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# Synthesis and Characterization of Poly(glutaraldehyde). A Potential Reagent for Protein Immobilization and Cell Separation

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**ABSTRACT:** The aldol condensation of aqueous glutaraldehyde in the pH range 7–13.5 yielded water-soluble and water-insoluble poly(glutaraldehyde) (PGL), the molecular weight of which was of the order of 12 to 20 000. The structure of PGL was elucidated by means of UV, IR spectrophotometry, electrochemical studies, and the analysis of reaction products of PGL with hydroxylamine hydrochloride. The structure of PGL polymers prepared in a wide pH range was found to be similar. The main differences consisted of changes in the concentration of functional groups. Sufficient evidence was obtained to explain the presence in PGL of the primary hydroxyl and of the carboxyl groups as due to a Cannizzaro reaction. Electrochemical studies confirmed the spectrophotometric evidence of the presence of conjugated aldehyde groups. Recent investigations of water-soluble or -insoluble PGL or of PGL in the form of microspheres indicate that these polymers may yield important immunoreagents for biological research.

Glutaraldehyde has many applications in different areas. It is used as a protein cross-linking agent,<sup>1</sup> for fixation of living cells<sup>2</sup> or tissues,<sup>3</sup> and for sterilization of hospital equipment;<sup>4</sup> it was also found to serve as an efficient binding agent of antibodies to microspheres.<sup>5</sup>

Glutaraldehyde was used in a variety of reactions for relatively long periods of time at physiological pH. It was only recently realized that under these conditions a considerable amount of glutaraldehyde is polymerized through the aldol condensation mechanism,<sup>6</sup> and many reported reactions of glutaraldehyde are actually reactions of glutaraldehyde as well as of poly(glutaraldehyde). Although a large number of studies were carried out in the past on the nature of aqueous glutaraldehyde,<sup>7–11</sup> little information is available on the structure of the solid aldol condensation product which was frequently considered to be an impurity associated with glutaraldehyde.<sup>12</sup> The present study showed that polymerization of glutaraldehyde under basic conditions results in the production of water-soluble and -insoluble polymers and that these polymers contain nonconjugated aldehyde, conjugated aldehyde, hydroxyl, and carboxyl groups. The weight ratio of the soluble to the insoluble polymer and the concentration of the functional groups were found to be dependent on the pH of the polymerization medium.

Poly(glutaraldehyde) might be used as a new reagent in protein chemistry and other areas. Only recently the polymer was found to constitute a valuable new reagent for the immobilization of antibodies on solid substrates.<sup>13</sup> Furthermore, a new method was developed for the preparation of poly(glutaraldehyde) in the form of microspheres.<sup>14</sup> The microspheres were used for cell labeling and cell separation and are suitable also for immobilization of enzymes, drugs, and proteins.

## Experimental Section

**(a) Reagents.** The following materials were acquired from commercial sources and were used without any further treatment: pyridine, phthalic anhydride, and hydroxylamine hydrochloride (Matheson Coleman and Bell), fluorescein isothiocyanate (Polysciences), tetraethylammonium perchlorate (Southwestern Analytical Co.), and ferrofluid (Ferrofluidic Co., Burlington, Mass.). Aqueous glutaraldehyde (Aldrich) was purified by treatment with activated carbon followed by filtration. Dimethylformamide (DMF), dimethyl sulfoxide (Me<sub>2</sub>SO) and crotonaldehyde were vacuum distilled.

**(b) Apparatus.** Infrared and UV spectra were obtained with a Fourier transform IR (FTS-15C, Houston Instruments) and a Cary 219 spectrophotometer (Varian), respectively.

Gel permeation chromatography (GPC) was carried out with the high-pressure liquid chromatography Model 6000, fitted with

a refractive index detector (Water Associates). The GPC was carried out with dimethylformamide as a solvent and a column of  $\mu$  styragel 10<sup>5</sup>, 10<sup>4</sup>, 10<sup>3</sup> Å (pore size); polystyrene was used for calibration.

Dupont thermal analyzer 900, Model 950 TGA, was used for thermogravimetric analysis.

Polarograms were obtained with a Princeton Applied Research (PAR) Model 174 polarographic analyzer and recorded with a Hewlett Packard Model 7004 x-y recorder. Cyclic voltammograms were obtained by means of a PAR Model 173 potentiostat driven by a conventional signal generator. The voltammograms were recorded with the x-y recorder. Controlled potential coulometry was studied with the PAR Model 173 potentiostat equipped with a Model 179 digital coulometer. The reference calomel electrode was isolated from the main cell compartment by means of a fritted glass disk. The auxiliary electrode was a platinum wire (diameter 0.076 cm) sealed in soft glass. The working electrode was a hanging mercury drop electrode.

