

Association of Synthetic Anionic Polymers with Gluten

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ABSTRACT

Synthetic anionic polymers—sodium polyvinyl sulfate (Na-PVS), sodium polyacrylate (Na-PA), and polyvinyl acetate sodium *s*-sulfonate (Na-PVSSO₃)—were used in this study. The precipitates, obtained by mixing dispersions of wheat gluten with Na-PVS and Na-PVSSO₃ in various proportions, contained approximately a fixed amount of nitrogen regardless of mixing ratios. Na-PA showed some results that differed from the others. The farinograms of dough mixed with Na-PVS in equivalent amounts to the total positive charge of dough at pH 4.8 and 4.3 showed a distinct difference from those of the control dough at the same pH levels. However, they were similar to the farinogram of dough at pH 5.8 where the total net charge was negligible. The result obtained with Na-PVSSO₃ was identical. Extensigrams of dough mixed with Na-PA, Na-PVS, and Na-PVSSO₃ showed a marked increase in resistance and decrease in extensibility in comparison with the control dough. From these results, the association of these polymers with gluten was postulated to be brought about by a saltlike linkage between positive and negative charges.

The physical properties of dough have been studied by many workers from the point of view of the sulfhydryl-disulfide exchange reaction. However, dough may also be considered as a polyelectrolyte, since the electric charge is assumed to be one of the elements affecting its properties. The effect of pH on dough properties reported by the authors (1) indicated the important role of ionized groups of the proteins as well as of the SH-SS system.

The stoichiometric reaction between oppositely charged groups of polymers, such as Na-polyvinyl sulfate (Na-PVS) or chitosan • HCl salt, has been used by Terayama as a basis for his colloid titration (2); this titration was applied to wheat protein by Yoshino and Matsumoto (3). This approach may prove useful for quantitative research on the association between gluten and anionic polymers.

Glabe *et al.* (4,5) have reported on the effects of carrageenan, an extract from Irish moss, on dough and bread. The interaction between polysaccharides and gluten was also reported by Udy (6) and by Wilham *et al.* (7), and in a similar paper Hamer (8) dealt with the reaction of nucleic acid, heparin, dextran sulfate, alginic acid, and bacterial polyglutamic acid with bovine plasma albumin. These papers are cited because the polysaccharides or polymers used have anionic groups such as sulfonate, sulfate, and carboxylate in their molecules.

In another study on the association of proteins with synthetic anionic polymers, Morgan (9) introduced sulfonated polystyrene as a reagent for stripping protein from yeast ribosomes. Morawetz and Hughes (10) studied the precipitation of bovine serum albumin by synthetic polyelectrolytes such as polymethacrylic acid, methacrylic acid-vinylpyridine copolymer, polyvinylamine hydrobomate, maleic anhydride-styrene copolymer, and methacrylic acid-diethylaminoethylmethacrylate copolymer.

In addition to the discussion in these papers, it should be noted that anionic surfactants have an improving effect on the mixing properties of dough, as was shown by Sullivan (11), by Swanson and Johnson (12), and by Thompson and Buddemeyer (13). These workers indicated that wheat proteins may be denatured by a combination between cationic groups of the proteins and anionic surfactants.

The authors have utilized the observation that gluten dispersions are precipitated by anionic compounds, to examine more fully the association of synthetic anionic polymers with gluten. The effect of these polymers on the physical properties of dough provided a second, rheological, approach for the study. The results are summarized in this report.

MATERIALS AND METHODS

The flour used was a commercial blend of hard red spring and hard red winter wheats containing 0.38% ash and 11.8% protein at 14.0% moisture.

Sodium polyvinyl sulfate (Na-PVS) was synthesized by sulfonation of polyvinyl alcohol in a pyridine bath with chlorosulfonic acid according to the method of Terayama (2). The charged group of this compound was found to be 5.06×10^{-3} eq./g. by analysis of sulfur content.

Polyvinyl acetate sodium *s*-sulfonate (Na-PVSSO₃) was prepared by esterification of polyvinyl alcohol with monochloroacetic acid, followed by thio-sulfonation by the method of Izard and Morgan (14). The charge of this compound was 3.42×10^{-4} eq./g. at pH 4.0. The polyvinyl alcohol used as starting material for these compounds was purchased from Wako Pure Chemicals Co. and had a polymerization degree of 500.

