Characterization of Coagulant–Protein Complexes Produced by Chemical Coagulation of Industrial Whole Animal Blood

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Protein–coagulant complexes were prepared from industrial whole animal blood by using zinc sulfate, aluminum sulfate, ferric chloride, ferric sulfate, sodium polyphosphate, and sodium lignosulfonate, under conditions which resulted in quantitative removal of protein from the blood. The amount of coagulant found with protein in the complexes produced represented from about 80% (polyphosphate, lignosulfonate) to greater than 95% (zinc, aluminum, or iron ion) of input. The nitrogen and coagulant compositions of the aluminum sulfate and iron chloride generated complexes were significantly lower than expected compared to those of complexes generated by using zinc sulfate and ferric sulfate. Sodium lignosulfonate–protein complexes were characterized by a method using sulfur analysis, since nitrogen was found to be present in the commercial preparation used. The results demonstrate that all of the coagulant–protein complexes studied form under conditions that require only a small excess of coagulant and that the complexes contain low percentages of heavy metal ions or nutritionally beneficial (sodium polyphosphate) or nontoxic (sodium lignosulfonate) coagulants.

Blood meal produced industrially from whole animal blood is of interest as a component of animal diets due to its high protein content. Conventional methods of blood meal production include procedures, such as steam coagulation, vat drying, ring drying, and spray drying, that utilize large amounts of heat energy and may affect the quality of protein in the meal (Waibel et al., 1977; Kramer et al., 1978). As an alternative, we have proposed and investigated the use of chemical coagulants to effect protein removal from industrial animal blood (Vandegrift and Ratermann, 1979; Ratermann et al., 1980; Vandegrift et al., 1981). Our results have demonstrated that sodium polyphosphate, ferric chloride, aluminum sulfate, and zinc sulfate quantitatively remove protein from animal blood and that sodium lignosulfonate treatment results in near-quantitative protein removal, under specified conditions.

In this report we have extended our studies of coagulants, utilizing ferric sulfate as a coagulant that quantitatively removes protein from industrial animal blood. In addition, we have characterized protein–coagulant complexes produced by treatment of animal blood with zinc sulfate, aluminum sulfate, ferric chloride, ferric sulfate, sodium polyphosphate, and sodium lignosulfonate.

EXPERIMENTAL SECTION

PRA-1 and CBOS-6, sodium lignosulfonates produced by sulfite wood pulping, were obtained from the American Can Co. (Greenwich, CT). Sodium polyphosphate (sodium hexametaphosphate, average chain length = 15, SPP) and purified porcine albumin were obtained from the Sigma Chemical Co. (St. Louis, MO). Ferri-floc was obtained from Cities Service Co. (Atlanta, GA). All other chemicals used were reagent grade and were obtained from commercial suppliers. Industrial whole animal blood samples, held no longer than 4 h past slaughter, were obtained from the holding tanks of the Emge Packing Co. (Fort Branch, IN) and used immediately or stored frozen at −20 °C until use. Blood used immediately was treated with sodium oxalate anticoagulant (1.5 mg/mL). Some clots were occasionally present in thawed blood samples and were removed by filtration prior to characterization. Control experiments, performed by using blood samples that differed only as to whether or not they had undergone freezing and thawing prior to coagulant treatment, demonstrated that freezing and thawing of blood did not alter the coagulant concentrations or pH conditions required to effect quantitative protein removal (Vandegrift and Ratermann, 1979). Storage at −20 °C for up to 3 months did not affect the quality of the blood. Blood samples and centrates were characterized for total Kjedahl nitrogen and nonprotein nitrogen by using 10% trichloroacetic acid as described previously (Vandegrift and Ratermann, 1979). All blood samples used had minimal protein degradation, since they contained low levels of nonprotein nitrogen (420 ± 50 mg of N/L) relative to the total nitrogen content (3000 ± 200 mg of N/L).