**(c) Synthesis of PGL.** Glutaraldehyde was added to an appropriate deaerated buffer solution and then the mixture was placed on a mechanical shaker for 72 h. The white-yellowish precipitated polymer was filtered, washed with water, and then dried under vacuum at 45 °C. The mother liquor was dialyzed extensively against distilled water and lyophilized in order to obtain the soluble PGL in solid form. The synthesis of PGL microspheres was already described.<sup>14</sup>

**(d) Aldehyde Group Determination.** The aldehyde content of PGL was determined from the percent nitrogen of the oxime prepared by the heterogeneous reaction of PGL with aqueous hydroxylamine hydrochloride.<sup>15,16</sup> PGL (50 mg) was shaken at room temperature for 24 h with 500 mg of hydroxylamine hydrochloride. The polymer was then filtered, washed with water, and dried under vacuum at 45 °C. A similar nitrogen content was obtained when the reaction was carried out at 60 °C or at room temperature but at pH 6.0.

**(e) Carboxyl Group Determination.** The carboxyl content of the polymer in the salt form was determined by ashing the samples and in the acid form by titration of the polymer dissolved in DMF/H<sub>2</sub>O (1:1) with 0.3 M NaOH. Similar results were obtained when the titration was carried out in warm pyridine. The agreement between the ashing method and the titration method was of the order of 15%.

**(f) Hydroxyl Group Determination.** The determination of the hydroxyl content was based on the phthalation method.<sup>17–19</sup>

## Results

**(a) General.** Monoglutaraldehyde polymerizes slowly in an aqueous media of pH as low as 7, and the polymerization rate increases markedly with the increase of hydroxyl ion and monomer concentration as well as temperature.<sup>6,20</sup> The rate changes at high dilutions can be monitored by the near UV absorption spectra bearing in mind that side reactions may alter the absorption maxima. Monoglutaraldehyde absorbs strongly at 285 nm ( $\epsilon = 4.2$

Table I  
Yield of Insoluble and Soluble PGL as a Function of pH  
and Concentration of Monomer<sup>a</sup>

run no.	monomer concn, v/v %	pH	% conversion to	
			insoluble polymer	soluble polymer
1	50.0	10.5	45.0	
2	20.0	10.5	35.0	1.4
3	12.0	10.5	26.0	
4	5.0	10.5	10.0	
5	1.0	10.5	1.0	
6	12.0	11.5	23.0	
7	12.0	12.2	14.0	
8	12.0	12.5	4.0	
9	12.0	13.3	0.0	
10	20.0	13.5	0.0	15.0

<sup>a</sup> Polymerization in a buffer solution in a total volume of 100 cm<sup>3</sup> at 27 °C for 24 h.

Table II  
Carboxyl and Hydroxyl Content of PGL Prepared  
in the pH Range 9.3–13.3

pH	no. of carboxyl groups/g × 10 <sup>-20</sup>	no. of hydroxyl groups/g × 10 <sup>-20</sup>
9.3	<0.6	8.5
10.5	0.6	8.5
11.5	1.2	8.5
12.5	2.8	16.5
10.8 <sup>a</sup>	6.4	27.5
13.3 <sup>a</sup>	6.6	21.0
13.3 <sup>b</sup>	9.8	

<sup>a</sup> Soluble polymer, precipitated at pH 5.0 (polymerization in the presence of argon). <sup>b</sup> Soluble polymer, precipitated at pH 5.0 (polymerization in the presence of air).

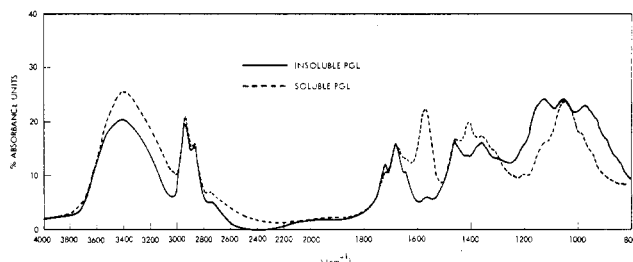


Figure 1. IR spectra of water-soluble and -insoluble PGL polymers (polymerization at pH 10.8 and 20% v/v glutaraldehyde).

× 10<sup>-2</sup> L g<sup>-1</sup> cm<sup>-1</sup>), and the condensation polymer exhibits an additional peak at 233 nm with a considerably higher extinction coefficient ( $\epsilon = 18.6$  L g<sup>-1</sup> cm<sup>-1</sup>).