Sodium polyacrylate (Na-PA) was a commercial product (Aronvis) provided by Nippon Pure Chemicals Co. The net charge was found to be 4.83, 5.29, 7.34, and 7.78×10^{-3} eq./g. at pH 4.0, 4.2, 4.8, and 5.2, respectively. The equivalent value of Na-PVSSO₃ and Na-PA was determined by colloid titration (3), with the use of standard chitosan.

Interaction between Gluten Dispersion and Synthetic Anionic Polymers

Wet crude gluten was obtained by centrifuging a flour slurry (flour:water ratio 1:2); it was collected as a coagulum which separated above the layer of starch in the centrifuge tube. This wet gluten was dispersed in a Waring Blender in 0.01*N* acetic acid at a concentration of 2.5%.

A solution of polymer which contained the same equivalent of charge per ml. as that of the gluten dispersion was prepared. This solution was mixed with the dispersion at the ratios shown in Table I, and the precipitates obtained were analyzed. The results are shown in this table.

RESULTS

The nitrogen contents in Table I, A and B, remained constant for each polymer regardless of the ratio of the components in the mixture. However, larger amounts of precipitate were obtained in the reaction when an equivalent amount or excess of polymer was used. From these results, the reaction

TABLE I
REACTION OF SYNTHETIC ANIONIC POLYMERS WITH GLUTEN DISPERSION

A Na-PVS, pH 2.65			B Na-PVSSO ₃ , pH 4.0			C Na-PA, pH 4.0		
Ratio of Equiva- lent ^a	Precipi- tate ^b	Nitrogen in Pre- cipitate	Ratio of Equiva- lent ^a	Precipi- tate ^b	Nitrogen in Pre- cipitate	Ratio of Equiva- lent ^a	Precipi- tate ^b	Nitrogen in Pre- cipitate
	%	%		%	%		%	%
4.2	16.7	15.1	4.9	15.3	14.6	2.2	1.4	8.2
2.2	35.7	15.7	3.2	23.6	14.3	1.5	1.0	5.8
1.1	64.7	15.1	1.6	47.5	14.3	0.3	3.4	11.1
0.7	76.1	15.0	0.8	88.7	14.3	0.4	21.9	11.1
0.5	89.1	14.6	0.5	68.4	14.3	0.2	88.6	13.2

^aGluten/polymer; calculated from the charge: gluten dispersion for exp. 1, 4.40×10^{-4} (pH 2.65); exp. 2, 2.92×10^{-4} (pH 4.0); exp. 3, 2.54×10^{-4} eq./g. (pH 4.0). Na-PVS 5.06×10^{-3} , Na-PVSSO₃ 3.42×10^{-4} , Na-PA 4.83×10^{-3} eq./g.

^bPercent of precipitated nitrogen per total nitrogen in the medium.

between polymer and gluten was presumed to proceed as an ionic neutralization between positive and negative charges. The neutralization of the charge seems to lead to gluten precipitation. As an exception, the nitrogen content of the precipitate with Na-PA (Table I, C) did not remain constant.

Effects of Synthetic Anionic Polymers on Farinograms of Dough

Flour was mixed in the farinograph at a fixed absorption of 61.5%. Figure 1 shows farinograms for a control dough and for doughs containing

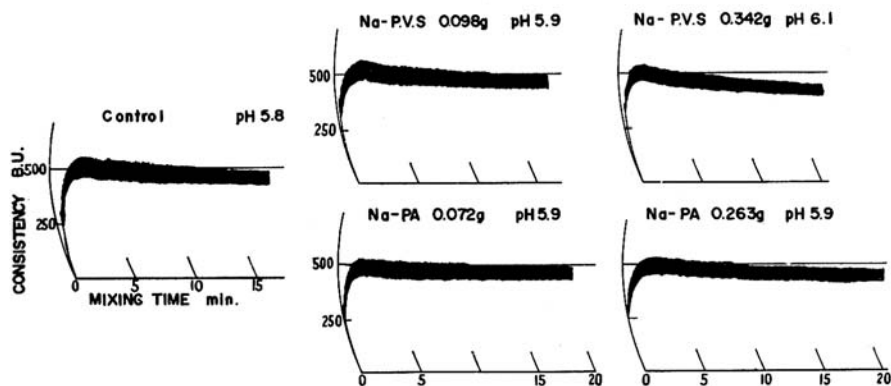


Fig. 1. Control experiment, farinograph, with synthetic anionic polymers without acid: Na-PVS, sodium polyvinyl sulfate; Na-PA, sodium polyacrylate. The number in the figure is g. incorporated per 300 g. flour. Absorption: 61.5% fixed.

