

# Statistical Analysis of Nitrogen-Containing Vinyl Copolymers: Radiation-Induced Copolymerization of Vinyl Acetate and *N*-Vinyl-2-Pyrrolidone

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## Synopsis

Radiation-induced copolymerization of vinyl acetate and *N*-vinyl-2-pyrrolidone was carried out at 5°C using  $\gamma$ -irradiation of 1450 rads/min. Copolymers prepared at conversions lower than 5% were analyzed by a saponification technique. Various linear and nonlinear statistical analysis techniques were used to determine the reactivity ratios of this system as  $r_1 = 0.348$  and  $r_2 = 3.108$ . These data were examined and analyzed in relation to problems of elemental analysis involving nitrogen-containing copolymers and to discrepancies in the reactivity ratios obtained by previous investigators. The presence of oxygen and a higher dose rate did not affect the copolymer composition within statistical error. Hydrolyzed copolymers prepared by this method have potential applications as biocompatible materials.

## INTRODUCTION

Copolymers of vinyl acetate (VAc, monomer 1) and *N*-vinyl-2-pyrrolidone (NVP, monomer 2) have been used recently as potential biomaterials on partial hydrolysis, crosslinking, and covalent heparinization of the surface hydroxyl groups of the resulting poly(vinyl alcohol) copolymers.<sup>1</sup> A radiation-induced copolymerization method has been used for this application, due to the virtual lack of undesirable toxic impurities such as initiator and solvents. Both homopolymers have been used as potential biomaterials due to their low toxicity, good mechanical properties, stability, and inert behavior in the physiological environment.<sup>2-4</sup> In addition, copolymers or grafted polymers of these monomers have been studied in connection with membrane diffusion and related biomedical applications.<sup>5-7</sup> Their copolymers incorporate the physical properties of both materials, as well as a controlled content of surface hydroxyl groups on hydrolysis.

Copolymers of VAc and NVP have been prepared by free radical bulk copolymerization using an azobisisobutyronitrile (AIBN) initiator at 50,<sup>8</sup> 60,<sup>9</sup> 70,<sup>9,10</sup> and 75°C<sup>11</sup> and unspecified temperatures<sup>7,12,13</sup>; in benzene using a *tert*-butyl perbenzoate initiator at 75°C<sup>14</sup>; and by emulsion polymerization at 55°C.<sup>15</sup> Considerable disagreement concerning the reactivity ratios  $r_1$  and  $r_2$  was noted by these investigators; it was attributed to the various methods of elemental analysis used for the determination of the copolymer composition and the statistical error of these analyses. Preliminary radiation-induced copolymerization of this system both in bulk and in aqueous dispersion systems has been reported

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by Ellinger.<sup>16</sup> In this study  $\gamma$ -irradiation at dose rates of 60–10,000 rads/min and high-voltage x-rays at  $5 \times 10^4$ – $8 \times 10^5$  rads/min at 60°C were used. No analysis of reactivity ratios was reported.

Here we report a new method of radiation-induced copolymerization of this system, as well as an investigation of the reactivity ratios and effect of oxygen and dose rate on this system.

## EXPERIMENTAL

Vinyl acetate (Aldrich Chemical Co., 99% pure), inhibited with hydroquinone and diphenyl amine, and NVP (Fisher Co., 99.5% pure) were distilled at 72 and 94°C/15mm Hg, respectively. Both monomers were degassed in flasks at –40°C for 6–8 hr, and mixtures for the copolymerization reaction were prepared in test tubes under oxygen-free atmosphere and sealed with paraffin wax. After thermal equilibrium in a constant-temperature chamber at  $5 \pm 1^\circ\text{C}$ , the chamber was placed in a  $^{60}\text{Co}$   $\gamma$ -irradiation unit (Department of Bionucleonics) and irradiated for 30 min at 1450 rads/min. Mixtures with a mole fraction of VAc in the comonomer feed  $f_1$  of 0.2, 0.4, 0.6, and 0.8 were irradiated. Polymerizations of pure monomers were also carried out at the same temperature for comparison.

