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Polymerization of Polyacrylamide Gels: Efficency and Reproducibility as a Function of Catalyst Concentrations

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Polymerization of Polyacrylamide Gels: Efficency and Reproducibility as a Function of Catalyst Concentrations

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Summary

A method has been developed for estimation of the extent of polymerization of acrylamide by measurement of the amide–nitrogen of residual extractable acrylamide monomer. This method has been applied to the evaluation of polymerization efficiency, its reproducibility, and its dependence on the concentrations of three catalysts (persulfate, riboflavin, and TEMED) at pH 3.9 and 0°C. The effects of TEMED on gel pH, on the relative mobility of dyes and proteins, and on the "stacking limits" of multiphasic buffer systems have also been studied. These studies permit development of guidelines by which polymerization conditions can be optimized.

INTRODUCTION

To date, the concentration of polyacrylamide (PA) gels used for either polyacrylamide gel electrophoresis (PAGE) or gel filtration has been defined in terms of the initial concentrations of the acrylamide

monomer and cross-linking agents (*1*). This makes the implicit assumption that the polymerization reaction is complete (100% conversion of monomer to polymer), or, at least reproducible. The present study was undertaken to evaluate the validity of this assumption. The use of initial monomer concentrations (%T, %C; see Table 1) to define "gel concentration" (*1*) is justifiable when PAGE is used as a qualitative method, e.g., for visual comparison of migration distances of two proteins subjected simultaneously to PAGE in the same gel. However, for "quantitative PAGE" (*1*) and when precise reproducibility is required, it is essential to know the exact polymer concentration in every gel. The use of the relative mobility for a macromolecule in the gel (R_f) as a physical constant (*2*) requires knowledge of the final, rather than the initial, monomer concentration (%T). Accordingly, all parameters derived from R_f also depend on the final polymer concentration. These include the slope or retardation coefficient (K_R), and the y-intercept (Y_0 or M_0) of the Ferguson plot, estimates of molecular size and net charge (*2*), and optimal gel concentration for either analytical (*3*) or preparative (*4*) PAGE and the predicted instantaneous velocities in gel gradients (*5*). Quantitative comparison of PAGE data derived from

TABLE 1

List of Abbreviations

Bis	*N,N'*-Methylenebisacrylamide
%C	Bis × 100/(acrylamide + Bis)
CV	Coefficient of variation
df	Degrees of freedom
KP	Potassium persulfate
K_R	Retardation coefficient
R_f	Relative electrophoretic mobility
LGB	Lower gel buffer
n	Number of observations
%EM	Percent extractable monomer
PA	Polyacrylamide
PAGE	Polyacrylamide gel electrophoresis
%PE	Percent polymerization efficiency
%T	(Acrylamide + Bis) × 100 (w/v)
RN	Riboflavin
σ	Standard deviation of %EM
TEMED	*N,N,N',N'*-Tetramethylethylenediamine
UGB	Upper gel buffer
[X]	Molar concentration of species X

separate experiments or laboratories depends on reproducible and accurate mobility values and, therefore, on a knowledge of polymerization efficiency and reproducibility.

Methods previously used to measure the degree of conversion of acrylamide monomer into linear polymer (*6*) are not applicable to cross-linked gels. A method for determining polymerization efficiency by measurement of amide–nitrogen extractable in 66% methanol has, therefore, been developed. This new method makes it possible to measure effective gel concentration directly. Until now, the apparent pore size (and thus extent of polymerization) could only be indirectly estimated of the basis of the R_f. Although R_f does provide a very sensitive and precise indication of effective gel concentration and pore size, it is also dependent on several other factors.

TABLE 2

Composition and Properties of Multiphasic Buffer Systems 35, 35.11, K, and L

```
                              SYSTEM NUMBER
      INPUT DATA

DATE = 01/26/72     COMPUTER SYSTEM NUMBER =  Chrambach    35
POLARITY = + (MIGRATION TOWARD CATHODE)    TEMPERATURE =    0  DEG. C.


          SPECIFIED CONSTITUENTS
CONSTITUENT 1 = NO.    2 , BETA ALANINE
CONSTITUENT 2 = NO.    4 , PYRIDINE
CONSTITUENT 3 = NO.   97 , POTASSIUM +
CONSTITUENT 4 = NO.   97 , POTASSIUM +
CONSTITUENT 5 = NO.   97 , POTASSIUM +
CONSTITUENT 6 = NO.   18 , ACETIC ACID

          SPECIFIED CONCENTRATIONS
PHASE ALPHA(1) -    C1 =      0.04000        C6 =      0.00980
PHASE  BETA(2) -    C2 =      0.04880        C6 =      0.01860
PHASE GAMMA(3) -    C3 =      0.03770        C6 =      0.28870

          PHASE DELTA(10) - ELUTION BUFFER
RATIO IONIC STRENGTHS IS(10)/IS(9) =      3.0
MIN PH =       2.5
MAX PH =       4.5

          PHASE EPSILON(11) - LOWER BUFFER
IS =          0.05
PHI(6) =      0.80

          PHASE PSI(5) AND TAU(6) - RESTACKING PARAMETERS
RFMAX =  0.90
MAX ABS(PH(5) - PH(9)) =  2.00
```

(*continued*)

TABLE 2 (continued)

SYSTEM NUMBER

DATE = 01/26/72 COMPUTER SYSTEM NUMBER = Chrambach 35
POLARITY = + (MIGRATION TOWARD CATHODE) TEMPERATURE = 0 DEG. C.

CONSTITUENT 1 = NO. 2 , BETA ALANINE
CONSTITUENT 2 = NO. 4 , PYRIDINE
CONSTITUENT 3 = NO. 97 , POTASSIUM +
CONSTITUENT 6 = NO. 18 , ACETIC ACID

	PHASES					
	ALPHA(1)	ZETA(4)	BETA(2)	PI(9)	LAMBDA(8)	GAMMA(3)
C1	0.0400	0.0400		0.0247		
C2			0.0488		0.0301	
C3						0.0377
C6	0.0098	0.0098	0.0186	0.2757	0.2811	0.2887
THETA	0.245	0.246	0.381	11.177	9.348	7.658
PHI(1)	0.105	0.106		0.608		
PHI(2)			0.349		0.980	
PHI(3)						1.000
PHI(6)	0.430	0.430	0.915	0.054	0.105	0.131
RM(1)	0.062	0.062		0.359		
RM(2)			0.300		0.843	
RM(3)						1.490
RM(6)	-0.340	-0.340	-0.723	-0.043	-0.083	-0.103
PH	4.62	4.62	5.77	3.50	3.81	3.92
ION.STR.	0.0042	0.0042	0.0170	0.0150	0.0295	0.0377
SIGMA	0.562	0.563	2.709	1.996	4.692	8.295
KAPPA	144.	144.	659.	488.	1115.	1950.
NU	0.111	0.111	0.111	0.180	0.180	0.180
BV	0.014	0.014	0.029	0.046	0.062	0.075

RECIPES FOR BUFFERS OF PHASES ZETA(4),BETA(2),GAMMA(3),PI(9)

CONSTITUENT		1X PHASE 4	4X PHASE 2	4X PHASE 3	4X PHASE 9
BETA ALANINE	GM	3.57			0.88
PYRIDINE	GM		1.54		
1N KOH	ML			15.08	
ACETIC ACID	GM	0.59	0.45	6.93	6.62
H2O TO		1 LITER	100 ML	100 ML	100 ML
AT FINAL CONCENTRATION =					
PH(25 DEG.C.)		4.55	5.52	3.90	3.46
KAPPA(25 DEG.C.)		251.	1172.	3606.	867.