Na-PVS and Na-PA in the same amounts as those used at pH 5.1 and 4.8 and shown in Fig. 2; i.e., the amount required to neutralize the net charge on dough at these pH values. A slight increase in the pH of dough containing the higher concentration of Na-PVS is shown.

Figure 2 shows farinograms for doughs with enough acetic acid to bring the pH to 5.2, 4.8, and 4.2 with the calculated amount of anionic polymer to contain a net charge equivalent to that of dough at a given pH value.

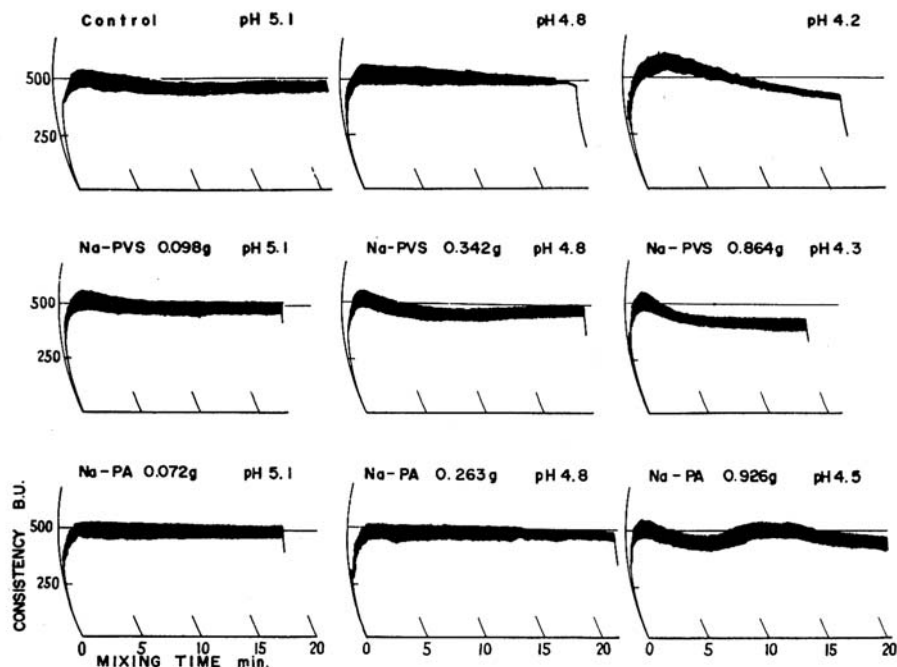


Fig. 2. Effects of synthetic anionic polymers (named in Fig. 1) on farinograms. Amount incorporated: equivalent to neutralize the net charge of dough at pH values employed, g. per 300 g. flour. The pH was adjusted with 1*N* acetic acid, 5 ml. (pH 5.1), 10 ml. (pH 4.8), and 30 ml. (pH 4.2) per 300 g. flour. Absorption: 61.5% fixed.

The characteristic increase in the farinograph consistency of dough by acid, which can be observed by comparing the control farinogram at pH 5.8 in Fig. 1 with those at pH 4.8 and 4.2 (upper row, Fig. 2), was reduced by addition of polymer as shown in the middle and bottom rows. Without acid, these polymers did not produce the marked effect on the farinogram at pH 5.8 to 6.1 as shown in the experiments (Fig. 1). Another phenomenon which should be noted is that the more viscous polymer (Na-PA) did not increase the consistency more than the less viscous one. This indicates a possible interaction between polymers and gluten. Na-PVSSO₃ caused a similar effect on the farinogram and extensigram (data not included in this paper).

Effects of Synthetic Anionic Polymers in Extensigrams of Dough

The flour was mixed to standard consistency, 500 B.U., with the same ingredients as were used in experiments with the farinograph. No salt was added to this dough. The dough was mixed for 1 min., allowed to react for 5 min., and again mixed for 3 min. Extension tests were done three times at 45-min. intervals for each sample, according to the routine method. In Figs. 3 and 4, only the extensigrams taken after 135 min. are shown.