The yields of water-soluble and -insoluble poly(glutaraldehyde) (PGL) as a function of pH and monomer concentration are shown in Table I. The yield of the PGL is considerably less than 100% since low molecular weight polymers are lost during dialysis. Investigations to be described below led to the conclusion that PGL prepared at different pHs although generally of similar structure contained a variable concentration of aldehyde, hydroxyl, and carboxyl functions as well as variable amounts of carbon carbon double bonds conjugated with the aldehyde groups. The water-soluble PGL was found to contain a larger number of carboxyl functions than the insoluble PGL, and the carboxyl content was still greater when the polymerization was carried out in the presence of air instead of argon (Table II). The IR spectrum shown in Figure 1 is characteristic of a number of IR spectra of water-soluble and -insoluble PGL polymers obtained at

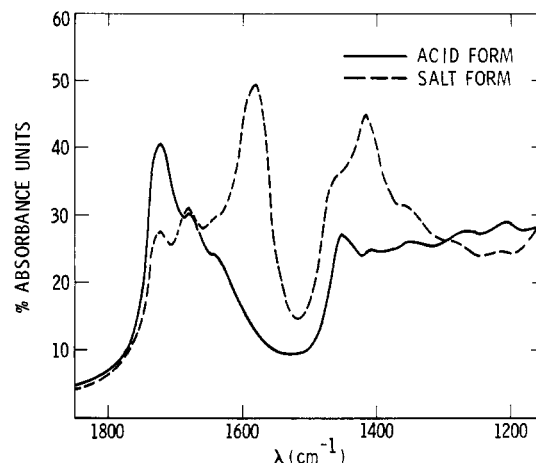


Figure 2. IR spectra of water-soluble PGL in salt and acid form (polymerization at pH 10.8 and 20% v/v glutaraldehyde).

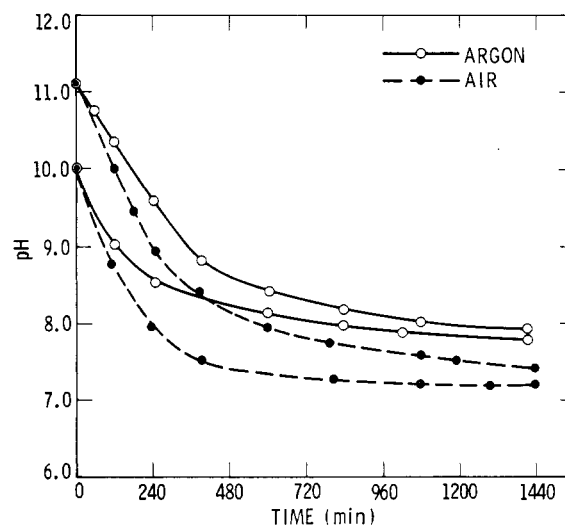
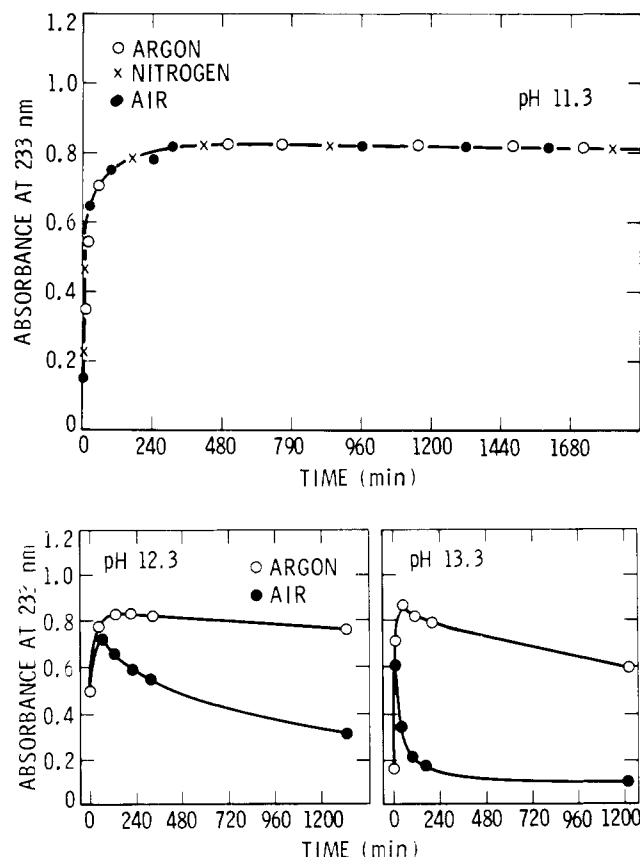


Figure 3. Change of pH during the polymerization of glutaraldehyde (glutaraldehyde 0.7% v/v, temperature 27 °C).

the initial pH between 7 and 12. The main difference between the two spectra is the intense peak at 1575 cm<sup>-1</sup> which was observed for the soluble polymer and is due to the presence of carboxyl functions in the form of sodium salts. By acidification of the soluble polymer the carboxylate peak at 1575 cm<sup>-1</sup> shifts to 1725 cm<sup>-1</sup> (Figure 2), and this fact confirms the assignment of the carboxylic acid group.<sup>29</sup>

Determination of pH as a function of time during the polymerization reaction revealed a gradual decrease in pH which was more pronounced when the reaction was conducted in the presence of air than in the presence of argon (Figure 3). The decrease of pH is due to the formation of carboxylic groups because of a Cannizzaro reaction and air oxidation (see Discussion).