After the reaction, the copolymer–monomer mixtures were washed with methanol. After distillation, the copolymers were precipitated with ethyl ether, and dried under vacuum at 25°C for 24 hr. During all these studies the conversion was kept under 5%.

The copolymer composition was determined by a modification of the saponification of acetate groups method, using methanol at elevated temperatures.<sup>12</sup> Copolymer samples of 25 mg were dissolved in 3 ml of KOH/methanol solution, fitted with reflux condensers, and boiled at 63°C for 11 hr. Because of a significant amount of reaction between the glass apparatus and the KOH solution at these long digestion times, blank reactions without copolymer were used for this correction. Following digestion the samples were washed three times with 4 ml methanol, titrated with standardized HCl solutions, and the conversion of the VAc units in the copolymer to hydroxyl groups was determined; this method was reproducible within 0.05%.

Copolymer composition was also determined using  $^1\text{H}$ -NMR spectroscopy. The NMR spectra were obtained in deuterated chloroform (32 mg/ml) at 28°C. The composition of the copolymers was determined by comparison of the integrated peak areas of 2.81–3.52  $\delta$  and 4.21–4.93  $\delta$  for the protons of NVP and VAc, respectively, as shown also in Figure 2.

## RESULTS

The mole fraction of VAc  $F_1$  in a series of copolymers of VAc and NVP prepared at low conversions (<5%) and at a total dose of  $4.35 \times 10^4$  rads was determined by saponification analysis. Figure 1 reports the variation of  $F_1$  as a function of the mole fraction of VAc  $f_1$  in the comonomer feed; at least five samples were analyzed at each mole fraction of comonomer feed. In most copolymer composition data reported before, there is significant error due to inadequacies in various steps of the chemical analysis. Because of steric hindrance

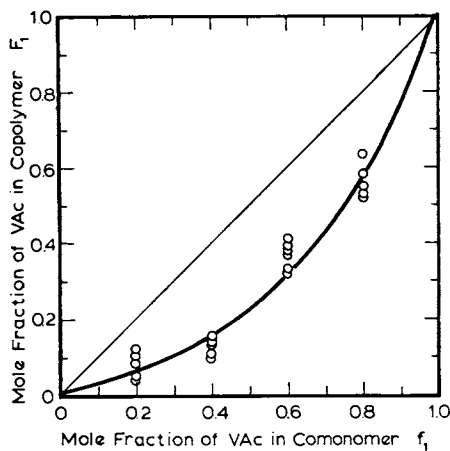


Fig. 1. Copolymerization curve for the VAc-NVP system.

problems due to the pyrrolidone ring, the polymeric chain is rather difficult to saponify completely. Ushakov's method<sup>12</sup> seems the most accurate for this analysis, while various methods of nitrogen analysis based on the Kjeldahl and Dumas techniques fail to lead to reproducible data.<sup>17</sup> By carrying out the saponification at elevated temperatures the process may be accelerated and a high-boiling solvent such as amyl alcohol can be used. Though amyl alcohol and similar solvents are adequate for copolymers high in NVP, they are inapplicable as the VAc fraction increases. Therefore, the low-boiling methanol was used as the solvent.

Copolymerizations carried out in the presence of oxygen using  $f_1 = 0.4, 0.6,$  and  $0.8$  did not result in any appreciable change of the copolymer composition. Infrared spectroscopic analysis (Perkin Elmer 267) showed no evidence of carbonyl and peroxidic bonds in the copolymer chain, despite previous speculations by Ellinger<sup>16</sup> that oxygen might be an inhibitor for this reaction.

The NMR analysis of spectra of the copolymers obtained in deuterated chloroform was also used to confirm the composition data obtained by elemental analysis. Figure 2 shows the peaks characterizing the protons A and B of vinyl acetate and NVP, respectively. Shifting of the peak of proton A downfield is observed as the VAc composition decreases below 20%, making the use of NMR spectra at this range quantitatively impossible. Otherwise the data agree with the previously calculated compositions.