(*continued*)

By use of the new methodology, we were able to evaluate reproducibility of polymerization in PA gels, to examine the relationship between polymerization efficiency (%PE) and catalyst (initiator) concentrations, and to develop guidelines for the selection of initiator concentrations capable of providing efficiently and reprodubibly polymerized gels.

The relative catalytic properties of riboflavin (RN), persulfate (KP), and TEMED (see Table 1) were evaluated in terms of polymerization efficiency in a buffer system at acid pH and 0°C—a relatively difficult problem in polymerization. This provides some promise that these three most commonly used catalysts, in combination, can provide satisfactory %PE throughout the temperature and pH range used in PAGE.

TABLE 2 (continued)

SYSTEM NUMBER

DATE = 01/26/72 COMPUTER SYSTEM NUMBER = Chrambach 35

PHASE DELTA(10) - ELUTION BUFFER

IS = 0.045

0 DEG.C.		25 DEG.C.			
PH	KAPPA	PH	KAPPA	C6	C4
2.50	2307.	2.48	4259.	7.8603	0.0450
3.00	2307.	2.98	4259.	2.5164	0.0450
3.50	2307.	3.48	4259.	0.8265	0.0450
4.00	2307.	3.98	4259.	0.2921	0.0450
4.50	2307.	4.48	4259.	0.1231	0.0450

PHASE EPSILON(11)-LOWER BUFFER

IS = 0.050

0 DEG.C.		25 DEG.C.			
PH	KAPPA	PH	KAPPA	C6	C5
5.34	2552.	5.32	4705.	0.0625	0.0500

STACKING AND UNSTACKING RANGES

PHASE ZETA(4) OR PI(9)					PHASE BETA(2) OR LAMEDA(8)					PHASE GAMMA(3)			
RM(1)	PHI(1)	C(1)	C(6)	PH	RM(2)	PHI(2)	C(2)	C(6)	PH	C(3)	C(6)	PH
0.006	0.010	0.0400	0.0004	5.69	0.16	0.182	0.0488	0.0092	6.15	0.0	0.0	0.0
0.035	0.060	0.0400	0.0041	4.88	0.21	0.250	0.0488	0.0129	5.98	0.0	0.0	0.0
0.065	0.110	0.0400	0.0105	4.60	0.31	0.360	0.0488	0.0193	5.75	0.0	0.0	0.0
0.094	0.160	0.0400	0.0201	4.41	0.43	0.503	0.0488	0.0289	5.49	0.0	0.0	0.0
0.124	0.210	0.0400	0.0335	4.27	0.56	0.653	0.0488	0.0422	5.23	0.0	0.0	0.0
0.153	0.260	0.0400	0.0514	4.14	0.67	0.774	0.0488	0.0602	4.97	0.0881	0.1046	5.47
0.183	0.310	0.0400	0.0750	4.04	0.73	0.853	0.0488	0.0837	4.74	0.0739	0.1161	4.98
0.212	0.360	0.0400	0.1054	3.94	0.78	0.902	0.0488	0.1141	4.54	0.0637	0.1316	4.71
0.242	0.410	0.0400	0.1444	3.85	0.80	0.932	0.0488	0.1531	4.37	0.0559	0.1512	4.51
0.271	0.460	0.0400	0.1944	3.76	0.82	0.951	0.0488	0.2032	4.21	0.0498	0.1755	4.34
0.301	0.510	0.0400	0.2588	3.67	0.83	0.964	0.0488	0.2676	4.07	0.0449	0.2056	4.19
0.330	0.560	0.0400	0.3425	3.59	0.84	0.974	0.0488	0.3513	3.93	0.0409	0.2432	4.05
0.360	0.610	0.0400	0.4529	3.50	0.84	0.980	0.0488	0.4617	3.80	0.0376	0.2911	3.91
0.389	0.660	0.0400	0.6018	3.40	0.85	0.985	0.0488	0.6106	3.67	0.0347	0.3535	3.78
0.419	0.710	0.0400	0.8091	3.30	0.85	0.989	0.0488	0.8179	3.54	0.0323	0.4380	3.64
0.448	0.760	0.0400	1.1113	3.19	0.85	0.992	0.0488	1.1201	3.39	0.0302	0.5581	3.50
0.478	0.810	0.0400	1.5834	3.06	0.86	0.995	0.0488	1.5921	3.24	0.0283	0.7418	3.34
0.507	0.860	0.0400	2.4071	2.90	0.86	0.996	0.0488	2.4159	3.05	0.0266	1.0574	3.15
0.537	0.910	0.0400	4.1689	2.69	0.86	0.998	0.0488	4.1777	2.81	0.0252	1.7244	2.91

RESTACKING PARAMETERS

	PHASE PSI(5)						PHASE TAU(6)				
CT?	IS	RM(7)	PHI(7)	C(7)	C(6)	PH	C(7)	C(6)	PH	PHI(7)	KAPPA
1	0.005	0.128	0.214	0.0249	0.2759	3.03	0.0404	0.0102	3.93	0.034	48.
42	0.005	0.118	0.223	0.0232	0.2742	3.02	0.0376	0.0074	3.99	0.030	38.

(*continued*)

MATERIALS AND METHODS

Gels

Acrylamide, Bis (see Table 1), and TEMED were purified and polymerization was carried out as described previously (*2*) except as noted. Potassium persulfate, RN, and TEMED were used as catalysts. Most studies used 10% acrylamide, 0.2% Bis gels (= 10.2%T, 2%C). The duration of the photopolymerization reaction was held constant at 60 min. Some studies involved 5%T, 2%C and 15%T, 2%C gels. Gels were made either in distilled water or in the lower gel (separation gel, phase GAMMA) buffer of multiphasic buffer systems B and F (*2*),

TABLE 2 (continued)

```
                    SYSTEM NUMBER
      INPUT DATA

DATE = 01/26/72    COMPUTER SYSTEM NUMBER = Chrambach      35.11
POLARITY = + (MIGRATION TOWARD CATHODE)   TEMPERATURE =   0  DEG. C.


          SPECIFIED CONSTITUENTS
CONSTITUENT 1 = NO.   2 , BETA ALANINE
CONSTITUENT 2 = NO.   4 , PYRIDINE
CONSTITUENT 3 = NO.  97 , POTASSIUM +
CONSTITUENT 4 = NO.  97 , POTASSIUM +
CONSTITUENT 5 = NO.  97 , POTASSIUM +
CONSTITUENT 6 = NO.  18 , ACETIC ACID

          SPECIFIED CONCENTRATIONS
PHASE ALPHA(1) -   C1 =      0.04000        C6 =      0.25860
PHASE  BETA(2) -   C2 =      0.04880        C6 =      0.26740
PHASE GAMMA(3) -   C3 =      0.04500        C6 =      0.20570

          PHASE DELTA(10) - ELUTION BUFFER
RATIO IONIC STRENGTHS IS(10)/IS(9) =      3.0
MIN PH =      2.7
MAX PH =      4.7

          PHASE EPSILON(11) - LOWER BUFFER
IS =         0.05
PHI(6) =     0.80

          PHASE PSI(5) AND TAU(6) - RESTACKING PARAMETERS
RPMAX =  0.90
MAX ABS(PH(5) - PH(9)) =  2.00
```

(*continued*)

and 35, and 35.11 (*7*), K, and L. A complete listing of the properties of the latter four buffer systems is given in Table 2. Noncross-linked linear PA (Gelamide 250) was obtained from the American Cyanamid Co.