Protein coagulation was effected by adding the coagulant as a solid (SPP) or as a solution (zinc sulfate, aluminum sulfate, ferric chloride) to diluted blood at the optimal pH and coagulant concentration previously described (Vandegrift and Ratermann, 1979; Ratermann et al., 1980). Ferric sulfate was added as a 10% solution [9.5% $Fe_2(SO_4)_3$ plus 0.5% $FeSO_4$ to enhance solubility], whereas ferri-floc was added as a 10% slurry. The ferric sulfate or ferri-floc was added to blood diluted with doubly distilled water by a dilution factor of about 1 to 2. The pH of the blood–coagulant mixture was adjusted to pH 4.5 with 2.5 M NaOH. The final dilution of blood was 3-fold. Coagulation experiments were performed at room temperature (25 °C), with 1-h settling times prior to centrifugation at 3000*g* and analysis of the centrate. All coagulant–protein complexes were dried at 125 °C for 4 h to determine the percentage of water and stored until further analysis in a vacuum desiccator.

From four to twelve separate coagulation experiments each were performed by using the coagulants (except PRA-1). The analytical results of these independent experiments are presented as the arithmetic mean, with the standard deviation included. Due to the tedious nature of the analysis of PRA-1 complexes only two independent experiments were performed. The results of the experiments were in good agreement with each other and were averaged. For all of the coagulant–protein complexes studied, an average of 98.5% of input coagulant was accounted for by analysis of the complexes and the supernatants. Percentage nitrogen in the dried complexes was determined by Kjeldahl analysis.

All determinations of metals associated with coagulants were preceded by digestion of the protein complex at the decomposition temperature of H_2SO_4 with periodic additions of concentrated HNO_3 and finally 30% H_2O_2 to produce a clear digestate. Iron determinations were per-

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Table I. Chemical Coagulation of Animal Blood: Analysis of Protein-Coagulant Complexes

coagulant	dilution of whole blood	final coagulant concn	pH	% H_2O[a]	% N in dried complex[b]	% coagulant in dried complex[c]	% of input coagulant in complex
$ZnSO_4 \cdot 7H_2O$	1:3	8.0 (4.5)[e]	8.0	85	13.83 ± 0.85	2.64 ± 0.05 (8)	95.1 ± 1.9
$Al_2(SO_4)_3 \cdot 16H_2O$	1:3	56.0 (30.4)	4.5	80	8.91 ± 0.89	4.61 ± 0.06 (12)	96.9 ± 1.3
$FeCl_3 \cdot 6H_2O$	1:5	33.3 (20.0)	4.5	83	10.28 ± 0.22	12.20 ± 0.42 (6)	98.5 ± 3.4
SPP	1:3	10.8	3.0	73	12.63 ± 0.35	9.40 ± 0.20 (7)	78.9 ± 1.5
PRA-1	1:3	20.7	3.5	74	11.55 ± 0.05	19.9 (2)	86.4
$Fe_2(SO_4)_3 \cdot 4H_2O$[d]	1:3	20.0 (16.9)	4.5	85	11.67 ± 0.32	6.39 ± 0.45 (4)	96.8 ± 6.7

[a] Water determinations based on complexes prepared by centrifugation at 3000g for 10 min. [b] Based on at least five determinations in each case. [c] In instances where metal salts are used in coagulation, values are expressed as percent metal ion. The number of determinations in each case is given in parentheses. [d] Added as ferri-floc (Cities Service Co., Atlanta, GA), a commercial flocculant. [e] Values in parentheses represent final concentration based only on the dry salt (grams per liter).

formed by titration with standard dichromate following reduction to Fe(II) with Sn(II) (Harris and Kratochvil, 1974).

Zinc determinations were made by titration with standard EDTA, after buffering to approximately pH 10 with NH_3–NH_4Cl, by using Eriochrome Black T as the indicator (Pietrzyk and Frank, 1974). The fluoride complexometric–alkalimetric method of Watts (1958) was used, with minor modifications, for the determination of aluminum. Metal determinations were occasionally cross-checked by atomic absorption procedures using a Jarrell-Ash Model 82-500 air–acetylene flame spectrophotometer. Phosphate analyses were performed by the vanadomolybdophosphoric acid method (American Public Health Association, 1971) following digestion of the complexes with H_2SO_4 and HNO_3 and clarification of the resulting solution using H_2O_2.