To investigate the effect of dose rate, additional experiments were carried out using another <sup>60</sup>Co unit with dose rate of 8900 rads/min. Samples of  $f_1 = 0.4$  and  $0.6$  irradiated with a total dosage of  $4.35 \times 10^4$  rads were analyzed, and it was found that within statistical limits there was no effect of the dose rate on the copolymer composition.

## DISCUSSION

Various problems concerning the analytical techniques for determination of the copolymer composition, the applicability of the classical copolymerization equation, the extent of reaction, the possible crosslinking, and the method of statistical analysis of the results have made the determination of the reactivity

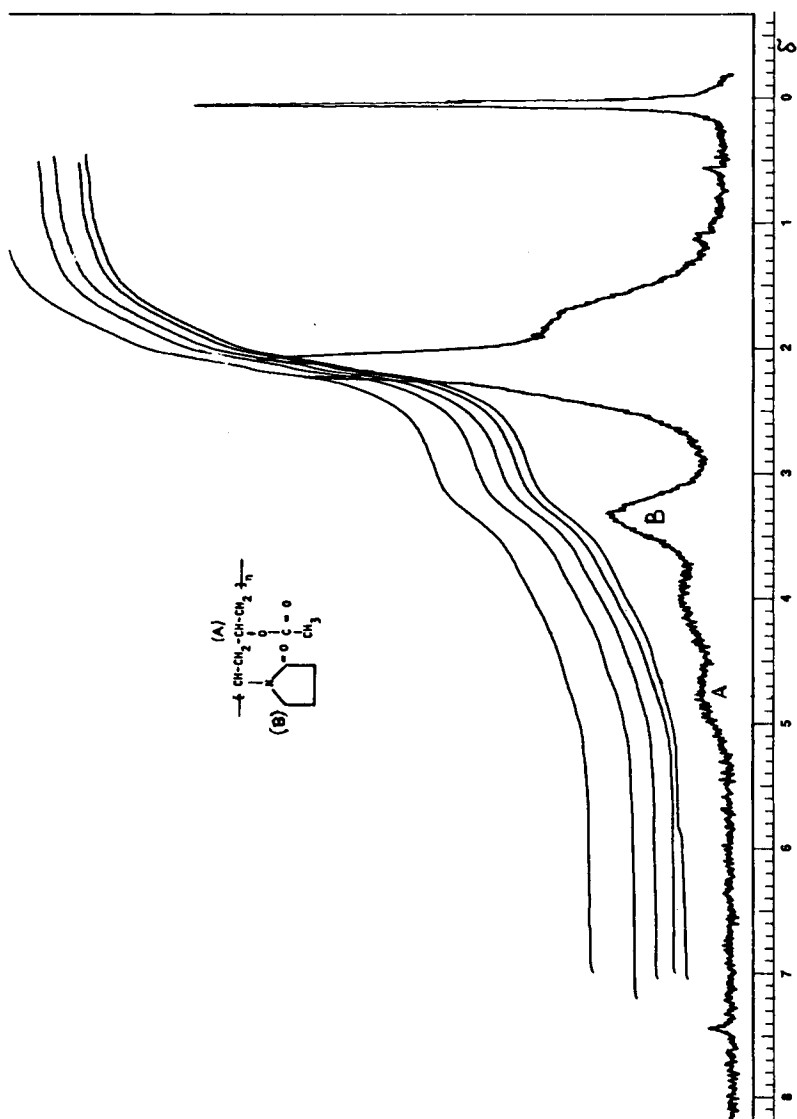


Fig. 2.  $^1\text{H}$ -NMR spectrum of VAc-NVP copolymer in  $\text{CDCl}_3$ .

ratios for this particular system rather cumbersome. Previous authors have reported different values for the copolymerization reactivity ratios of the system VAc ( $r_1$ ) and NVP ( $r_2$ ). Table I compares the results of various reported studies.