Determination of Percent Extractable Monomer in PA Gels

Figure 1 shows the sequence of steps comprising the extraction of acrylamide from PA (Section 1), the removal of amines on Dowex 50 (Section 2), dilution, and alkaline hydrolysis and microdiffusion analysis for ammonia (Section 3).

1. Extraction of PA. Polyacrylamide gels corresponding to a polymerization mixture of 1 ml were sliced transversely into 1.3-mm sections using the device and method previously described (*8*). The slices were suspended in 2-ml absolute methanol in screw-capped vials

TABLE 2 (continued)

1 SYSTEM NUMBER

DATE = 01/26/72 COMPUTER SYSTEM NUMBER = Chrambach 35.11
POLARITY = + (MIGRATION TOWARD CATHODE) TEMPERATURE = 0 DEG. C.

CONSTITUENT 1 = NO. 2 , BETA ALANINE
CONSTITUENT 2 = NO. 4 , PYRIDINE
CONSTITUENT 3 = NO. 97 , POTASSIUM +
CONSTITUENT 6 = NO. 18 , ACETIC ACID

	PHASES					
	ALPHA(1)	ZETA(4)	BETA(2)	PI(9)	LAMBDA(8)	GAMMA(3)
C1	0.0400	0.0400		0.0294		
C2			0.0488		0.0359	
C3						0.0450
C6	0.2586	0.2586	0.2674	0.1901	0.1966	0.2057
THETA	6.465	6.461	5.480	6.459	5.478	4.571
PHI(1)	0.510	0.510		0.510		
PHI(2)			0.964		0.964	
PHI(3)						1.000
PHI(6)	0.079	0.079	0.176	0.079	0.176	0.219
RM(1)	0.301	0.301		0.301		
RM(2)			0.829		0.829	
RM(3)						1.490
RM(6)	-0.062	-0.062	-0.139	-0.062	-0.139	-0.173
PH	3.67	3.67	4.07	3.67	4.07	4.19
ION.STR.	0.0204	0.0204	0.0471	0.0150	0.0346	0.0450
SIGMA	2.717	2.718	7.492	1.999	5.510	9.901
KAPPA	656.	656.	1743.	489.	1300.	2309.
MU	0.111	0.111	0.111	0.150	0.150	0.150
BV	0.066	0.066	0.093	0.049	0.069	0.081

RECIPES FOR BUFFERS OF PHASES ZETA(4),BETA(2),GAMMA(3),PI(9)

CONSTITUENT		1X PHASE 4	4X PHASE 2	4X PHASE 3	4X PHASE 9
BETA ALANINE	GM	3.57			1.05
PYRIDINE	GM		1.54		
1M KOH	ML			18.00	
ACETIC ACID	GM	15.53	6.42	4.94	4.57
H20 TO		1 LITER	100 ML	100 ML	100 ML
AT FINAL CONCENTRATION =					
PH(25 DEG.C.)		3.62	4.03	4.17	3.62
KAPPA(25 DEG.C.)		1157.	3167.	4261.	862.

(*continued*)

equipped with polyethylene-lined caps (A. H. Thomas Cat. No. 2392-C70). The resulting 66% methanolic solution was designated as the "extract." A sample of 1.5 ml of the extract was withdrawn and added to 0.5 ml of 0.1 *M* potassium acetate buffer pH 4.7, yielding 2 ml in buffered 50% methanol.

2. Removal of Amines on Dowex 50. The 2-ml fraction of buffered, 50% methanolic extract was filtered through a small bed (approxi-

TABLE 2 (continued)

```
1                    SYSTEM NUMBER

DATE = 01/26/72    COMPUTER SYSTEM NUMBER = Chrambach      35.11

              PHASE DELTA(10) - ELUTION BUFFER
IS = 0.045
      0 DEG.C.              25 DEG.C.
    PH        KAPPA      PH        KAPPA      C6         C4
  2.70        2310.    2.68        4263.    4.9819     0.0450
  3.20        2310.    3.18        4263.    1.6062     0.0450
  3.70        2310.    3.68        4263.    0.5387     0.0450
  4.20        2310.    4.18        4263.    0.2011     0.0450
  4.70        2310.    4.68        4263.    0.0944     0.0450

              PHASE EPSILON(11)-LOWER BUFFER
IS = 0.050
      0 DEG.C.              25 DEG.C.
    PH        KAPPA      PH        KAPPA      C6         C5
  5.34        2552.    5.32        4705.    0.0625     0.0500

              STACKING AND UNSTACKING RANGES
PHASE ZETA(4) OR PI(9)                    PHASE BETA(2) OR LAMBDA(8)          PHASE GAMMA(3)
 RM(1) PHI(1)   C(1)     C(6)     PH    RM(2) PHI(2)   C(2)     C(6)     PH     C(3)     C(6)     PH
 0.006 0.010 0.0400 0.0004   5.69   0.16 0.182 0.0488 0.0092   6.15 0.0      0.0      0.0
 0.035 0.060 0.0400 0.0041   4.88   0.21 0.250 0.0488 0.0129   5.98 0.0      0.0      0.0
 0.065 0.110 0.0400 0.0105   4.60   0.31 0.360 0.0488 0.0193   5.75 0.0      0.0      0.0
 0.094 0.160 0.0400 0.0201   4.41   0.43 0.503 0.0488 0.0289   5.49 0.0      0.0      0.0
 0.124 0.210 0.0400 0.0335   4.27   0.56 0.653 0.0488 0.0422   5.23 0.0      0.0      0.0
 0.153 0.260 0.0400 0.0514   4.14   0.67 0.774 0.0488 0.0602   4.97 0.0882   0.1047   5.47
 0.183 0.310 0.0400 0.0750   4.04   0.73 0.853 0.0488 0.0837   4.74 0.0740   0.1163   4.98
 0.212 0.360 0.0400 0.1054   3.94   0.78 0.902 0.0488 0.1141   4.54 0.0637   0.1319   4.71
 0.242 0.410 0.0400 0.1444   3.85   0.80 0.932 0.0488 0.1531   4.37 0.0560   0.1514   4.51
 0.271 0.460 0.0400 0.1944   3.76   0.82 0.951 0.0488 0.2032   4.21 0.0499   0.1757   4.34
 0.301 0.510 0.0400 0.2588   3.67   0.83 0.964 0.0488 0.2676   4.07 0.0450   0.2058   4.19
 0.330 0.560 0.0400 0.3425   3.59   0.84 0.974 0.0488 0.3513   3.93 0.0410   0.2435   4.05
 0.360 0.610 0.0400 0.4529   3.50   0.84 0.980 0.0488 0.4617   3.80 0.0376   0.2914   3.91
 0.389 0.660 0.0400 0.6018   3.40   0.85 0.985 0.0488 0.6106   3.67 0.0348   0.3539   3.78
 0.419 0.710 0.0400 0.8091   3.30   0.85 0.989 0.0488 0.8179   3.54 0.0323   0.4385   3.64
 0.448 0.760 0.0400 1.1113   3.19   0.85 0.992 0.0488 1.1201   3.39 0.0302   0.5587   3.50
 0.478 0.810 0.0400 1.5834   3.06   0.86 0.995 0.0488 1.5921   3.24 0.0283   0.7427   3.34
 0.507 0.860 0.0400 2.4071   2.90   0.86 0.996 0.0488 2.4159   3.05 0.0267   1.0587   3.15
 0.537 0.910 0.0400 4.1689   2.69   0.86 0.998 0.0488 4.1777   2.81 0.0252   1.7264   2.91

              RESTACKING PARAMETERS
     PHASE PSI(5)                                          PHASE TAU(6)
CT7  IS    RM(7)  PHI(7) C(7)    C(6)    PH                C(7)    C(6)    PH  PHI(7) KAPPA
  1 0.005  0.100 0.167 0.0297 0.1904  3.17                0.0404 0.2590  3.17 0.167  228.
 42 0.005  0.092 0.174 0.0276 0.1883  3.16                0.0376 0.2562  3.16 0.174  210.
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(*continued*)

mately 0.2 ml wet volume) of Dowex 50 × 8 (200–400 mesh), using a sintered glass funnel (A. H. Thomas Cat. No. 5220-F-28). The resin had been previously equilibrated and washed with buffered 50% MeOH (prepared as in Section 1). A wash of 0.5 ml of diluent was put through the Dowex bed to displace the last of the extract and was collected with it. The combined eluate and wash are subsequently referred to as "filtrate." The 2.5-ml filtrate could be stored in screw-capped tubes for weeks without change in amide content. The filtrate was diluted, either immediately or after a period of storage, to yield amounts of ammonia compatible with the capacity of the buret used in amide analysis (see below). The range of dilutions varied from 1:2.5 to 1:40 depending on

TABLE 2 (continued)

