(NOE) is −3.93 (Witanowski and Webb, 1973). The signal to noise (S/N) enhancement will be maximum if dipole–dipole interactions dominate the relaxation mechanism. If the dipole–dipole interactions are not dominant, the NOE enhancement factor will range from 1 to −3.93, proportional to the magnitude of the dipole–dipole interactions' contribution to the relaxation mechanism. In general, a single bond dipole–dipole interaction gives rise to maximum NOE enhancement. In Figure 2, the α- and ϵ-nitrogen would be expected to have identical intensities as they do in Figure 1. The α-nitrogen signal, however, is only half that of the ϵ-nitrogen. The binding of lysine in the polymer may alter the importance of the various relaxation mechanisms. The ϵ-nitrogen bound in the matrix has intramolecular dipole–dipole interactions, whereas the α-nitrogen has only the intermolecular interactions which are limited in the polymer. These NOE differences are useful for deducing structural changes but make quantitation difficult. Numerous other peaks occur downfield from the urea reference and probably are from urea substituted with methylene derived from formaldehyde. No assignments have been made yet, but one could speculate that the peaks occurring at 0.4 and 1.0 ppm are due to unsubstituted urea nitrogen resonances. The peaks between 4.9–5.6 and 18.7–20.6 ppm likewise could be assigned to mono- and disubstituted urea nitrogen resonances, respectively, since substitution of electron donors larger than methyl is known to cause downfield displacement of the nitrogen resonance in ureas and amines (Witanowski et al., 1973).

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Chemical Preservation of Protein in Industrial Whole Animal Blood

Fresh industrial whole animal blood samples obtained from a packer were found to be low in nonprotein nitrogen (~500 mg of N/L) but were contaminated by microbes. Industrial blood samples were incubated for 48 h at 35 °C in the presence of putative chemical preservatives, and the nonprotein nitrogen levels of the samples were compared to those of controls. Chemicals tested were sodium bisulfite, sodium polyphosphate, succinic acid, propyl gallate, benzoic acid, D-isoascorbic acid, propionic acid, sorbic acid, sulfuric acid, acetic acid, and phosphoric acid. Sodium bisulfite or sodium polyphosphate added as solids without pH adjustment to a final concentration of 0.8–1.0 g/100 mL was most effective in preventing blood protein degradation to nonprotein nitrogen. Phosphoric acid added to a final concentration of 0.7 g/100 mL, acetic acid (0.5 g/100 mL), propionic acid (0.75 g/100 mL), and succinic acid (0.75 g/100 mL) were also effective blood protein preservatives.

Fresh whole animal blood, derived from bovine and porcine species, is characterized by low nonprotein nitrogen levels relative to the total nitrogen content. Blood samples obtained from renderers are often high in nonprotein nitrogen and ammonia levels, reflecting considerable degradation of blood protein (Vandegrift and Ratermann,

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1979). Blood protein degradation presents an economic loss for the meat packer and renderer since degradation results in a loss of protein ordinarily recovered for use as an animal feed supplement. In addition, the low molecular weight nonprotein nitrogen compounds produced during protein degradation are not easily recoverable and thus contribute to the biochemical oxygen demand of the resulting waste water.

The use of open vessels to collect and store animal blood after slaughter, and prior to treatment, promotes contamination of the blood by bacteria, thus enhancing protein degradation. However, more hygienic forms of blood collection are not always economically feasible. The chemical preservation of blood offers an economically attractive solution to the problem of blood stabilization. Akers (1973) has suggested that sodium metabisulfite at a 1% final concentration be used at pH 3.2 to preserve blood for several days. Patgiri and Arora (1978) have shown that the addition of 1 part of CaO to 8 parts of fresh blood produces a blood meal which is significantly devoid of microbial load.

In the work reported here, several chemicals were screened for effectiveness as blood protein preservatives. Our approach was to choose compounds which are listed as being generally recognized as safe (GRAS) for animal feeds (Association of American Feed Control Officials, 1976). Sodium bisulfite, which is not listed as GRAS, was chosen for comparison because of its established ability to act as a blood preservative (Akers, 1973). Many of the chemicals tested are used as preservatives, presumably because of their microbicidal or microbiostatic properties. Our results suggest that sodium bisulfite, sodium polyphosphate, or simple pH adjustment of blood using phosphoric, acetic, or propionic acids may act to preserve blood samples for up to 2 days.

