethyl ketone with filtration after each successive soaking. This treatment produces complete replacement of the water in the swollen chitin with formation of chitin methyl ethyl ketonate. The complex is superficially dried in air at room temperature with mechanical working of the product with a spatula.

The differential thermal analysis thermogram of the ketonate of this Example is given in FIG. 5. With the chitin complex and the inert control material heated at a constant rate of increase of 20° C per minute a sharp endothermic heat change begins at 77° C.

EXAMPLE VII

This Example illustrates the use of the process of forming a chitin alcohol complex as an intermediate in the formation of a phosphate salt of chitin.

One part by weight of chitin and 40 parts by volume of a 50:50 (by volume) mixture of phosphoric acid and 2-propanol are stirred together at room temperature for two hours. The mixture if filtered through wool felt and the filtrate poured in a fine stream into a large excess of 2-propanol to precipitate the chitin in the form of a chitin 2-propanol complex. The solvent-swelled chitin is filtered through paper and resuspended with vigorous stirring in fresh 2-propanol for about 15 minutes. This wash and filtration is repeated three more times, at which point the system is neutral. The filter cake is then spread in an open tray with good ventilation at room temperature and the product worked with a spatula to break up the caked material and continually present new surfaces for solvent evaporation. The product is a fine, light-colored powder that passes readily through a 100-mesh sieve (openings of about 157 microns).

Since chitin may contain one free amino group for about every six N-acetylglucosamine units, and the acid in this system was not neutralized, it is believed that the product is a chitin phosphate salt.

Properties of the sample are as follows:

Sp. gravity	1.422	
Nitrogen	6.57%	(6.57%) calcd.
Phosphorus	2.54%	(2.43%) calcd.

It was reported by an outside laboratory that this product was effective as a wound-healing accelerator in preliminary tests in rats.

The examples given above are selected to illustrate significant aspects of the invention, but are not to be considered limiting. Various modifications and equivalents will be obvious to one skilled in the art. Some of these variations are described below.

Chitin from many sources can be used to make the products of this invention. Chitin from arthropods, in particular from the shells of crabs, shrimp, crayfish, lobsters and other crustaceans is operable. Crabs such as, for example, blue, red, rock, king, and Dungenes crabs are especially good sources of chitin. Chitin from other sources such as cell walls of fungi, e.g., the fungal residues from fermentation processes involving such fungi as *Asperigillus niger* or Penicillium. Chitins of high molecular weight are preferred for use in this invention.

The water-swelled chitin, or hydrated chitin, used in Examples I and II, is an especially preferred form of chitin for use in preparing the chitin complexes of this invention. A convenient method of making hydrated chitin is as follows:

Five parts of chitin (8 mesh) is added to a mixture of 63 parts of 2-chloroethanol and 82 parts of 73% sulfuric acid, with mechanical stirring at room temperature. Small additional portions of solvent may be added if necessary to reduce the viscosity. After one hour the mixture is filtered through felt. A solution of 88 parts of conc. ammonium hydroxide and 100 parts of water is cooled in an ice bath in a vessel equipped for mechanical stirring. The above chitin solution is then poured in a fine stream, with rapid stirring, into the ammonia solution. Additional ammonia may be added if necessary to make alkaline. When the addition is complete and the solution alkaline, the system is stable and may be kept for several days, if necessary. The precipitated chitin is decanted, filtered or centrifuged, and the precipitate reslurried in water made slightly alkaline with ammonia. After 15 minutes the precipitate is again filtered, and the slurry-washing repeated with plain water until it is free of sulfate ion (barium chloride test). Two to four washes may be required. The resultant product is a hydrated chitin and may be kept as such in a stoppered bottle.

As indicated above, alcohols, aldehydes and ketones having up to 10 carbon atoms are useful in forming the chitin complexes of this invention. Specific examples of alcohols that can be used are the aliphatic alcohols: methanol, ethanol, propanol, 2-propanol, chloroethanol, and butanol. Cyclohexanol is a useful alicyclic alcohol. Aliphatic and alicyclic ketones that are useful include; acetone, methyl ethyl ketone, diethyl ketone, methyl propyl ketone, methyl isobutyl ketone, acetyl acetone, cyclopenatanone, and camphor. Suitable aliphatic aldehydes include: formaldehyde, acetaldehyde, propionaldehyde, n-butyaldehyde, isobutyaldehyde and chloral. As indicated previously, the alcohol, aldehyde and ketone reactants can have halogen substituents, e.g., one or more chlorine substituents.