PGL is soluble in DMF and polymerization in the latter results in a homogeneous solution. The structures of PGL whether obtained from water or DMF appear identical as judged from their IR spectra and elemental analysis. The intrinsic viscosity and the molecular weight distribution determined by gel permeation chromatography (GPC) for the polymers prepared in different pHs were found to be approximately the same. The intrinsic viscosity was about 0.1 dL/g, and the molecular weight derived from GPC is between 12 000 and 20 000. When heated in N<sub>2</sub> at a rate of 5 °C/min it undergoes a weight loss of approximately 3.6% in the temperature range 75–160 °C and regains most of the original weight when exposed to air. Between 160

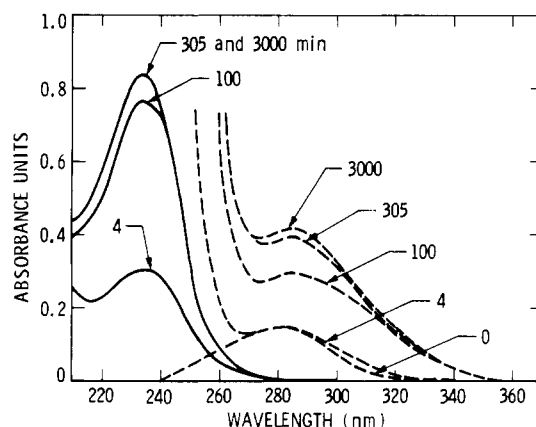


**Figure 4.** Change in absorbance at 233 nm during the polymerization of glutaraldehyde at pH 11.3 (glutaraldehyde 0.7% v/v, temperature 27 °C) 12.3 and 13.3.

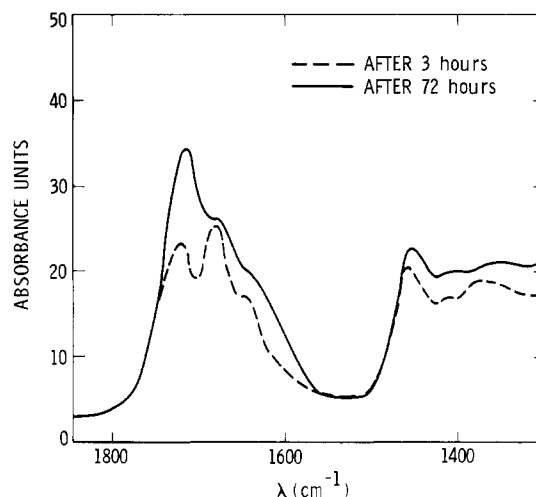
and 300 °C the weight loss amounted to 37%. PGL synthesized in the pH range 9–12 forms viscous solutions in amines<sup>13</sup> (e.g., methylamine, allylamine, butylamine, hydrazine, liquid  $\text{NH}_3$ , etc.) and in aqueous solutions of sodium bisulfite. These solution processes are associated with a chemical reaction involving the aldehyde groups.

**(b) Spectrophotometric Data.** The absorbance at 233 and 285 nm of aqueous solutions of glutaraldehyde was monitored as a function of time and pH in argon, nitrogen, and air. Figure 4 shows the change in absorbance at 233 nm. At pH 11.3 the absorbance increased gradually to a maximum and remained almost constant for at least 28 h. However, at pH 12.3 the absorbance reached a maximum and then a significant decrease in absorbance was observed. This phenomenon was more pronounced the higher the pH. It was also noted that the decrease in the absorbance in the presence of air was faster than that observed under argon. The 233-nm peak expresses the concentration of ethylenic linkages conjugated with aldehyde groups (see Discussion). As the polymerization proceeds, the number of ethylenic linkages increases, thus the optical density at 233 nm increases with time. The observed decrease of optical density at elevated pH is believed to be due to the occurrence of a Cannizzaro reaction which is expected to shift the absorbance of the ethylenic bond to a lower wavelength.

The absorbance at 285 nm is due to aldehyde groups (see Discussion), the concentration of which is expected to decrease with time during the aldol condensation reaction. However, experimental evidence proves that this is not the case (Figure 5), and the 285-nm peak actually increases during polymerization. This fact may be explained by the appearance of acrolein type structures and/or by a shift in the equilibrium between hydrated and nonhydrated



**Figure 5.** Change in the absorbance spectrum during the polymerization of glutaraldehyde at pH 11.3 (glutaraldehyde 0.7% v/v, temperature 27 °C).