```
DATE = 01/31/72     COMPUTER SYSTEM NUMBER =  Chrambach     K
POLARITY = - (MIGRATION TOWARD ANODE)      TEMPERATURE =    0  DEG. C.


          SPECIFIED CONSTITUENTS
CONSTITUENT 1 = NO.  18 , ACETIC ACID
CONSTITUENT 2 = NO.  99 , CHLORIDE -
CONSTITUENT 3 = NO.  99 , CHLORIDE -
CONSTITUENT 4 = NO.  99 , CHLORIDE -
CONSTITUENT 5 = NO.  99 , CHLORIDE -
CONSTITUENT 6 = NO.   2 , BETA ALANINE

          SPECIFIED CONCENTRATIONS
PHASE ALPHA(1) -   C1 =      0.04000        C6 =      0.04190
PHASE  BETA(2) -   C2 =      0.05380        C6 =      0.05570
PHASE GAMMA(3) -   C3 =      0.02370        C6 =      0.98960

          PHASE DELTA(10) - ELUTION BUFFER
RATIO IONIC STRENGTHS IS(10)/IS(9) =      3.0
MIN PH =       4.5
MAX PH =       6.5

          PHASE EPSILON(11) - LOWER BUFFER
IS =          0.05
PHI(6) =      0.80

          PHASE PSI(5) AND TAU(6) - RESTACKING PARAMETERS
RFMAX =  0.90
MAX ABS(PH(5) - PH(9)) =  2.00
```

(*continued*)

the polymerization efficiency and gel concentration. Diluent was buffered 50% methanol (as in section 2). Subsequently, the diluted filtrate will be referred to as a "dilution."

3. Hydrolysis and Microdiffusion Analysis of Dilutions. Fractions of the dilutions were hydrolyzed by 2 *N* KOH in the outer well of sealed Conway microdiffusion cells (A. H. Thomas Cat. No. 3806-H10) and the ammonia resulting from hydrolysis was allowed to diffuse into boric acid contained in the inner well of the sealed cell and was titrated there with standardized HCl. The procedure followed that previously described (*9, 10*) except as explicitly stated. The porcelain Conway dishes were carefully cleaned and immersed in a silicone solution (1% Siliclad, Clay-Adams, Inc.). The dishes were dried overnight in a 110°C oven. Before use, molten paraffin–Vaseline was applied to the outer rim (in a 1:3 weight ratio), using a Pasteur pipet. Then 0.2 ml of boric acid indicator (0.0005% methyl red–0.0025% bromcresol green in 2% boric acid) was pipetted into the center well, and 0.2 ml of a dilution of the filtrate (containing approximately 20 μg of amide nitrogen) was pipetted

TABLE 2 (continued)

1 SYSTEM NUMBER

DATE = 01/31/72 COMPUTER SYSTEM NUMBER = Chrambach K
POLARITY = - (MIGRATION TOWARD ANODE) TEMPERATURE = 0 DEG. C.

CONSTITUENT 1 = NO. 18 , ACETIC ACID
CONSTITUENT 2 = NO. 99 , CHLORIDE -
CONSTITUENT 3 = NO. 99 , CHLORIDE -
CONSTITUENT 6 = NO. 2 , BETA ALANINE

	PHASES					
	ALPHA(1)	ZETA(4)	BETA(2)	PI(9)	LAMBDA(8)	GAMMA(3)
C1	0.0400	0.0420		0.0185		
C2			0.0538		0.0237	
C3						0.0237
C6	0.0419	0.0439	0.0557	0.9844	0.9896	0.9896
THETA	1.047	1.045	1.035	53.238	41.755	41.755
PHI(1)	0.235	0.235		0.847		
PHI(2)			1.000		1.000	
PHI(3)						1.000
PHI(6)	0.225	0.225	0.966	0.016	0.024	0.024
RM(1)	-0.186	-0.186		-0.669		
RM(2)			-1.626		-1.626	
RM(3)						-1.626
RM(6)	0.133	0.133	0.570	0.009	0.014	0.014
PH	4.23	4.23	2.24	5.48	5.30	5.30
ION.STR.	0.0094	0.0099	0.0538	0.0157	0.0237	0.0237
SIGMA	1.253	1.314	11.505	2.084	5.068	5.068
KAPPA	312.	326.	2659.	509.	1216.	1216.
NU	-0.148	-0.141	-0.141	-0.321	-0.321	-0.321
BV	0.033	0.035	0.004	0.041	0.053	0.053

RECIPES FOR BUFFERS OF PHASES ZETA(4),BETA(2),GAMMA(3),PI(9)

CONSTITUENT		1X PHASE 4	4X PHASE 2	4X PHASE 3	4X PHASE 9
ACETIC ACID	GM	2.52			0.44
IN HCL	ML		21.52		
IN HCL	ML			9.48	
BETA ALANINE	GM	3.91	1.98	35.27	35.08
H2O TO		1 LITER	100 ML	100 ML	100 ML
AT FINAL CONCENTRATION =					
PH(25 DEG.C.)		4.17	2.14	5.20	5.39
KAPPA(25 DEG.C.)		569.	4800.	2212.	927.

(*continued*)

into a sector of the outer well. This was followed by 0.2 ml of 4 *N* KOH–20% KHB_4O_7 (potassium tetraborate), pipetted into an opposite sector of the outer well. A drop of concentrated detergent solution (Column Coat, Canalco) was placed in the outer well between the sample and KOH–borate drops. A 2×2 in.^{2} glass cover was pressed onto the Vaseline–paraffin-coated rim sufficiently firmly to assure vapor-tight sealing. The sample and KOH–KHB_4O_7 drops were mixed by gently rotating the sealed dish. After standing overnight at room temperature the samples were titrated. A 200-μl Grunbaum–Kirk microburet (Micro-