Na-PVS and Na-PA increased extensigram resistance, especially at low

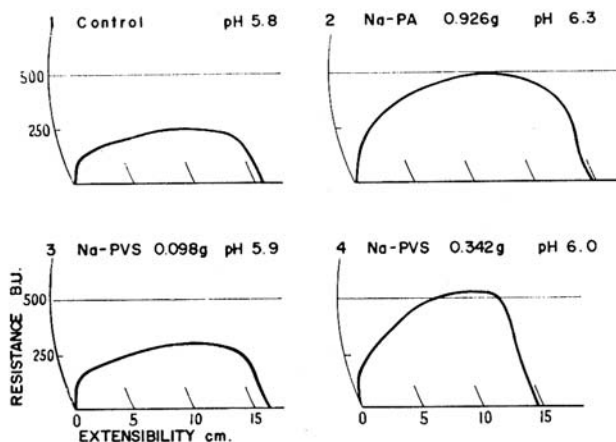


Fig. 3. Control experiment, extensigraph, with the synthetic anionic polymers without acid (named in Fig. 1). Absorption: (1) 60.8%, (2) 61.8%, (3) 60.8%, (4) 60.5%.

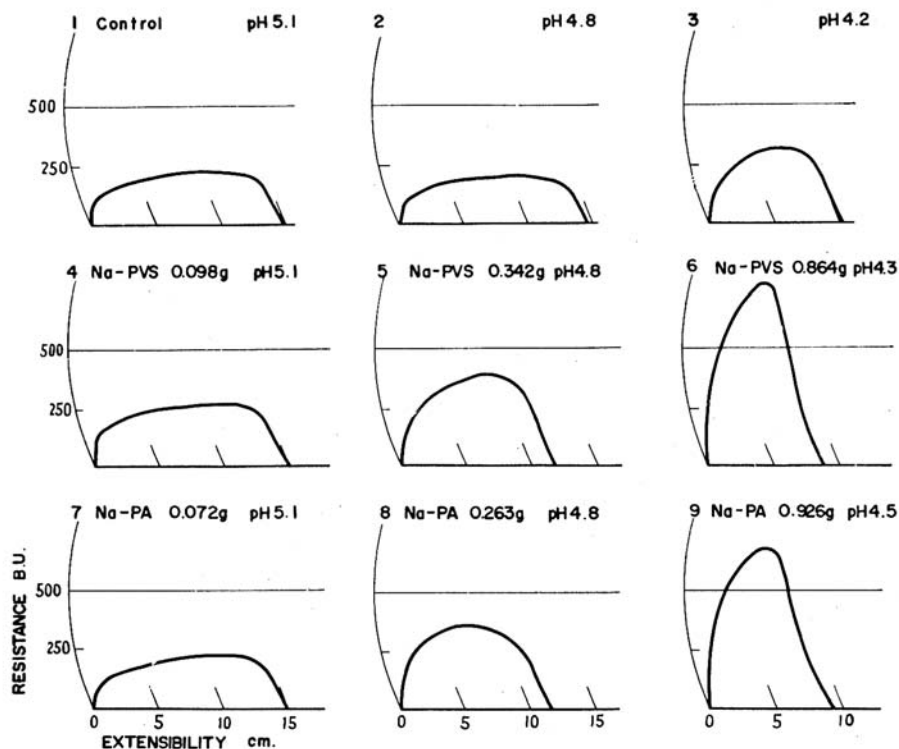


Fig. 4. Effects of synthetic anionic polymers (named in Fig. 1) on extensigram. Amount incorporated: equivalent to neutralize the net charge of dough at pH values employed, g./300 g. flour. The pH was adjusted with 1N acetic acid, 5 ml. (pH 5.1), 10 ml. (pH 4.8), 30 ml. (pH 4.2) per 300 g. flour. Absorption: (1) 60.5%, (2) 61.5%, (3) 63.1%, (4) 60.8%, (5) 56.3%, (6) 52.3%, (7) 60.5%, (8) 59.5%, (9) 60.5%.

pH values; however, they decreased the extensibility markedly under these conditions (see Fig. 4). Na-PVSSO₃ showed a similar effect to that of Na-PVS (data not shown). Both polymers caused an increase in resistance but without affecting extensibility when they were incorporated without acid in the range of pH 5.8 to 6.3 (Fig. 3).