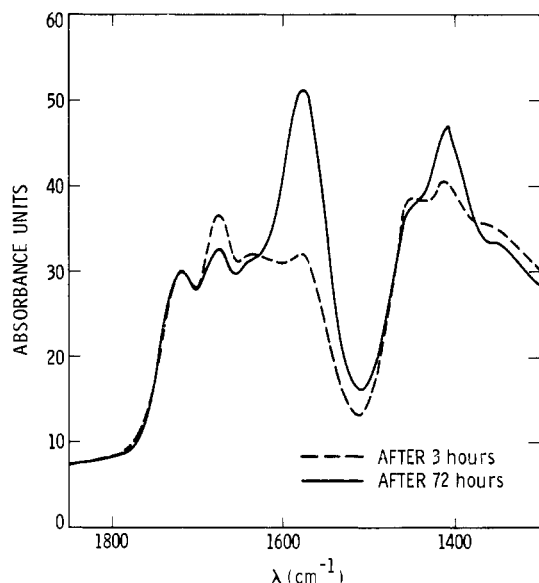
**Figure 6.** IR spectra of PGL (acidic form) prepared under argon at pH 13.3 (glutaraldehyde 20% v/v, temperature 27 °C). The PGL isolated by acidification with acetic acid to pH 5.0.

aldehyde groups (see Discussion).

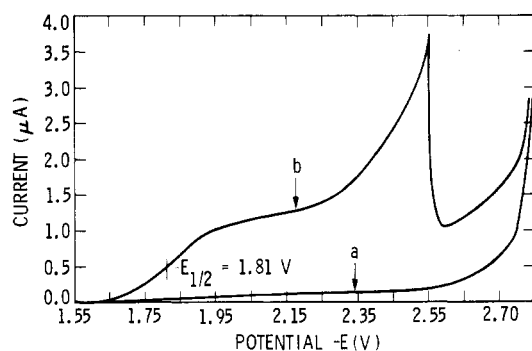
The observed changes in IR during the polymerization and as a function of pH are consistent with the above interpretation. The PGL prepared at pH 13.3 and isolated after 72 h has a different structure than the PGL isolated after 3 h (Figure 6). The main change is the increase in the optical density at 1725  $\text{cm}^{-1}$ . The PGL isolated in the initial reaction stage (after 3 h at pH 13.3) appears to have a practically identical structure as the insoluble polymer synthesized at pH 11.3. However, with increased polymerization time (72 h) a definite structural change occurs shown by the increase in optical density at 1725  $\text{cm}^{-1}$ , and this conclusion is confirmed by the examination of the IR spectra of the same samples in the salt form (Figure 7). The spectrophotometric data therefore present strong evidence for the Cannizzaro reaction which was also confirmed by chemical analysis.

**(c) Chemical Analysis.** The carboxyl, hydroxyl, and aldehyde contents of PGL polymers obtained at different pHs are shown in Tables II and III. The decrease of the number of aldehyde groups with an increase of pH is associated with the increase of the number of carboxyl and hydroxyl groups.

**(d) Electrochemical Analysis.** The UV absorption spectra of PGL provided strong evidence for the presence of acrolein units in the polymer. Since polymeric molecules containing electroactive centers yield voltammetric waves with a magnitude determined by the concentration of these



**Figure 7.** IR spectra of PGL (salt form) prepared under argon at pH 13.3 (glutaraldehyde 20% v/v, temperature 27 °C). The PGL isolated by lyophilization after dialysis.



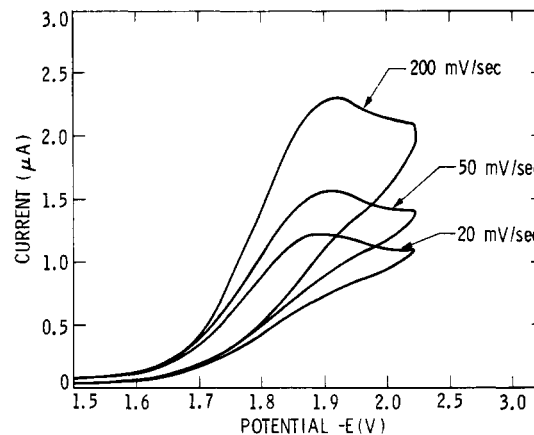
**Figure 8.** Normal pulse voltammogram for the reduction of PGL: PGL, -5 mg, solvent, Me<sub>2</sub>SO-H<sub>2</sub>O (4:1); supporting electrolyte, tetraethylammonium perchlorate; drop time 1 s; scanning rate, 10 mV/s.

**Table III**  
Aldehyde Content of PGL Prepared in the pH Range 10–13.5

pH	% N in reaction product <sup>b</sup>	no. of Aldehyde groups/g × 10 <sup>-21</sup>
10	7.8	3.4
11.5	7.3	3.1
12.3	4.8	1.8
10.8 <sup>a</sup>	3.4	1.3
13.3 <sup>a</sup>	2.2	0.9

<sup>a</sup> Soluble polymer, reaction of PGL in the acidic form with hydroxylamine hydrochloride. <sup>b</sup> Corrected, % N found before reaction = 0.05%.