It is apparent that changes and modifications may be made without departing from the invention in its broader aspects. The aim of the appended claims, therefore, is to cover all such changes and modifications as fall within the true spirit and scope of the invention.

I claim:

1. A complex of chitin with an oxygen-containing complexing agent containing up to 10 carbon atoms selected from the group consisting of saturated aliphatic and alicyclic alcohols, aldehydes, and ketones, said complex having a sharp endothermic heat change when subjected to differential thermal analysis.

2. A complex of claim 1 wherein the oxygen-containing complexing agent contains up to 5 carbon atoms.

3. The complex of claim 1 wherein the mole ratio of N-acetylglucosamine unit of chitin to the oxygen-containing complexing atent ranges from 1:6 to 5:1.

4. The complex of claim 3 wherein the oxgen-containing complexing agent is methanol.

5. The complex of claim 3 wherein the oxygen-containing complexing agent is acetone.

6. The complex of claim 3 wherein the oxygen-containing complexing agent is chloral.

7. The complex of claim 1 wherein the oxygen-containing complexing agent is camphor.

* * * * *

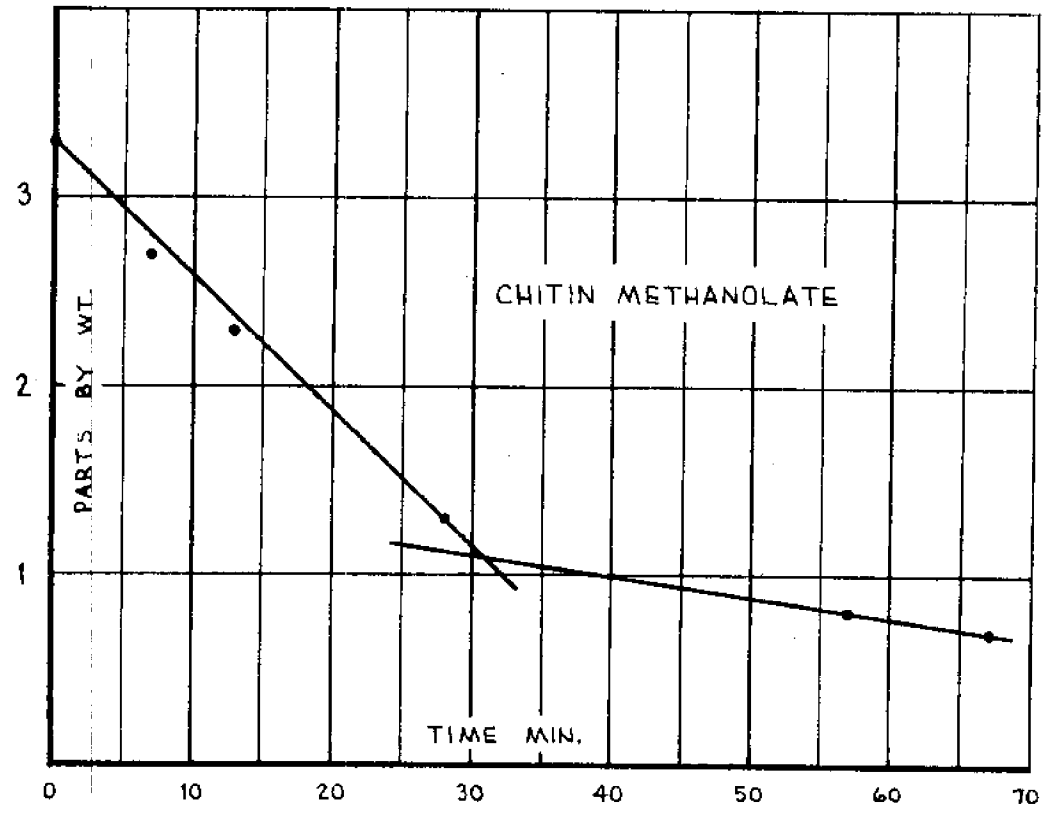
CHITIN METHANOLATE
PARTS BY WT.
TIME MIN.
3
2
1
0
10
20
30
40
50
60
70