TABLE 2 (continued)

1 SYSTEM NUMBER

DATE = 01/31/72 COMPUTER SYSTEM NUMBER = Chrambach E

PHASE DELTA(10) - ELUTION BUFFER

IS = 0.047

0 DEG.C. PH	KAPPA	25 DEG.C. PH	KAPPA	C6	C4
4.50	2337.	4.40	4224.	0.3501	0.0470
5.00	2337.	4.90	4224.	1.0057	0.0470
5.50	2337.	5.40	4224.	3.0788	0.0470
6.00	2337.	5.90	4224.	9.6345	0.0470
6.50	2337.	6.40	4224.	30.3655	0.0470

PHASE EPSILON(11)-LOWER BUFFER

IS = 0.050

0 DEG.C. PH	KAPPA	25 DEG.C. PH	KAPPA	C6	C5
3.09	2481.	2.99	4481.	0.0625	0.0500

STACKING AND UNSTACKING RANGES

PHASE ZETA(4) OR PI(9) RM(1)	PHI(1)	C(1)	C(6)	PH	PHASE BETA(2) OR LAMBDA(8) RM(2)	PHI(2)	C(2)	C(6)	PH	PHASE GAMMA(3) C(3)	C(6)	PH
-0.183	0.231	0.0420	0.0425	4.22	-1.63	1.000	0.0538	0.0543	1.69	0.0867	0.0876	1.69
-0.222	0.281	0.0420	0.0637	4.33	-1.63	1.000	0.0538	0.0755	3.30	0.0713	0.1001	3.30
-0.262	0.331	0.0420	0.0913	4.44	-1.63	1.000	0.0538	0.1031	3.65	0.0605	0.1160	3.65
-0.301	0.381	0.0420	0.1267	4.53	-1.63	1.000	0.0538	0.1386	3.89	0.0526	0.1355	3.89
-0.341	0.431	0.0420	0.1722	4.62	-1.63	1.000	0.0538	0.1840	4.07	0.0465	0.1591	4.07
-0.380	0.481	0.0420	0.2306	4.71	-1.63	1.000	0.0538	0.2424	4.23	0.0417	0.1878	4.23
-0.420	0.531	0.0420	0.3061	4.79	-1.63	1.000	0.0538	0.3179	4.38	0.0378	0.2231	4.38
-0.459	0.581	0.0420	0.4046	4.88	-1.63	1.000	0.0538	0.4165	4.52	0.0345	0.2671	4.52
-0.499	0.631	0.0420	0.5358	4.97	-1.63	1.000	0.0538	0.5476	4.65	0.0318	0.3234	4.65
-0.538	0.681	0.0420	0.7148	5.07	-1.63	1.000	0.0538	0.7266	4.79	0.0294	0.3977	4.79
-0.578	0.731	0.0420	0.9685	5.17	-1.63	1.000	0.0538	0.9803	4.93	0.0274	0.4998	4.93
-0.617	0.781	0.0420	1.3480	5.29	-1.63	1.000	0.0538	1.3598	5.08	0.0257	0.6490	5.08
-0.657	0.831	0.0420	1.9652	5.43	-1.63	1.000	0.0538	1.9771	5.24	0.0241	0.8868	5.24
-0.696	0.881	0.0420	3.1209	5.61	-1.63	1.000	0.0538	3.1327	5.45	0.0228	1.3255	5.45

RESTACKING PARAMETERS

CT7	PHASE PSI(5) IS	RM(7)	PHI(7)	C(7)	C(6)	PH	PHASE TAU(6) C(7)	C(6)	PH	PHI(7)	KAPPA
19	0.014	-0.520	0.826	0.0167	0.9826	5.54	0.0379	0.0398	4.29	0.211	236.
20	0.005	-0.189	0.356	0.0153	0.9812	5.94	0.0347	0.0366	4.96	0.054	53.
23	0.001	-0.025	0.061	0.0132	0.9791	6.77	0.0301	0.0320	5.84	0.008	6.
24	0.001	-0.022	0.077	0.0104	0.9763	6.77	0.0236	0.0255	5.79	0.009	5.
25	0.000	-0.010	0.032	0.0114	0.9773	7.12	0.0258	0.0277	6.16	0.004	2.
28	0.000	-0.006	0.010	0.0167	0.9826	7.46	0.0379	0.0398	6.59	0.001	2.
31	0.003	-0.103	0.224	0.0142	0.9800	6.18	0.0321	0.0340	5.22	0.031	26.
35	0.004	-0.135	0.306	0.0138	0.9797	6.05	0.0313	0.0332	5.06	0.043	35.
36	0.002	-0.055	0.116	0.0143	0.9802	6.46	0.0325	0.0344	5.53	0.015	13.
41	0.000	-0.005	0.008	0.0164	0.9823	7.59	0.0373	0.0392	6.71	0.001	1.

(*continued*)

chemical Specialties Co., Berkeley, Calif.) was used, with a standard HCl solution adjusted so that one major division on the buret corresponded to 1 μg of ammonia nitrogen. Our buret was used with 0.0056 N HCl. This provided a buret capacity of 35 μg N and a limit of detectability of 0.1 μg N.

Microdiffusion without alkaline hydrolysis was done by the same procedure, substituting 0.2 ml of 20% KHB_4O_7 for the KOH–KHB_4O_7 mixture. No Dowex filtrate yielded ammonia in the absence of KOH.

4. Calculations. When the method is carried out using extraction and dilution volumes as given above, the calculation reduces to

mg acrylamide/ml gel =
(μg N titrated) $\times$ (dilution factor)/8 (extractable monomer)

TABLE 2 (continued)

```
                    SYSTEM NUMBER
     INPUT DATA

DATE = 01/30/72    COMPUTER SYSTEM NUMBER = Chrambach   L
POLARITY = - (MIGRATION TOWARD ANODE)      TEMPERATURE =   0  DEG. C.


          SPECIFIED CONSTITUENTS
CONSTITUENT 1 = NO.  23 , TES
CONSTITUENT 2 = NO.  82 , PHOSPHATE-DIBASIC
CONSTITUENT 3 = NO.  99 , CHLORIDE -
CONSTITUENT 4 = NO.  99 , CHLORIDE -
CONSTITUENT 5 = NO.  99 , CHLORIDE -
CONSTITUENT 6 = NO.   5 , 4-PICOLINE

          SPECIFIED CONCENTRATIONS
PHASE ALPHA(1) -   C1 =      0.04000        C6 =      0.04400
PHASE  BETA(2) -   C2 =      0.04860        C6 =      0.05350
PHASE GAMMA(3) -   C3 =      0.05580        C6 =      0.95650