DISCUSSION

The interaction between polymers (Na-PVS and Na-PVSSO₃) and wheat gluten dispersed in acetic acid seems to be brought about as an ionic combination between oppositely charged groups of both macromolecules. The precipitate contained approximately the same amount of nitrogen (Table I, A and B) as that calculated from the equivalence of anionic groups of polymers and cationic groups of gluten, independent of the ratio of the components in the mixture. However, one should also consider the fact that the nitrogen content of the precipitate is nearly the same as that of crude gluten (13.1%) used in this experiment. The crude gluten may possibly be reprecipitated under the influence of a small amount of anionic polymer. This possibility is supported by the finding of Hamer (8) that only several parts of a polyacid associate with 300 parts of albumin.

Nevertheless, the authors favor the interpretation that the precipitation occurs as a result of formation of a polymer-gluten complex which contains an amount of nitrogen similar to that of crude gluten as a strange coincidence. The following results also support this assumption: 1) Na-PVS was found to combine with gluten stoichiometrically as reported by the authors (3). 2) When the ratio of anionic polymer to gluten was increased, the total amount of precipitate increased (Table I). 3) The precipitate obtained by mixing anionic polymers and gluten is dissolved again when the pH is raised. A phenomenon similar to 2 and 3 was observed by Morawetz and Hughes (10) in the precipitation of bovine serum albumin with synthetic polyelectrolytes.

The results with Na-PA (Table I) differed from those with other polymers. This may be ascribed to the carboxyl groups which dissociate weakly.

The fact that precipitation seems to proceed with neutralization of opposite charges suggests that anionic polymers could function as an "improver"—as a reagent for modification of dough properties on the basis of quantitative considerations—that is, an equivalent amount of polymer to gluten with respect to their net charges, which may be determined by colloid titration. Thus the problem to be studied is whether the proper amount for dough improvement should be higher or lower than this level.

The positive charge of gluten increases as pH decreases (3), and the association of gluten with anionic polymers will proceed more effectively at low pH. Rheological tests were therefore carried out at a low pH.

In the farinograph test, anionic polymers decreased the consistency of dough at low pH. One should first consider the effect of the sodium ion dissociated from the polymer, since polymers are used as the sodium salt. How-

ever, the highest concentration of this ion from the polymer was calculated as 26.6×10^{-3} g. for Na-PVS and 34.2×10^{-3} g. for Na-PA, both per 100 g. flour, even assuming their complete dissociation. These amounts of the sodium ion may not affect the farinogram, as flour contains more than these levels. Thus, the anionic polymer itself is assumed to react with the dough in a way similar to that of salt, possibly through a combination with cationic groups of gluten, and may cause partial dehydration of swelled gluten.

The viscous and hygroscopic polymer incorporated in dough does not increase the consistency, but decreases or maintains it at a fixed value, at the same absorption. This is another reason for suggesting an interaction.

The dough at pH 4.8 and 4.2, whose net charge was neutralized to approximately zero with polymers (Fig. 2: Na-PVS 0.342 g. at pH 4.8 and 0.864 g. at pH 4.2), showed farinograms similar to that of the control dough which is at the isoelectric point.

With the extensigraph, the anionic polymer increased resistance and decreased extensibility, which is similar to the effect of an oxidizing agent and the effects of salt and acid (1). This implies that there is only a small capacity for yielding before the larger-scale molecular slip occurs, and that the large molecule of the polymer prevents dough from returning to its original extensigraph, even when the positive net charge is neutralized. While these properties of high resistance and low extensibility are also brought about by oxidizing agents, the modification by anionic polymers could be applied to dough improvement by adjustment of the amount to a suitable level.

The farinograms and extensigraphs of dough with Na-PVSSO₃ have characteristics identical with those with Na-PVS (not illustrated). Na-PVSSO₃ has a disulfide linkage in the molecule and may have specific affinity for the SH-SS system of proteins.

The experiments in this paper were carried out in the absence of salt in order to obtain clear results; however, data with salt would be of interest in future studies.

Acknowledgment

This research work was supported by funds of the U.S. Department of Agriculture, P.L. 480.

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[Received March 6, 1967. Accepted January 16, 1968]