FIG. 1

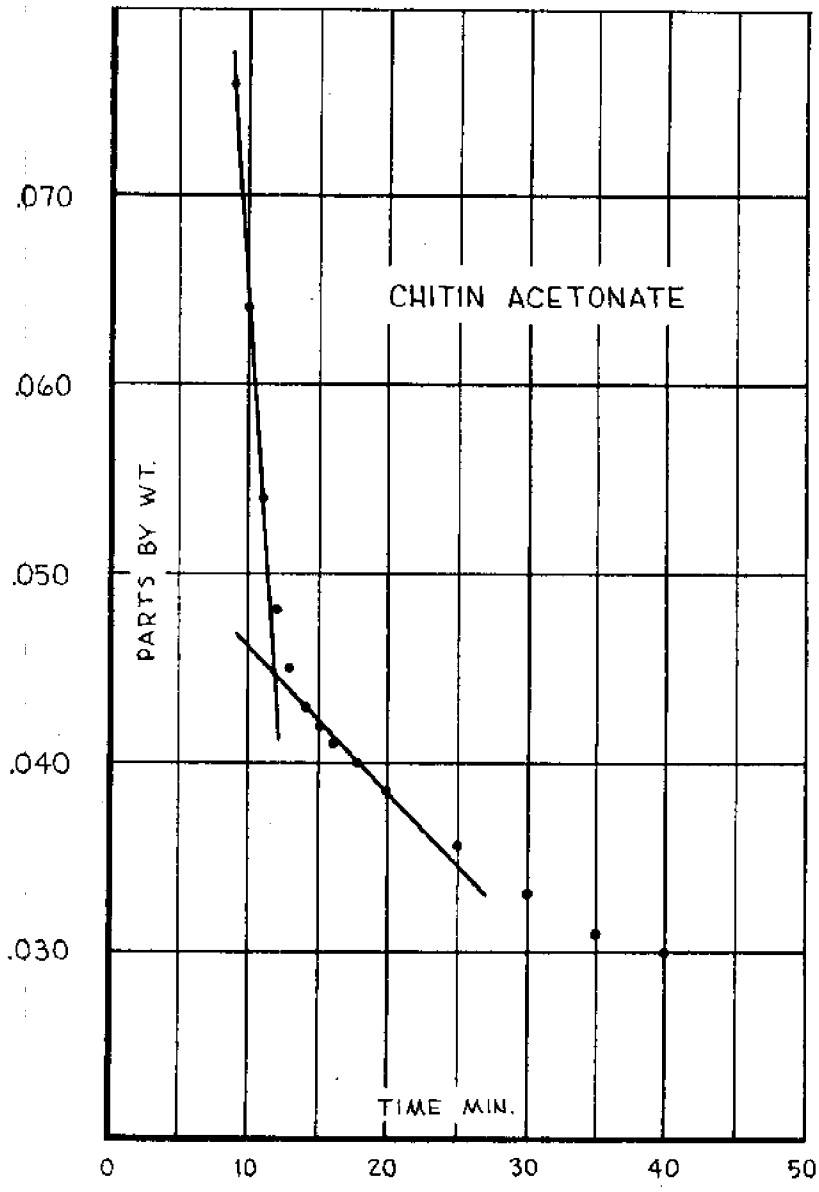
.070
.060
.050
.040
.030
PARTS BY WT.
CHITIN ACETONATE
TIME MIN.
0
10
20
30
40
50