          PHASE DELTA(10) - ELUTION BUFFER
RATIO IONIC STRENGTHS IS(10)/IS(9) =      3.0
MIN PH =      7.0
MAX PH =      9.0

          PHASE EPSILON(11) - LOWER BUFFER
IS =         0.05
PHI(6) =     0.80

          PHASE PSI(5) AND TAU(6) - RESTACKING PARAMETERS
RFMAX =  0.90
MAX ABS(PH(5) - PH(9)) =  2.00
```

(*continued*)

In detail;

$$\text{mg acrylamide (mol wt} = 70.8) = \text{mg N (AW} = 14)/\text{gel} \times 5$$
$$\text{mg N/gel} = \mu\text{g N/gel} \times 10^{-3}$$
$$\mu\text{g N/gel} = \mu\text{g N/2.5-ml filtrate} \times 2$$
$$\mu\text{g N/2.5-ml filtrate} = \mu\text{g N/0.2-ml filtrate} \times 12.5$$
$$\mu\text{g N/0.2-ml filtrate} = \mu\text{g N titrated/0.2 dilution} \times \text{dilution factor}$$

The %EM was calculated as

$$\%\text{EM} = \frac{\text{mg extractable monomer/ml gel}}{\text{total mg monomer/ml gel}}$$

the %PE as

$$\%\text{PE} = 100 - \%\text{EM}$$

TABLE 2 (continued)

SYSTEM NUMBER

DATE = 01/30/72 COMPUTER SYSTEM NUMBER = Chrambach L
POLARITY = - (MIGRATION TOWARD ANODE) TEMPERATURE = 0 DEG. C.

CONSTITUENT 1 = NO. 23 , TES
CONSTITUENT 2 = NO. 82 , PHOSPHATE-DIBASIC
CONSTITUENT 3 = NO. 99 , CHLORIDE -
CONSTITUENT 6 = NO. 5 , 4-PICOLINE

	PHASES					
	ALPHA(1)	ZETA(4)	BETA(2)	PI(9)	LAMBDA(8)	GAMMA(3)
C1	0.0400	0.0400		0.0293		
C2			0.0486		0.0247	
C3						0.0558
C6	0.0440	0.0439	0.0535	0.9300	0.9458	0.9565
THETA	1.100	1.098	1.101	31.692	38.366	17.142
PHI(1)	0.123	0.123		0.517		
PHI(2)			0.012		0.744	
PHI(3)						1.000
PHI(6)	0.112	0.112	0.919	0.016	0.045	0.058
RM(1)	-0.051	-0.051		-0.212		
RM(2)			-0.585		-0.870	
RM(3)						-1.626
RM(6)	0.080	0.080	0.653	0.012	0.032	0.041
PH	7.11	7.11	5.15	7.99	7.53	7.42
ION.STR.	0.0049	0.0049	0.0498	0.0152	0.0613	0.0558
SIGMA	0.533	0.533	6.166	1.641	6.730	12.579
KAPPA	136.	136.	1431.	401.	1545.	2902.
NU	-0.095	-0.095	-0.095	-0.129	-0.129	-0.129
BV	0.020	0.020	0.010	0.051	0.105	0.121

PHASE ETA(7) X1= 1.332 X2= 0.031 X3= 1.971 X4= 0.046

RECIPES FOR BUFFERS OF PHASES ZETA(4),BETA(2),GAMMA(3),PI(9)

CONSTITUENT		1X PHASE 4	4X PHASE 2	4X PHASE 3	4X PHASE 9
TES	GM	9.17			2.69
1M PHOSPHORIC ACID	ML		19.44		
1N HCL	ML			22.32	
4-PICOLINE	GM	4.09	1.99	35.63	34.64
H2O TO		1 LITER	100 ML	100 ML	100 ML
AT FINAL CONCENTRATION =					
PH(25 DEG.C.)		6.77	5.01	7.27	7.73
KAPPA(25 DEG.C.)		404.	2832.	5445.	1063.

(*continued*)

The reproducibility of %EM was evaluated by standard methods of analysis of variance (ANOVA). The relationship between %EM and catalyst concentrations was analyzed by multiple regression analysis using several models, cluster analysis, and rank correlation methods.

5. Determination of PA by Total Amide–Nitrogen Obtained After Acid Hydrolysis. Polyacrylamide (Gelamide 250) was determined quantitatively by hydrolysis in 6 *N* HCl for 3 hr at 110°C, followed by microdiffusion analysis of the hydrolyzate for ammonia. In order to prevent diffusion of 6 *N* HCl into the boric acid indicator, the hydrolyzate was

TABLE 2 (continued)

SYSTEM NUMBER

DATE = 01/30/72 COMPUTER SYSTEM NUMBER = Chrambach L

PHASE DELTA(10) - ELUTION BUFFER

IS = 0.046

0 DEG.C.		25 DEG.C.			
PH	KAPPA	PH	KAPPA	C6	C4
7.00	2393.	6.85	4499.	0.3264	0.0455
7.50	2393.	7.35	4499.	0.9335	0.0455
8.00	2393.	7.85	4499.	2.8537	0.0455
8.50	2393.	8.35	4499.	8.9256	0.0455
9.00	2393.	8.85	4499.	28.1267	0.0455

PHASE EPSILON(11)-LOWER BUFFER

IS = 0.050

0 DEG.C.		25 DEG.C.			
PH	KAPPA	PH	KAPPA	C6	C5
5.61	2615.	5.46	4912.	0.0625	0.0500

STACKING AND UNSTACKING RANGES

PHASE ZETA(4) OR PI(9)				PHASE BETA(2) OR LAMBDA(8)					PHASE GAMMA(3)			
RM(1)	PHI(1)	C(1)	C(6)	PH	RM(2)	PHI(2)	C(2)	C(6)	PH	C(3)	C(6)	PH
-0.050	0.122	0.0400	0.0433	7.10	-0.58	0.010	0.0487	0.0528	5.07	0.2359	0.2460	4.84
-0.068	0.167	0.0400	0.0818	7.26	-0.62	0.110	0.0449	0.0944	6.16	0.1730	0.2681	5.95
-0.086	0.210	0.0400	0.1336	7.38	-0.66	0.210	0.0419	0.1485	6.49	0.1376	0.3070	6.30
-0.104	0.254	0.0400	0.2044	7.49	-0.70	0.310	0.0397	0.2210	6.72	0.1137	0.3595	6.54
-0.123	0.301	0.0400	0.3036	7.59	-0.74	0.410	0.0378	0.3217	6.91	0.0959	0.4282	6.75
-0.145	0.353	0.0400	0.4486	7.70	-0.78	0.510	0.0363	0.4680	7.09	0.0817	0.5205	6.94
-0.170	0.414	0.0400	0.6748	7.81	-0.82	0.610	0.0350	0.6952	7.26	0.0697	0.6515	7.13
-0.200	0.488	0.0400	1.0660	7.94	-0.86	0.710	0.0339	1.0872	7.46	0.0591	0.8569	7.34
-0.240	0.585	0.0400	1.8827	8.11	-0.90	0.810	0.0330	1.9046	7.70	0.0493	1.2438	7.59