centers,<sup>21</sup> an electrochemical study was used as a technique to obtain quantitative information on the number of conjugated aldehydes (electroactive centers) of PGL. The polarogram of PGL prepared at pH 11 is shown in Figure 8. The half-wave potential  $E_{1/2} = -1.81$  V is most probably due to the presence of conjugated double bonds. The second wave may be interpreted as arising from the reduction of aldehyde groups. The cyclic voltammograms corresponding to the first wave (Figure 9) show irreversible behavior characteristic of acrolein and substituted acroleins. The electrolysis of PGL (20 mg) prepared at pH 11.0 and 13.3 consumed 5 and 1.3 C, respectively. It is known that substituted acrolein molecules consume one electron

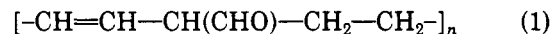


**Figure 9.** Cyclic voltammograms for the reduction of PGL: PGL, 20 mg; solvent, Me<sub>2</sub>SO-H<sub>2</sub>O (4:1); supporting electrolyte, tetraethylammonium perchlorate.

**Table IV**  
Nitrogen Content of PGL Microspheres Prepared in the Presence of Variable Amounts of Aerosol 604

aerosol 604, % w/v	% N
1	2.19
2	3.79
7	5.57
20	8.05

per molecule,<sup>22</sup> i.e., 96 540 C. Therefore, if we assume a structure for PGL consisting of units shown below



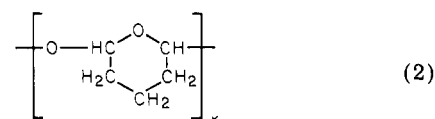
it can then be calculated from the consumed number of coulombs that PGL prepared at pH 11.0 contain a larger number of conjugated aldehyde functions (21%) than PGL prepared at pH 13.3 (8.0%).

**(e) Reaction of PGL with *m*-Aminophenol.** The presence of acrolein units (double bonds conjugated with aldehyde groups) in the PGL polymer was also ascertained by the reaction of the PGL with *m*-aminophenol which yielded a highly fluorescent polymer with fluorescence emission at 470 nm.<sup>6,23</sup>

## Discussion

It is known that glutaraldehyde can be polymerized in different ways. Distilled glutaraldehyde alone or dissolved in chloroform undergoes rapid polymerization to a glassy solid,<sup>10</sup> which reverts to the monomer on heating at 45 °C under vacuum. Glutaraldehyde also polymerizes in the presence of organometallic compounds<sup>25</sup> or acid catalysts.<sup>10</sup>

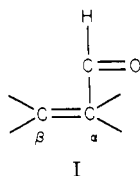
The structure of PGL prepared thermally by means of organometallic compounds or cationic catalysts was assumed to consist mainly of the following units



The presence of aldehyde groups formed during this "intra-inter-molecular" polymerization is still questionable. The facile degradation at relatively low temperature and its spectral properties leave little doubt that its structure is entirely different from PGL obtained by aldol condensation.

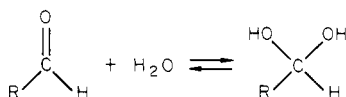
The present work is restricted to the aldol condensation-PGL product and to the elucidation of structures of PGL water-soluble and -insoluble fractions as a function of pH in the range of pH 7–13.5.

(a) **Interpretation of UV and IR Results.** An isolated ethylenic linkage gives a high intensity  $\pi\text{-}\pi^*$  band near 190 nm and an isolated carbonyl group gives a low intensity  $n\text{-}\pi^*$  band near 275 nm.<sup>26</sup> However, when a carbonyl group is conjugated with an ethylenic linkage both bands undergo shifts to longer wavelengths. The absorbance peaks of PGL at 233 and 285 nm were assigned to a  $\pi\text{-}\pi^*$  transition of  $\text{C}=\text{C}$  and  $n\text{-}\pi^*$  transition of the  $\text{C}=\text{O}$  bonds, respectively. These assignments are substantiated by the use of Woodward's rules which postulate a red shift of 11 nm for  $\alpha$  as well as  $\beta$  substitution in structure I.<sup>26</sup>



The simplest structure representing I is acrolein which in ethanolic solution has a  $\pi\text{-}\pi^*$  transition expressed by an absorbance at 209 nm. For the PGL structure characterized by both  $\alpha$  and  $\beta$  substitution, a shift of 22 nm is expected in accordance with Woodward's rules, predicting an absorbance at 231 nm. Since water is a more polar solvent than ethanol, a bathochromic shift from 231 nm is expected. Therefore, the observed absorbance at 233 nm is in relatively good agreement with Woodward's rules. The  $n\text{-}\pi^*$  transition of acrolein results in an absorption at 328 nm in ethanol. Alkyl substitution shifts this transition to shorter wavelengths.<sup>27</sup> Thus crotonaldehyde, a  $\beta$ -substituted acrolein, has a  $n\text{-}\pi^*$  transition at 322 nm and 2-ethyl-2-hexenal, an  $\alpha, \beta$  substituted acrolein, at 313 nm. In highly polar solvents, e.g., water, the  $n\text{-}\pi^*$  transition of acrolein exhibits a blue shift from 328 (in ethanol) to 312 nm (in water) and that of crotonaldehyde from 322 (in ethanol) to 302 (in water). An additional shift to the lower end of the spectral range would therefore be expected for PGL (which is substituted in position  $\alpha$  as well as in  $\beta$ ) measured in water. The assignment of the 285 nm to a  $n\text{-}\pi^*$  transition of a carbonyl conjugated with an ethylenic linkage is therefore plausible.