PRA-1 was characterized, prior to complex formation, for total nitrogen and nonprotein nitrogen by using Kjeldahl analysis. Whole blood and PRA-1 samples were also assayed for total sulfur content by combustion in a bomb calorimeter, followed by precipitation as $BaSO_4$ and gravimetric analysis (Harris and Kratochvil, 1974). In addition, noncombusted whole blood and PRA-1 samples were analyzed for sulfate gravimetrically and found to contain only trace levels of free sulfate. By using standardized H_2SO_4 to produce the optimum pH for complex formation (pH 3.5), it was possible to monitor the amount of PRA-1 actually present in the complex. The use of other acids for pH adjustment was precluded, since other acids altered the optimum conditions for coagulation previously characterized (Vandegrift and Ratermann, 1979).

Centrifugation of PRA-1–protein complexes (3000g for 10 min) was performed in preweighed Corex tubes, and the supernatant was carefully decanted. The total volume of supernatant was recorded and divided into five equal aliquots. Two aliquots were analyzed for residual nitrogen via Kjeldahl, two for sulfate sulfur via the gravimetric method, and the fifth aliquot, to be used for total sulfur content, was dried at 125 °C for 4 h. After the tubes containing the wet complex were weighed, a portion was removed and weighed, and the wet mass was extracted with hot water. The sulfate extracted from the complex was determined gravimetrically. The remaining complex was weighed in the Corex tubes following drying at 125 °C for 4 h, and the weight of the dry complex was corrected for the portion removed earlier. The dry complex was then divided into small samples and analyzed for total sulfur.

PRA-1 samples were also assayed for proteinaceous compounds by using biuret (Cooper, 1977) and dye binding (Bradford, 1976) techniques. A determination of the amount of lignin present in PRA-1 was made by utilizing a Folin–Denis assay (American Can Company, 1976) as compared to a highly purified lignin preparation (CBOS-6).

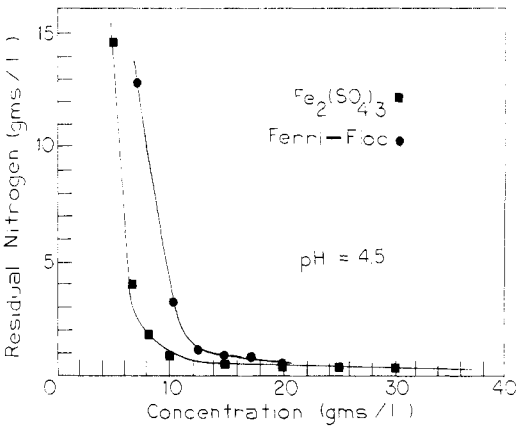

Figure 1. Normalized residual total Kjeldahl nitrogen after treatment of diluted blood vs. the concentration of the coagulants ferric sulfate or ferri-floc [$Fe_2(SO_4)_3 \cdot 4H_2O$]. All data points represent the average of at least three independent experiments performed at pH 4.5.

RESULTS AND DISCUSSION

Data comparing the effectiveness of the coagulants ferric sulfate and ferri-floc for removal of protein from blood are displayed in Figure 1. Each data point represents an average of at least three independent experiments. The final volume of blood in the experiments represents a dilution of 3 times the original volume. All residual nitrogen values plotted have been normalized by multiplying the observed residual nitrogen levels by 3, to enable a direct comparison of the *total* amount of residual nitrogen in the effluent relative to the *total* amount of nitrogen in whole blood. Values plotted along the abscissa represent the actual concentration of coagulant in the blood *after* the blood had been diluted to its final treatment volume.