FIG. 2

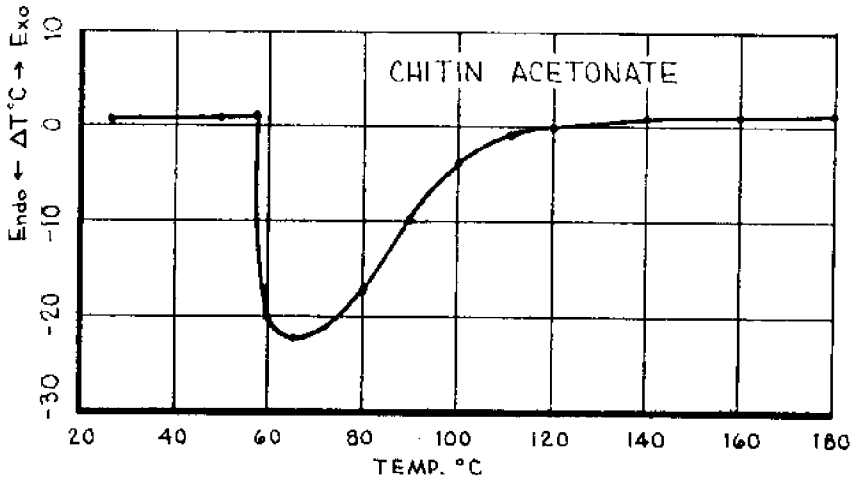
CHITIN ACETONATE
Endo ← ΔT°C → Exo
10
0
-10
-20
-30
20
40
60
80
100
120
140
160
180
TEMP. °C

FIG. 3

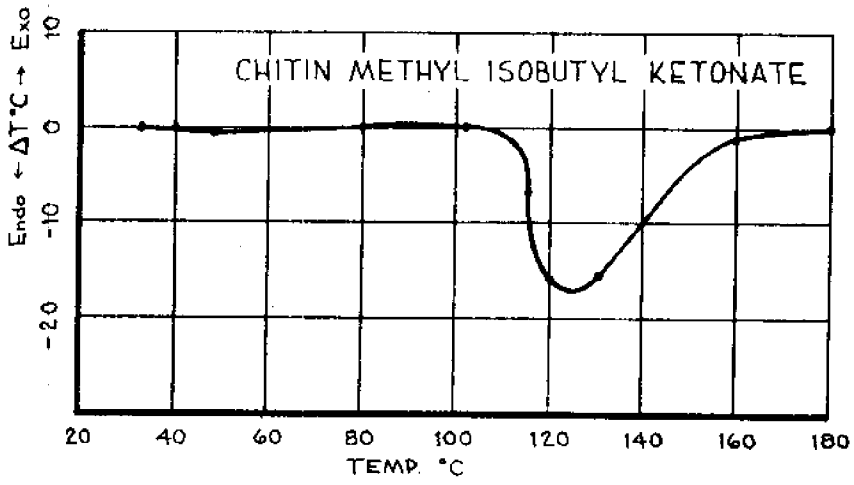
CHITIN METHYL ISOBUTYL KETONATE
Endo ← ΔT°C → Exo
10
0
-10
-20
20
40
60
80
100
120
140
160
180
TEMP. °C

FIG. 4

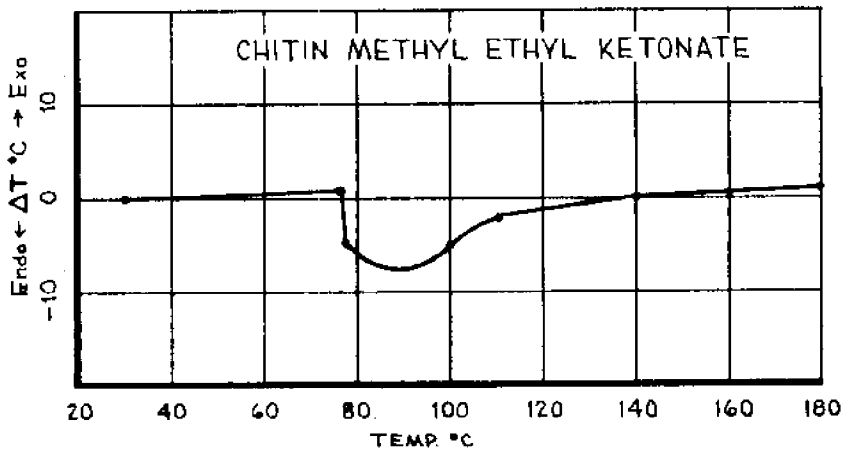
CHITIN METHYL ETHYL KETONATE
Endo ← ΔT °C → Exo
10
0
-10
20
40
60
80
100
120
140
160
180
TEMP. °C

FIG. 5