MATERIALS AND METHODS

Sodium bisulfite, sodium polyphosphate, succinic acid, propyl gallate, benzoic acid, D-isoascorbic acid, and propionic acid were reagent-grade chemicals obtained from Sigma Chemical Co. (St. Louis, MO). Sorbistat (sorbic acid) was obtained from Pfizer Inc. (New York, NY). Glacial acetic acid and phosphoric acid were reagent grade and were obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). Sulfuric acid was reagent grade and was obtained from Fisher Scientific Co. (Fair Lawn, NJ). Industrial whole blood samples were obtained from the Emge Co. (Fort Branch, IN). The blood samples contained 80 ± 1% water by weight.

Industrial blood samples were used to investigate the ability of several compounds to act as blood preservation agents. Blood samples, with and without putative preservatives, were incubated at 35 °C for 48 h. Chemical preservatives were normally added as liquids (those compounds which are commercially available as liquids were added without dilution) or as solids with vigorous agitation to ensure uniform distribution in the blood samples. All preservatives were added to produce a specific weight of pure chemical to volume of whole blood ratio. No pH adjustments were made following addition of the compounds to blood. Samples were not agitated during the incubation period. All samples were incubated in marble capped tubes under conditions which resulted in minimal evaporation of water from the blood. All experimental data represent an average of four independent experiments, with a variation of ±35 mg of N/100 mL. The blood was analyzed for nonprotein Kjeldahl nitrogen after incubation as described previously (Vandegrift and Ratermann, 1979). Nonprotein nitrogen levels represent the total Kjeldahl nitrogen remaining in a sample after all protein has been extracted with 10% trichloroacetic acid.

Table I. Comparison of Protein Preservatives for Treatment of Whole Animal Blood

compd	final concn, g/100 mL	nonprotein N, exptl[a]	effectiveness
(A) Compounds Added as Solids			
succinic acid	0.30	1245	poor
	0.40	1060	poor
	0.50	680	poor
	0.60	260	moderate
	0.75	150	very good
isoascorbic acid	0.10	1145	poor
	0.20	950	poor
	0.30	710	poor
sorbic acid	0.15	815	poor
	0.20	770	poor
	0.25	450	poor
benzoic acid	0.10	1000	poor
	0.20	970	poor
	0.30	995	poor
propyl gallate	0.20	1080	poor
	0.25	425	poor
	0.30	240	moderate
	0.35	260	moderate
sodium polyphosphate	0.60	260	moderate
	0.70	150	very good
	0.80	140	very good
	0.90	135	excellent
	1.0	125	excellent
sodium bisulfite	0.60	260	moderate
	0.70	220	moderate
	0.80	140	very good
	0.90	140	very good
	1.0	135	excellent
control, no addition	0	865	
(B) Compounds Added as Liquids			
phosphoric acid	0.50	185	good
	0.60	180	good
	0.70	150	very good
acetic acid	0.30	300	moderate
	0.40	300	moderate
	0.50	150	very good
propionic acid	0.50	180	good
	0.60	170	good
	0.75	155	very good
control, no addition	0	865	

[a] All nonprotein nitrogen values are in mg of N/100 mL. Each value is an average of four independent experiments. Nonprotein nitrogen levels of fresh whole blood prior to incubation for 48 h at 35 °C was 50 mg of N/100 mL.

RESULTS AND DISCUSSION

Animal blood samples obtained fresh from a packer have acceptable levels of nonprotein nitrogen (~500 mg of N/L) but are contaminated by microbes. Inoculation tests using blood obtained from a packer gave positive tests for bacterial growth on blood agar plates. Microbial inoculation brings about the degradation of blood rapidly, as compared to that of blood samples obtained under sterile conditions. The degradation of blood protein may be monitored by observing the increase in nonprotein nitrogen levels after 48 h of incubation at 35 °C. Inhibition of blood protein degradation, as a result of the addition of chemical compounds, may be taken as indirect evidence that the added

compounds act as microbicidal or microbiostatic agents.