Both ferric sulfate and ferri-floc (which contains approximately 95% hydrated ferric sulfate by weight) are extremely effective in the removal of protein from industrial animal blood. As displayed in Figure 1, ferric sulfate treatment (calculated as the anhydrous salt) results in quantitative removal of protein from blood at about 15 g/L at pH 4.5 (98% removal of N), whereas a concentration of 20 g/L is required for ferri-floc. The efficiency differential between these two coagulants is nominal, however, considering that ferri-floc is a hydrated preparation. In contrast to $FeCl_3$ (Vandegrift and Ratermann, 1979) both ferri-floc and ferric sulfate can be used at a 3-fold dilution without encountering clumping. A comparison of aluminum sulfate, ferric chloride, and ferric sulfate (Table I) reveals that the molar concentration of ferric ion as ferric sulfate is much lower than the molar

Table II. Typical Calculation for Sulfur in the PRA-1 Coagulation System[a]

sulfur input				sulfur recovery			
source	quantity, g	S from SO_4^{2-}	total S	source	quantity	S from SO_4^{2-}	total S
blood	52.39 g	trace	0.0529 g	complex	12.12 g	0.093 g	0.349 g
PRA-1	7.52 g	0.00074 g	0.228 g	centrate	110 mL	0.157 g	0.189 g
H_2SO_4	14.82 mL (0.558 M)	0.264 g	0.264 g				
	total input:	0.265 g	0.545 g		total recovery:	0.250 g	0.538 g

[a] Calculation of sulfur in a coagulation system consisting of blood, sodium lignosulfonate (PRA-1), and H_2SO_4 (pH 3.5). Summary relationship: total S(complex) = total input S - total S(centrate).

concentration of either aluminum ion or ferric ion (as $FeCl_3$) at the effective coagulant dose of each, when the dilution factors are taken into account. Anhydrous ferric chloride quantitatively removes protein at 20 g/L and pH 4.5 from blood diluted 5-fold, whereas anhydrous aluminum sulfate is effective at pH 4.5 at 30.4 g/L (Table I). Thus, a concentration corresponding to 0.62 mol of ferric ion/L of undiluted blood (from $FeCl_3$) and 0.54 mol of aluminum ion [from $Al_2(SO_4)_3$] per L of undiluted blood is required to effect quantitative protein removal. However, ferric ion as ferric sulfate is effective at a concentration of 0.225 mol/L of undiluted blood at pH 4.5. So that anion effects could be fully compared, experiments performed using $AlCl_3$ (data not shown) indicated that 0.65 mol of aluminum ion per L of undiluted blood is required to effect quantitative protein removal. Sulfate anion better stabilizes protein structure than chloride ion and is therefore presumably more effective in the salting out of the protein (Haschemeyer and Haschemeyer, 1973). However, why the sulfate effect is so much more pronounced in the case of ferric sulfate vs. ferric chloride, as compared to aluminum sulfate vs. aluminum chloride, is unclear. Coprecipitation of the anions by metal hydroxide–proteinate species and, therefore, competition for metal ion may be involved (see discussion on weight of complexes), but it is not clear why ferric sulfate would be much less subject to such an effect.

The analysis of complexes containing sodium lignosulfonate (PRA-1) and protein presented analytical problems arising from the difficulty in distinguishing contributions to the complex by the two components. PRA-1 is a commercial product derived from the sulfite pulping of wood. The commercial preparation is initially an ammoniated product that is later converted to the sodium salt, and is supplied as a nominal 50% w/w solution. Standard biuret assays showed that (on a dry weight basis), PRA-1 gave an absorbance of about 75% that of an equivalent weight of purified porcine albumin. Since minimal interference to this assay is caused by ammonium ion (Cooper, 1977), the presence of higher molecular weight nitrogen containing compounds in PRA-1 is indicated. Experiments performed by using the dye binding protein assay of Bradford (1976) indicate that PRA-1 (on a dry weight basis) yields an absorbance of about 4% that of an equivalent weight of porcine albumin. However, a standard protein nitrogen analysis of the commercial PRA-1 preparation using 10% Cl_3AcOH indicates that, in spite of a significant Kjeldahl nitrogen level (700 mg of N/L), no Cl_3AcOH-removable protein is present. This result suggests that lignosulfonate or other components in the PRA-1 preparation affect the dye binding assay. Taken together, the above data support the hypothesis that the nitrogen compounds present in PRA-1 are neither high molecular weight (i.e., protein), nor ammonium ion components. Thus, the commercial preparation presumably contains substantial levels of materials that are not lignin derived, since pure lignosulfonate should be free of nitrogen.