-0.300	0.733	0.0400	4.5430	8.40	-0.93	0.910	0.0322	4.5656	8.07	0.0394	2.3719	7.98

RESTACKING PARAMETERS

	PHASE PSI(5)						PHASE TAU(6)				
CT7	IS	RM(7)	PHI(7)	C(7)	C(6)	PH	C(7)	C(6)	PH	PHI(7)	KAPPA
24	0.014	-0.169	0.604	0.0227	0.9234	8.03	0.0309	0.0348	7.06	0.140	105.
25	0.008	-0.102	0.320	0.0249	0.9256	8.27	0.0340	0.0379	7.43	0.063	56.

neutralized (to a normality of 0.1 *N* acid or less) by KOH immediately after it was placed into the Conway cell. The normality of the KOH was selected to maintain a pH of less than 7 in the neutralized hydrolyzate, to avoid loss of ammonia prior to sealing of the cell. The buffering capacity of KHB_4O_7 was sufficient to neutralize any residual acid while maintaining a pH very close to 9.1 during the diffusion step. Other conditions of microdiffusion analysis were as described above.

6. Spectrophotometric Analysis. The extract was analyzed in a Gilford 2000 spectrophotometer (1 cm path length) with digital readout at 230 to 320 nm. Absorbance values were obtained against water blanks.

7. Electrophoretic Studies. The pH of the gel both before and after electrophoresis was measured as described previously (*2, 11*). In general, the position of the buffer discontinuity (PI–LAMBDA boundary) (*1, 2, 7*), frequently termed the "stack" or "front," could not be marked by use of tracking dyes such as Methyl Green and Brilliant Green in

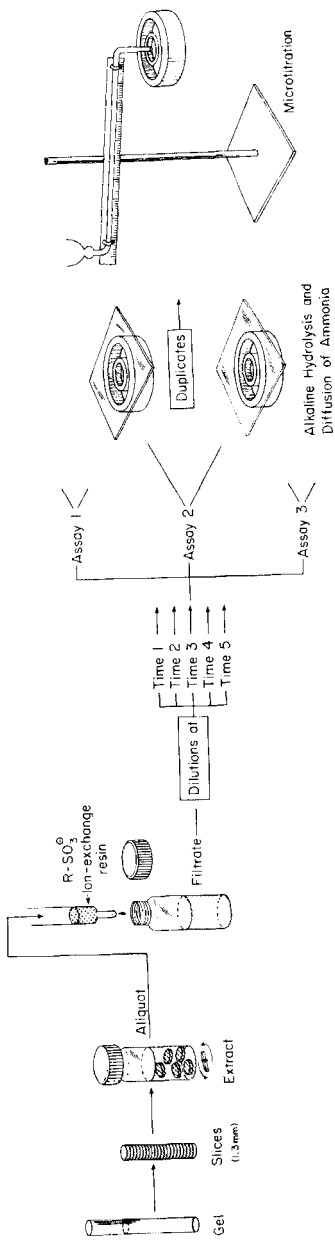

FIG. 1. Schematic illustration of the procedure for analysis of %EM in cross-linked polyacrylamide gels.

the buffer systems used here (systems 35 and 35.11). Therefore, the positions of the PI–LAMBDA moving boundaries were determined (*2*, *7*) as the points of inflection on curves of pH vs gel slice number. Alternatively, when pyridine was the "leading ion" (CONSTITUENT 2), curves of absorbance at 260 nm vs slice number were constructed. The R_f values for several proteins and dyes were then calculated with reference to the PI–LAMBDA boundary (*2*).

RESULTS

Validation of Method for Determining Percent Extractable Monomer

1. Determination of the Amide–Nitrogen of Acrylamide Monomer Solutions. Acrylamide was determined quantitatively by alkaline hydrolysis and microdiffusion analysis of amide–nitrogen using an acrylamide solution in water. Values obtained reached a maximum at 95 to 99% of the amide–nitrogen values calculated on the basis of weight, when the microdiffusion analysis was carried out overnight and at room temperature according to the standard procedure. After hydrolysis and diffusion the amide–nitrogen of acrylamide is quantitatively recovered from aqueous solutions in 36 hr (25°C) or 16 hr (40°C) and from 50% methanol within 16 hr (25°C).

2. Extraction of Residual Monomer from PA Gel. Preliminary studies used extraction by aqueous solvents. In such media the gel slices swell and are pulverized during stirring, even when very small magnetic stirring bars are used. Extraction was incomplete and irreproducible even after long periods and/or several changes of media. Accordingly, methanol was selected as a solvent for extraction since it had been successfully used by Chen (*6*) to separate methanol-insoluble PA from soluble monomer. Extraction of PA gel slices in 66% methanol was found to be reproducible. Extraction times between 1.5 and 42 hr gave identical amounts of extractable amide–nitrogen. Methanol at a concentration of 50% (obtained after passage through Dowex-50) does not interfere with the subsequent alkaline hydrolysis and microdiffusion analysis. Extracts in 66% methanol were stable if protected from evaporation. Since PA gel slices dehydrate, shrink, and harden in 66% methanol, they can be readily separated from the extract.

3. Removal of Volatile Amines from the Extract by Dowex 50. Ammonia, TEMED, and other volatile amines or amino compounds that might give rise to volatile amines after alkaline hydrolysis were removed

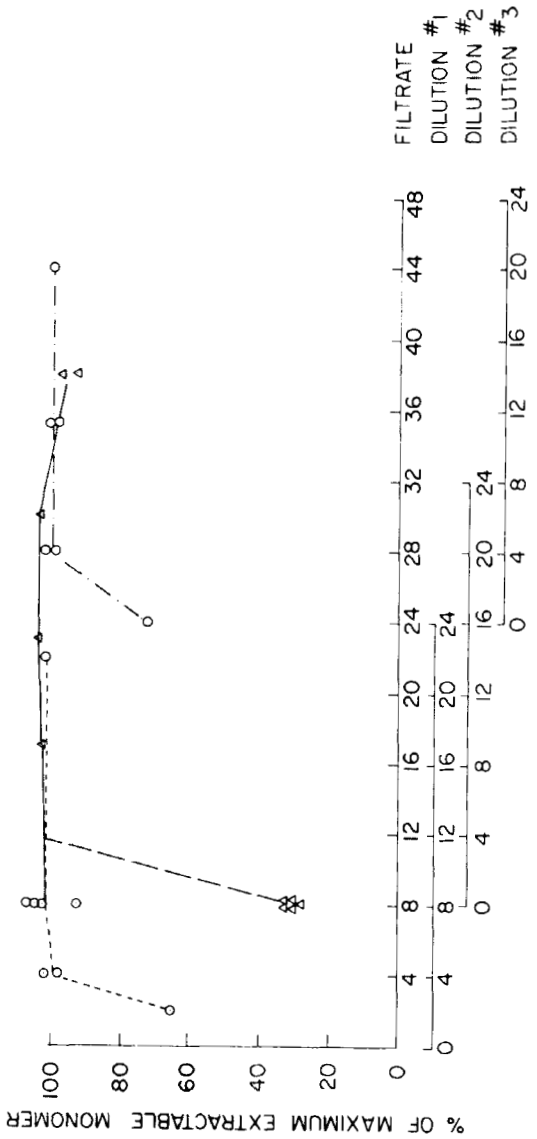

FIG. 2. Maximum %EM vs age of dilution at different ages of filtrate (single gel).

from the extract by Dowex 50 × 8 (200–400 mesh) at pH 4.7. Recovery of acrylamide after passage through Dowex 50 was quantitative. The capacity of the resin was adequate to remove quantitatively the maximal amine concentrations in gels used in the present studies (0.46 mmole TEMED or 0.95 mmole 4-picoline). Filtration through Dowex 50 did not increase absorbance of the extract at 260 nm.