The final concentrations of solids used to treat blood were chosen according to their solubilities in undiluted blood. Sorbic acid, D-isoascorbic acid, and benzoic acid added without pH adjustment to the blood were not effective as preservatives [Table I (A)]. Propyl gallate displayed moderate effectiveness at a concentration of 0.35 g/100 mL. Of the solids screened in this investigation, only succinic acid (0.75 g/100 mL) and sodium polyphosphate (sodium hexametaphosphate) or sodium bisulfite (0.8–1.0 g/100 mL) are effective. We have performed experiments (data not shown) on a wide variety of dilution conditions with several blood samples, and we conclude that the use of sodium polyphosphate or sodium bisulfite provides the cheapest and most effective form of blood preservation. However, neither sodium polyphosphate nor sodium bisulfite completely prevents the degradation of blood protein. At effective doses of 0.8–1.0 g/100 mL for sodium polyphosphate and sodium bisulfite, the nonprotein nitrogen level of the blood increases ~250% compared to the nonprotein nitrogen level of fresh blood. At very low concentrations of succinic acid, there appeared to be an increase in the breakdown of blood protein compared to that of the control. The mechanism by which low concentrations of a succinic acid may stimulate blood protein breakdown is not clear.

The enhanced effectiveness of succinic acid at higher concentrations may reflect a pH effect. Sodium succinate, when tested at concentrations up to 1.4 g/100 mL, was not an effective blood preservative (data not shown). In order to determine whether pH alone can preserve blood protein, we incubated blood with varying concentrations of the inorganic acids H_2SO_4 and H_3PO_4 and the organic acids acetic acid and propionic acid. Even at moderate concentrations these acids cause blood to congeal at about pH 4.0. In some cases the blood does not congeal immediately but does so after some period of incubation. Thus, the problem of blood coagulation necessitates constant stirring when adding acid to produce a pH below 4.0 (Akers, 1973). Although packers may be able to stir blood continuously at collection, renderers must often wait for delivery of blood which has been stored without stirring for up to 48 h. In our experiments, we arbitrarily discarded from consideration as a blood preservative any acid (or other chemical) which caused clumping or congealing of blood during 48 h of incubation, without stirring, at 35 °C.

Acetic acid (99.5% w/v), phosphoric acid (85% w/v), sulfuric acid (96%), and propionic acid were added directly to whole blood to determine their effectiveness as blood preservatives. Over a 48-h incubation period, no tested concentration of H_2SO_4 (0.5 g/100 mL or greater), added as an undiluted acid, is an effective blood preservation agent which does not cause blood to congeal. Phosphoric acid, at a final concentration of 0.7 g/100 mL, is a very good blood preservative as are acetic acid (0.5 g/100 mL) and propionic acid (0.75 g/100 mL) [Table I (B)].

In summary, due to the problems inherent in using acids and the apparent unacceptability of sodium bisulfite, we recommend that sodium polyphosphate be used to preserve raw industrial animal blood for periods up to 2 days before processing into animal feeds.

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Synthesis of *O,O*-Dimethyl 2,2-Dichloro-1-(acyloxy)ethenephosphonates, Major Constituents in Acylated Trichlorfons

In the synthesis of acylated trichlorfons, the dehydrohalogenated compounds are the major contaminants. Six of the compounds were synthesized by using 1,5-diazabicyclo[5.4.0]undec-5-ene (BDU) as the dehydrohalogenation reagent. The compounds were synthesized for structural confirmation and for assessment of their contribution to the toxicity of the parent compounds that are used as experimental insecticides on forest insect pests.

Acyl trichlorfons (I) enjoy some success in insect control (Casida and Arthur, 1959; Pieper and Richmond, 1976). They have superior solubility properties in ordinary solvents compared with the nonacylated trichlorfon and their low mammalian to high insect toxicity ratios are environmentally advantageous in forest applications. In a study with a homologous series of acyl trichlorfons, it was found that their syntheses were accompanied by large amounts (10% or more) of impurities with absorption in the 6.32-μm region of the infrared spectrum. These impurities were identified as the dehydrohalogenated acyl trichlorfons, *O,O*-dimethyl 2,2-dichloro-1-(acyloxy)ethenephosphonates (III) and their structural verification by synthesis (Figure 1) is described.

The impurities were difficult to separate from the acylated trichlorfons, especially in the scale needed in an experimental spray operation. Dehydrohalogenated compounds can be removed from acylated trichlorfons by vacuum distillation; however, the acylated trichlorfons undergo extensive decomposition in the process.