Due to the likelihood that the contaminating components in the PRA-1 preparation affect both biuret and dye binding assays, we chose not to use these to characterize the composition of the complexes. Kjeldahl nitrogen analysis could only be used to characterize the protein level in the complexes on the assumption that none of the Kjeldahl nitrogen present in the PRA-1 preparation coprecipitates with the PRA-1 protein complex. Although there is good reason to suspect that this is the case (see below), we sought to verify this by an independent method (sulfur analysis) that allows the direct determination of PRA-1 in the complexes.

Sulfur analysis allows a delineation of sulfur contributed to the system as PRA-1 sulfur, blood sulfur, and sulfate sulfur from H_2SO_4. Virtually all of blood sulfur is potentially removable by protein coagulants since it is removed (>99%) by standard 10% Cl_3AcOH treatment of whole blood. The trace level of nonremovable sulfur is contributed mostly by sulfate (Federation of American Societies for Experimental Biology, 1961). The sulfate level of PRA-1 is also low, representing only about 0.31% of the total sulfur present in the commercial preparation. Thus, since 99.7% of the sulfate contributed to the system is derived from H_2SO_4, sulfate contributed by blood or PRA-1 may be considered to be negligible during calculations. By using sulfur analysis we were able to account for 98.7% of all input sulfur in the coagulation system.

Treatment of the centrate, produced by PRA-1 coagulation of blood at a concentration of 20.7 g/L and at pH 3.5, reveals that no components (protein) are present that can be removed by 10% Cl_3AcOH. Since none of the Kjeldahl nitrogen present in PRA-1 itself is removed by 10% Cl_3AcOH, our present data suggest that the high residual Kjeldahl nitrogen observed previously (Vandegrift and Ratermann, 1979) in the effluent from PRA-1 treatment of blood is due principally to Kjeldahl nitrogen contributed by the PRA-1. Furthermore, the total residual nitrogen remaining after treatment of blood with PRA-1 approximates the total of the Kjeldahl nitrogen contributed by PRA-1 and the nonprotein nitrogen of the blood. Thus, we conclude, in contrast to our earlier report (Vandegrift and Ratermann, 1979), that under optimal conditions PRA-1 quantitatively removes protein from animal blood.

Average calculations resulting from sulfur analysis of PRA-1 treatment of blood are displayed as Tables II and III. The summary relationship, total S(complex) = total S(input) – total S(centrate), is illustrated in Table II, as are the distribution data between centrate and dry complex. Complexes prepared by PRA-1 treatment of whole blood contain, in addition to other components, 86.4% by weight of the sulfur originally present in the lignosulfonate preparation (Tables I and III). Since this value is based on weighed amounts of input PRA-1 and whole blood, and known amounts of added sulfate (from H_2SO_4), it is independent of the percentage of lignosulfonate present in the PRA-1 sample. To determine the percent lignosulfonate in PRA-1, we used as a standard a lignosulfonate

Table III. Distribution of PRA-1 in Complex and Centrate[a]

I.	centrate	
	total sulfur recovered, g	0.189
	sulfate sulfur, g	0.157
	PRA-1 sulfur, g	0.032
II.	dry complex	
	total sulfur recovered, g	0.349
	sulfate sulfur, g (0.093) + whole blood sulfur, g (0.053)[b]	0.146
	PRA-1 sulfur, g	0.203
III.	% of PRA-1 used found in dry complex: [0.203/(0.203 + 0.032)] × 100 = 86.4%	
IV.	composition of complex	
	lignosulfonate: [(0.864)(7.52)(0.372)[c]/12.12] × 100 = 19.9%	
	sulfate: [(0.093)(3)[d]/12.12] × 100 = 2.3%	
	blood protein[e] and other components: [(12.12 − 2.41 − 0.279)[f]/12.12] × 100 = 77.8%	