Both isolation and analytical errors abound in the literature. It has been reported that even after 8 hr of digestion Kjeldahl nitrogen analysis gave unsatisfactory results,<sup>12</sup> and an effort has been made to correct these analyses with various arbitrary correction factors.<sup>8,14</sup> In our technique we have overcome this problem by using a modified saponification method<sup>12</sup> with methanol. These results have been verified using infrared spectroscopic analysis of the —OH characteristic 916 and 1320  $\text{cm}^{-1}$  bands<sup>1,15</sup> (Fig. 3). The 849-, 1140- and 1425- $\text{cm}^{-1}$  bands are also characteristic of the saponified and hydrolyzed poly-(VAc) repeating units of the copolymer, while the 1570- $\text{cm}^{-1}$  band is characteristic of the poly(NVP) spectrum. The carbonyl-sensitive 1639- $\text{cm}^{-1}$  band was almost nonexistent, indicating that the presence of oxygen during irradiation does not affect the chemical structure of the copolymers.

Another problem exists in the assumed adequacy of the classical copolymerization relationship.<sup>18</sup> This relationship applies to only the simplest free-radical copolymerization reactions, and in a more complex system, intermolecular and intramolecular interactions may seriously alter the kinetics and composition of the resulting copolymer. Finally, the use of inadequate mathematical evaluation techniques has been a significant problem.

The reactivity ratios reported in Table I were obtained mainly via a Finemann-Ross analysis.<sup>19</sup> Bork and Coleman (8) noted the limitations of the

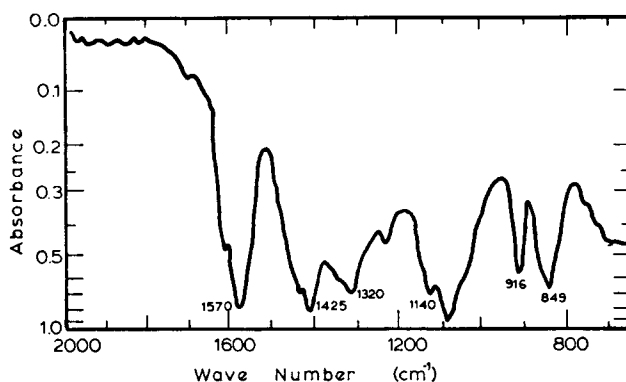


Fig. 3. Infrared spectrum of saponified (methanol/KOH) VAc-NVP copolymers.

TABLE I  
Reactivity Ratios of VAc ( $r_1$ ) and NVP ( $r_2$ ) by Free Radical Copolymerization

Initiation	Temperature, °C	Analysis of copolymer	$r_1$	$r_2$	Ref.
AIBN	50	Kjeldahl	0.205	3.300	8
AIBN	60, 70	Kjeldahl	0.380	0.440	9
AIBN	70	Modified Dumas-Pregel	0.205	3.300	10
AIBN	75	Unknown	0.237	2.280	11
Perbenzoate	75	Modified Kjeldahl	0.237	2.280	14
Radiation	5	Saponification	0.348	3.108	This work

classical Kjeldahl method for nitrogen analysis in polymers and attempted to compensate. Hayashi and Smets<sup>9</sup> used the Kjeldahl method without any modification and the discrepancy in the reported values is attributable to this analytical error. In all cases,<sup>8-11,14</sup> no more than a total of six samples were analyzed, rendering a statistical evaluation inadequate.