The polymerization of glutaraldehyde involves interactions with the aldehyde groups. Unexpectedly, the absorbance at 285 nm instead of decreasing was increasing during the polymerization process. This can be explained by the formation of conjugate aldehyde groups (i.e., substituted acrolein units) whose extinction coefficient for the transition  $n\text{-}\pi^*$  is much higher than that of glutaraldehyde [e.g.,  $\epsilon_{\text{max}}$  for  $n\text{-}\pi^*$  of crotonaldehyde is 28 L mol<sup>-1</sup> cm<sup>-1</sup> and that of glutaraldehyde is 4.1 L mol<sup>-1</sup> cm<sup>-1</sup>]. It also has to be remembered that the polymerization in basic conditions shifts the equilibrium of the reactions shown below to the left,



and, therefore, it is expected that under basic conditions more free aldehyde groups are available. Thus several effects are responsible for the spectral changes at about 285 nm, e.g., changes in substituted conjugated and nonconjugated aldehyde content as well as changes in the equilibrium between hydrated and nonhydrated aldehyde forms. It was also observed that at pH higher than 12 a new absorption band was formed at about 310 nm, the reason for which is not clear at the present.

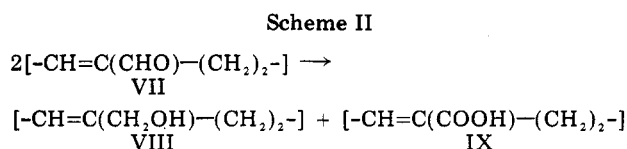
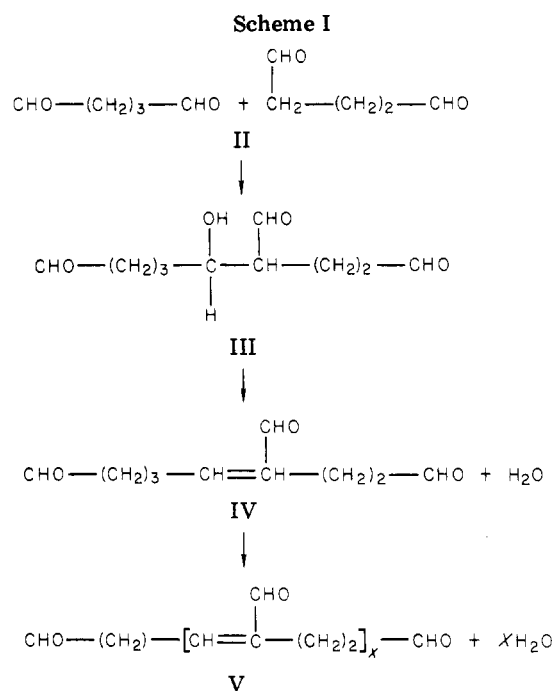
The absorbance at 233 nm of the conjugate aldehyde groups decreased during time (at pH 12.3 or 13.3). This

decrease is mainly due to the Cannizzaro reaction, as a consequence of which two aldehyde groups react to give one primary hydroxyl group and one carboxyl group. Tables II and III confirm this conclusion since with increasing pH the polymer contains less aldehyde molecules but more hydroxyl and carboxyl molecules. The soluble PGL prepared at pH 10.8 contains almost as many aldehyde, hydroxyl, and carboxyl functions as that prepared at pH 13.3; however, it has to be kept in mind that its yield is very low compared to that of the insoluble PGL prepared at pH 10.8 (Table I) and that the higher the pH the higher the yield of the soluble polymer. The decrease in pH during the polymerization of glutaraldehyde (Figure 3) confirms our conclusion that the Cannizzaro reaction occurs during this polymerization process. It should be noted that the pH decrease is faster in the presence of air than in argon. This, together with the fact that the decrease in the absorbance at 233 nm at pH 12.3 as well as pH 13.3 is enhanced in the presence of air (Figure 4) leads to the conclusion that air oxidation must also occur simultaneously and the number of carboxyl groups produced is a combination of air oxidation and Cannizzaro reaction. Analysis of the IR spectra may be used as a supporting evidence for the interpretation of the UV results. The interpretation of the IR is as follows:<sup>28,29</sup> 3430 cm<sup>-1</sup>, stretching of the OH due to water, carboxylic group, or alcoholic hydroxyl group; 2945 and 2875 cm<sup>-1</sup>, asymmetric and symmetric stretching of the CH<sub>2</sub>, respectively; 2740 cm<sup>-1</sup>, asymmetric stretching of the CH of the aldehyde group; 1720 cm<sup>-1</sup>, stretching of the CO groups of carboxylic acid and nonconjugated aldehyde; 1680 cm<sup>-1</sup>, stretching of the CO group of conjugated aldehyde; 1640 cm<sup>-1</sup>, stretching of the C=C group and the OH of water; 1575 cm<sup>-1</sup>, asymmetric stretching of the CO of the carboxylate group (sodium salt); 1410 cm<sup>-1</sup>, symmetric stretching of the CO of the carboxylate group (sodium salt); 1200–1500 cm<sup>-1</sup>, may be due to variable deformation vibrations of the CH<sub>2</sub> or CH groups; and 900–1200 cm<sup>-1</sup>, different C–O bends (1055 cm<sup>-1</sup> due to primary alcohol, 1125 cm<sup>-1</sup> due to secondary alcohol).