[a] Determination of the distribution of lignosulfonate sulfate, protein, and other components in complexes produced by PRA-1 treatment of blood at pH 3.5 (H_2SO_4). Based on 12.12 g of complex prepared by treatment of 50 mL of whole blood (30 000 mg of N/L) with 7.52 g of PRA-1. [b] All blood sulfur is assumed to be part of the complex. [c] PRA-1 contains 37.2% lignosulfonate (based on the Folin-Denis assay). [d] Conversion factor (SO_4^{2-}/S). [e] Data indicate 11.5% N × 6.25 = 72.2% protein (Table I). [f] The calculated weight of lignosulfonate (2.41 g) and sulfate (0.279 g) in the complex.

sample (CBOS-6) which was devoid of Kjeldahl nitrogen. This sample was a highly purified, low molecular weight lignosulfonate, which could not be used effectively to coagulate protein (Detroit, 1980). On the basis of a standard Folin–Denis assay, the PRA-1 sample contained 37.2% lignosulfonate by weight. The complexes were then calculated to be 19.9% lignosulfonate (Table III). Converting the nitrogen value to protein (11.55% N × 6.25) indicates that the complex contains about 72.2% protein (Table I). The remainder of the solids in the complexes are sulfate (2.3%) and presumably other counterions (see discussion below) and nonlignosulfonate solids present in PRA-1.

Results of analyses performed on the protein–coagulant complexes generated by chemical coagulation of whole animal blood with selected agents are displayed in Table III. Each coagulant was used at the optimum conditions of pH, dilution, and concentration previously characterized. The water content of the complexes ranged from 75% of the total weight (SPP) to 85% (zinc sulfate or ferri-floc). It is important to note that actual water retention within the complex is somewhat less, since some water remains physically entrapped by the precipitate as the centrate is decanted following centrifugation and since the complexes are prepared by centrifugation at only 3000*g*.

Of all the complexes characterized, those involving zinc contain the greatest nitrogen content (13.83%) and the lowest coagulant (2.65% Zn) content (Table III). About 95.1% of input coagulant (as Zn^{2+}) is present in the Zn^{2+}–protein–coagulant complex. The high nitrogen content of the complexes is reasonable, since on a dry weight basis, the required amount of coagulant is lower for zinc sulfate than for any of the other coagulants used. Only 0.084 mol of Zn^{2+} (as $ZnSO_4$)/L of undiluted blood are required for quantitative protein removal. The high percentage of nitrogen is also reflective of the lower dry weight of the complex produced by zinc sulfate coagulation per unit amount of blood.

The low percentage nitrogen values for aluminum sulfate (8.91%) and ferric chloride (10.28%) complexes and the lower than predicted percent metal composition (12.2% Fe, 4.6% Al) can be explained by an examination of the weight of the complexes. If the weight of the lowest weight complex produced per unit amount of blood is taken to be 1, then the approximate weight ratios are as follows: aluminum sulfate, 1.5:1; $FeCl_3$, 1.5:1; $Fe_2(SO_4)_3$, 1.2:1; $ZnSO_4$, 1:1 (Table IV). A theoretical weight and percent metal ion content can be calculated for each complex and compared to the experimentally determined values as follows (by assuming quantitative binding of metal ion by the protein and an original volume of whole animal blood containing 10 g of protein):

$$W = fcL + 10(\text{grams of protein})$$

$$M = [(W - 10)/W] \times 100$$

where W is the weight of the complex in grams, M is the percent metal ion in the complex, f is the fractional abundance expressed in decimal form of metal ion in the compound used in coagulation, c is the final coagulant concentration in grams per liter, and L is the final volume in liters. These values (for the metal ion–protein complexes) are displayed in Table IV. For zinc sulfate and ferric sulfate coagulants, the theoretical values agree very well with those values obtained through experiment. Ferric chloride and aluminum sulfate complexes, however, show significant deviation, principally as a result of the fact that the complexes produced from these coagulants have a significantly increased dry weight. The increased weight of the two complexes, coupled with the low values of nitrogen and coagulant ion composition, suggests significant contributions to the dry weight of the complexes by agents other than protein or metal ion. That this weight increase is not due to organic material is indicated by the comparable total organic carbon levels remaining in the centrates after treatment with any of the metal coagulants (Vandegrift and Ratermann, 1979; Ratermann et al., 1980). Thus, the weight of complex produced per unit of blood may be affected by the amount of associated ion (e.g., Na^+, Cl^-, or SO_4^{2-}) removed from solution with the coagulant used. It is interesting to note that $FeCl_3$ and $Al_2(SO_4)_3$ are the least effective coagulants on a weight basis, as well as in terms of moles of metal ion per liter of undiluted whole blood.