In the present study a thorough statistical analysis with various linearization techniques was performed. The classical copolymer equation contains one random dependent variable  $F_1$  and one nonrandom independent variable  $f_1$ . On linearization both dependent and independent variables become stochastic, and even under the assumption of normally distributed variables, the estimation of parameters in the linear model and the designation of suitable tests and confidence intervals become quite difficult.<sup>20</sup>

Table II compares the reactivity ratios for the VAc-NVP copolymer system using various statistical methods (described in Appendix A). As mentioned previously, the technique used originally by Finemann and Ross<sup>19</sup> has been used in all other investigations of this system.<sup>8,9,12,14</sup> The inadequacy of this technique for the data of this study is obvious, since on inversion a negative value of  $r_2$  is obtained. As pointed out by Kelen and Tudos,<sup>21</sup> this is not an uncommon phenomenon. Other linearization techniques of Table II are also not invariant on reindexing, though they do seem to fit the data better. A problem associated with all these linearization techniques is the unequal weighing of data obtained at the extreme ends of the comonomer feed axis. Introducing an arbitrary constant, an invariant symmetrical relationship was obtained,<sup>21</sup> which on least-squares analysis led to a variety of reactivity ratios. The fit attained in terms of the correlation coefficient  $r^2$  was the most satisfactory, but graphic evaluation showed that the data were simply brought closer, thus decreasing the residual sum of squares. In a previous publication describing the biomedical applications of these polymers, we reported preliminary  $r_1$  and  $r_2$  values obtained only by linear techniques.<sup>1</sup>

Though the different linear methods produced a variety of inconclusive estimates of the reactivity ratios, the estimates were useful as starting values for the

TABLE II  
Techniques of Statistical Analysis of Reactivity Ratios of VAc and NVP Copolymers

Statistical method	Equation of Appendix A	Ref.	Reactivity ratios		Correlation coefficients
			$r_1$	$r_2$	$r^2$
Linear					
Finemann and Ross	(A1)	19	0.292	3.376	0.387
Finemann and Ross	(A2)	19	-0.230	1.960	0.649
Linearization	(A3)	19	0.200	3.230	0.899
Linearization	(A4)	19	0.240	3.400	0.899
Linearization	(A5)	19	0.310	3.030	0.649
Kelen and Tudos	(A6)	21	0.210	3.410	0.425
Kelen and Tudos	(A7)	21	1.060	8.540	0.955
Kelen and Tudos	(A8)	21	0.470	4.200	0.917
Nonlinear					
Marquardt	—	22	0.348	3.108	$s_{res}^2$ 0.04756
Tidwell and Mortimer	—	24	0.348	3.108	0.04756

application of nonlinear techniques. The first nonlinear method used in this work was proposed by Marquardt,<sup>22</sup> and it is a compromise between the nonlinear techniques of Gauss<sup>20</sup> and Kittrell et al.<sup>23</sup> By repetitive linear least-squares analysis, a correction vector of the original values of the reactivity ratios is used until adequate convergence is obtained. Convergence by both techniques was rapid, yielding essentially identical values for the two parameters of the classical copolymerization equation  $r_1$  and  $r_2$ . Asymptotic 95% confidence intervals were calculated as between 0.18 and 0.517 for  $r_1$  and 2.06 and 4.16 for  $r_2$ .

The adequacy of the classical copolymerization equation was examined using the Kelen and Tudos<sup>21</sup> graphical evaluation of the adequacy method and a statistical analysis of the nonlinear relationship of Tidwell and Mortimer<sup>24</sup> (Appendix B). It was found that with a 95% certainty, the classical copolymerization equation is adequate for the description of the VAc-NVP system.

### CONCLUSIONS

From this statistical analysis it is concluded that the values of reactivity ratios are  $r_1 = 0.348$  and  $r_2 = 3.108$ . This communication further stresses the importance of the applications of nonlinear techniques for the analysis of copolymerization data, especially in cases of copolymer compositions obtained through elemental analysis with significant statistical error. In the case of the VAc-NVP system, it is concluded that previously reported values have been obtained through simplified models and inaccurate statistical methods.