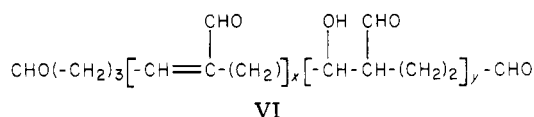
PGL always contains variable amounts of water, even after being dried at high vacuum. The absorbance in the IR due to the presence of water is at the same wavelength as that of the carbon carbon double bond, a fact which made the comparison of the different polymers, relating to the amount of the carbon carbon double bond, practically impossible. It also has to be kept in mind that the peaks of the carboxyl groups and that of the nonconjugate aldehyde groups are overlapping at 1725 cm<sup>-1</sup>. The intensity of the absorbance peak at 1575 cm<sup>-1</sup> (Figure 1) assigned to the carboxylate group confirms the conclusion that the soluble polymer contains more carboxylate groups than the insoluble polymer. By acidification of the PGL sodium salt the carboxylate peak at 1575 cm<sup>-1</sup> shifts to 1725 cm<sup>-1</sup> (Figure 2), and this shift is well known to be due to the generation of a carboxylic acid from its salt.<sup>29</sup> The increase in the content of the carboxyl group during time due to the Cannizzaro reaction is shown by the higher peak at 1725 cm<sup>-1</sup> of PGL isolated after 72 h compared to that isolated after 3 h (Figure 6). The same results only relating to the carboxylate salt (peak at 1575 cm<sup>-1</sup>) are shown in Figure 7.

(b) **Mechanism.** On the basis of previously reported reaction kinetics<sup>6</sup> and our recent investigations, the mechanism in Scheme I appears to fit most of the results obtained to date.

The aldol condensation involves a dehydration step yielding ethylene linkages conjugated with aldehyde



functions. According to chemical, spectroscopic, and electrochemical analysis, the number of aldehyde and double bonds conjugated with the aldehyde groups varied as a function of pH, and their concentration was considerably less than that theoretically calculated on the basis of structure V. Therefore a more likely PGL structure is VI.



VI implies that dehydration does not always occur at every consecutive step of the aldol condensation reaction at room temperature and would account for the reduced concentration of double bonds conjugated with aldehyde functions. (The latter are likely to be in equilibrium with hydrated forms.)<sup>7,10</sup> The decrease of the number of aldehyde functions was proven to result from a Cannizzaro reaction (Scheme II) most probably occurring intermolecularly since the intramolecular mechanism would require an eight-membered intermediate. It should be mentioned that although a few examples were reported of a Cannizzaro dismutation with aldehydes having a hydrogen atom on the carbon atom adjacent to the aldehyde group,<sup>30-32</sup> this is the first case of a Cannizzaro reaction taking place during an aldol condensation. A similar scheme to Scheme II would also apply to the saturated units of VI. With an increase of the pH of the polymerization medium, the water solubility of PGL polymers increased considerably. This fact is consistent with the increase of carboxyl group concentration (Table II) and the spectrophotometric data (Figures 1-3, 6, and 7) and justifies the proposed mechanism (Scheme II).

In conclusion, PGL prepared in the range of pH 7-13.5 whether soluble or insoluble in water is a reactive polymer. It contains variable numbers of hydrated and nonhydrated aldehyde groups which may or may not be adjacent to an ethylenic linkage, and it is also characterized by a variable

concentration of primary and secondary hydroxyl and carboxyl functions. The presence of secondary hydroxyl groups is explained by an incomplete aldol condensation and that of primary hydroxyl and a carboxyl group by the occurrence of a Cannizzaro reaction for which ample evidence was obtained. Air oxidation of aldehyde during and after the polymerization at high pH may yield additional carboxyl functions.

The high reactivity of PGL whether in solution, in solid state, or in the form of water-insoluble microspheres offers possibilities for important applications of PGL as a new immunoreagent in the biological and biomedical fields.<sup>13,14</sup>

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