Complexes prepared from SPP treatment of blood contain 12.63% nitrogen and 9.40% phosphate. Of all the coagulants tested, SPP is the only one for which less than 80% (78.9%) of the input (as phosphate) is removed with the complex. Ferric sulfate (as ferri-floc) treatment of

Table IV. Dry Weight, Metal Ion Content, and Nitrogen Composition of Selected Complexes[a]

coagulant	f	W(calcd), g	W(expt), g	M(calcd), %	M(expt), %	N, %
$ZnSO_4 \cdot 7H_2O$	0.228	10.3	9.8	2.7	2.6	13.8
$FeCl_3$	0.344	11.7	14.4	14.7	12.2	10.3
$Fe_2(SO_4)_3 \cdot 4H_2O$[b]	0.237	10.7	11.5	6.6	6.4	11.7
$Al_2(SO_4)_3 \cdot 16H_2O$	0.086	10.7	14.9	6.7	4.6	8.9

[a] Comparison of calculated dry weight (W), metal ion content (M), and nitrogen composition (percent N) of complexes produced by treatment of blood containing 10 g of protein with experimental results. f represents the fractional abundance expressed in decimal form of the metal ion in the compound used for coagulation. The final volume of treated blood was 0.15 L, except for $FeCl_3$ which was 0.25 L. [b] Added as ferri-floc (Cities Service Co., Atlanta, GA).

blood produces complexes that contain 11.67% N and 6.39% Fe. Almost 97% of the input iron ion is found associated with the complexes.

These results indicate that all of the coagulant–protein complexes studied form under conditions that require only a small excess of coagulant and that the complexes contain low percentages of heavy metals or nutritionally beneficial (sodium polyphosphate) or nontoxic (sodium lignosulfonate) protein coagulants. Thus, several of the coagulants tested may be feasible for industrial use in the "cold" processing of animal blood. Pilot projects designed to test utilization of the coagulants on an industrial scale, followed by feed testing of these complexes blended as a part of animal diets, are necessary. Results of these tests should enable industrial packers to evaluate the digestibility, amino acid availability, safety, and economic advantages or disadvantages of cold blood processing relative to conventional methods.

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Antioxidant Properties of Synthetic 5-Hydroxy-1,3-benzodioxole Derivatives

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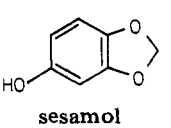
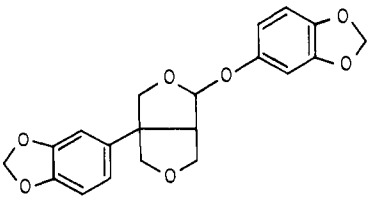
The sesamol analogues 2,2-disubstituted-5-hydroxy-1,3-benzodioxoles all show greater antioxidant effects than the parent sesamol. Cycloalkyl-substituted compounds are the most efficient, but alkyl derivatives are also good antioxidants with optimum activity at C_7. Protection is shown to a variety of lipid substrates, with best effects observed for lard. The most efficient analogues are comparable in activity to butylated hydroxyanisole and propyl gallate.

In spite of their proven utility, remarkably few antioxidants have been approved for use in foods. Stringent toxicity testing has limited the compounds permitted and has eliminated several formerly important commercial antioxidants such as nordihydroguairaretic acid (NDGA). There is a continuing need for new agents which will be highly effective without undesirable side effects.

Sesamol (5-hydroxy-1,3-benzodioxole), which occurs naturally in sesame oil (Budowski and Markley, 1951) both free and in bound form as sesamolin (Beroza, 1954, 1955),



sesamol



sesamolin

is responsible for the crude oil's considerable resistance to oxidation. The oil's resistance to autoxidation has been accurately correlated with the sesamol content (Budowski, 1950; Budowski et al., 1950). This property of the oil has been overshadowed by its insecticidal synergistic effects.

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