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### APPENDIX A: LINEAR TECHNIQUES OF COPOLYMERIZATION ANALYSIS

The intersection method<sup>25</sup> of plots of  $r_2$  versus  $r_1$  is not applicable in this system, and it should be avoided in general due to inaccuracy. Linearization using the Finemann-Ross technique<sup>19</sup> is based on the classical copolymerization equation rearranged as

$$r_1 X - r_2 = Y \quad (\text{A1})$$

or

$$r_1 - (1/X)r_2 = Y/X \quad (\text{A2})$$

with

$$X = \frac{f_1^2 (1 - F_1)^2}{f_2^2 F_1} \quad \text{and} \quad Y = \frac{f_1 (2F_1 - 1)}{f_2 F_1}$$

By plotting  $X$  versus  $Y$  and  $1/X$  versus  $Y/X$ , one should obtain identical values of  $r_1$  and  $r_2$ . This equation was plotted and fitted by least-squares analysis using the data of this system, but eq. (A1) gave negative value for  $r_1$ .

Other linearization equations used included the following (see also Table II):

$$\frac{1}{Y} = \left(\frac{r_1}{r_2}\right) \frac{X}{Y} - \frac{1}{r_2} \quad (\text{A3})$$

$$\frac{X}{Y} = \left(\frac{r_2}{r_1}\right) \frac{1}{Y} + \frac{1}{r_2} \quad (\text{A4})$$

$$\frac{1}{X} = \left(-\frac{1}{r_2}\right) \frac{Y}{X} + \frac{r_1}{r_2} \quad (\text{A5})$$

The Kelen and Tudos technique<sup>21</sup> produced equations invariant to inversion and yielded consistent values for constants in the equation

$$\frac{Y}{(a+X)} = \frac{(r_1 + r_2/a)X}{(a+X)} - \frac{r_2}{a} \quad (\text{A6})$$

with  $a = F_{1,\text{high}}F_{1,\text{low}} = 3.05$  and symmetrical distribution of the data between  $X/(X+a) = 0.0$  and  $1.0$ . By applying the same technique to eqs. (A3) and (A4), similar relationships were obtained:

$$\frac{(1/Y)}{2[b + (X/Y)]} = \frac{(r_1/r_2 + 1/br_2)(X/Y)}{2[b + (X/Y)]} - \frac{1}{2br_2} \quad (\text{A7})$$

$$\frac{(X/Y)}{2[g + (X/Y)]} = \frac{(r_2/r_1 - 1/gr_1)(1/Y)}{2[g + (X/Y)]} + \frac{1}{2gr_1} \quad (\text{A8})$$

with

$$b = -[(1/Y)_{\text{high}} + (1/Y)_{\text{low}}]/2$$

$$g = -[(X/Y)_{\text{high}} + (X/Y)_{\text{low}}]/2$$

Although both relationships produced good fits on least-squares analysis, the intercept contained significant variance affecting the values of the reactivity ratios.

## APPENDIX B: NONLINEAR TECHNIQUES OF COPOLYMERIZATION ANALYSIS

Stemming from the original linear estimates of the reactivity ratios, the Gauss technique<sup>20</sup> utilizes a repetitive linear least-squares analysis to obtain a correction vector, changing the original value of the parameter until adequate convergence is obtained. The Kitrell technique<sup>23</sup> of steepest descent uses the sum of squares as a surface in a space where the parameters are variables. The Marquardt technique<sup>22</sup> utilizes a correction vector which expedites the steepest-descent process.

The Tidwell and Mortimer nonlinear technique<sup>24</sup> is used to calculate a residual sum of squares  $s_{\text{res}}^2$  and a sum of squares due to experimental error  $s_{\text{err}}^2$ , yielding a sum of squares  $s_w^2$  which is distributed as  $\chi^2\sigma_1^2$  with  $(p-2)$  degrees of freedom. The fraction  $ks_w^2/(p-2)s_{\text{err}}^2$ , where  $k$  is the number of observations at each experimental condition and  $p$  is the number of experimental conditions, is approximately distributed as  $F$  with  $p-2$  and  $k$  degrees of freedom. The fraction above was calculated to be  $2.021 < F_{0.95(2.5)} = 5.781$ . Therefore with 95% certainty the classical copolymerization equation describes this system adequately.



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