4. Dependence of Amide Analysis on Age of Dilution of Dowex 50 Filtrate. The values of %EM obtained as a function of the age of the filtrate remain constant for a period of up to 35 days. In contrast, the value of %EM is highly dependent on the age of the dilution, irrespective of the age of the filtrate from which it was taken (Fig. 2): %EM increases with time and reaches a plateau value when the dilution is 3–4 days old. Although values obtained on the first day after making the dilution are extremely variable, the plateau value is reproducible for dilutions from a single filtrate. Figure 3 shows the time course for

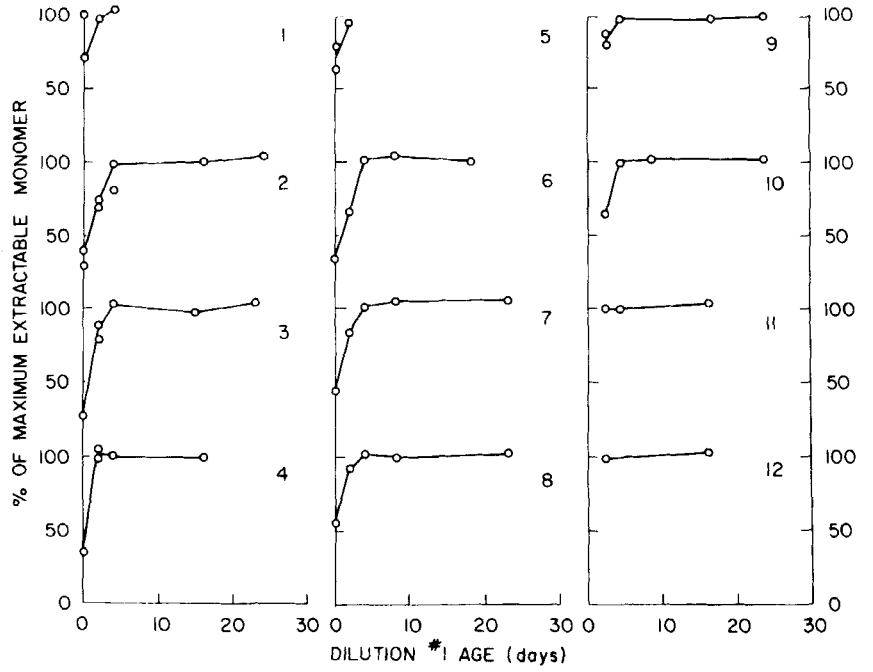

FIG. 3. %EM (as fraction of maximum plateau value) vs age of dilution at a single age of filtrate in twelve gels polymerized under markedly different conditions.

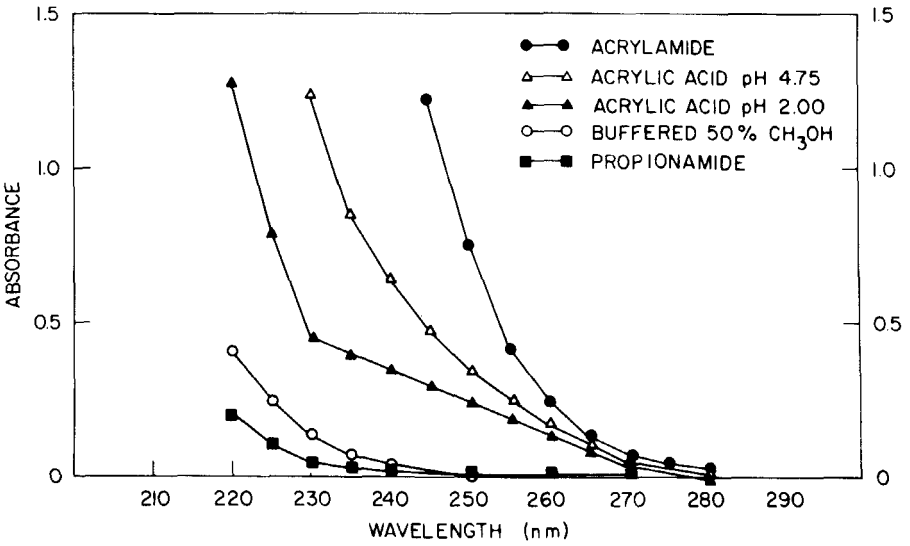

Fig. 4. Absorbance of acrylamide and related compounds (3.57 n*M*) vs wavelength (nm).

%EM expressed as a percentage of the final plateau value for twelve representative gels after making the dilution. The plateau is reached after 4 days in each case, irrespective of the final level of %EM.

5. Spectrophotometric Determination of Acrylamide in the Filtrate. Acrylamide can be differentiated from noncross-linked PA or propionamide by ultraviolet (UV) absorbance since its end absorption occurs at higher wavelengths. It is also possible to distinguish acrylamide from acrylic acid by the ratio of the absorbance at 260 nm at two ph values (pH 2.00 and 4.75), since absorbance of acrylamide is independent of pH, unlike acrylic acid (Fig. 4). Thus, in the absence of interference by other absorbing substances. acrylamide could be estimated by spectrophotometry. The absorbance at 260 nm of acrylamide solutions was found to correlate closely with amide content determined by microdiffusion analysis. However, the absorbance of the diluted filtrate of cross-linked PA in 66% methanol was about 30% higher than that of recrystallized acrylamide. This effect was assumed to be due to side-products of the polymerization reaction and was not further investigated. This evidence for UV-absorbing impurities invalidated the spectrophotometric method for quantitative analysis of monomer extractable from PA gel.

Applications

1. Reproducibility of Measurement of %EM and Reproducibility of Polymerization (phase GAMMA, system 35, 10.2%T, 2%C). Gels were made with a wide variety of catalyst concentrations to provide a wide range of %EM or %PE. Table 3 and Fig. 5 present the analysis of variance in reproducibility for the %EM.

Variance was calculated (see Fig. 1) between duplicates (microdiffusion assays same day), between assays (microdiffusion analyses on different days), between dilutions (of the same filtrates), between gels prepared on the same day, and, finally, between gels prepared on different days. The estimates of variance were expressed as standard deviations (σ) of %EM. In all cases, there was a direct proportionality between σ and %EM (Fig. 5). The slopes of the lines represent a mean coefficient of variation (CV) and are given in Table 3. As expected, the variance increased progressively throughout this hierarchy: from variation between duplicates, assays, and dilutions, which may be regarded as "method" errors, to variation between gels which is attributed to the polymerization process. The CV for gels polymerized on different days

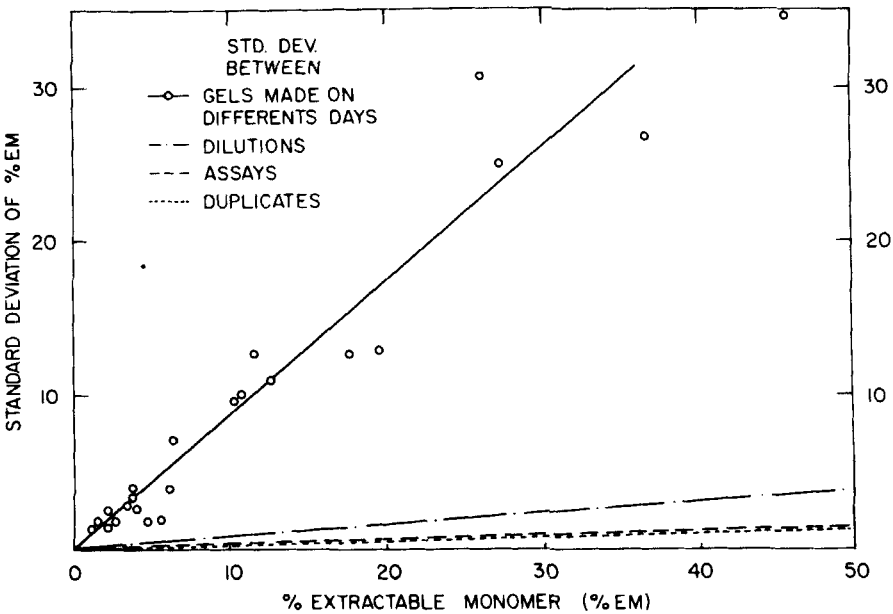

FIG. 5. Standard deviation of replicate measurements of %EM vs mean %EM for these measurement. A direct proportionality is observed; the slope of each line represents a coefficient of variation.

TABLE 3

Analysis of Variance for Reproducibility of %EM Measurements and of Polymerization (System 35, 10.2%T, 2%C)

Source of variation[a]	No. of gels	*df*	*CV*[b]
Duplicates	100	415	0.026
Assays	100	527	0.044
Dilutions	100	121	0.140
Gels, same day	42	21	0.220
Gels, different days	88	65	0.830

[a] Duplicates—variation between two microdiffusion analyses on the same dilution, same day; assays— variation beteen two microdiffusion analyses on the dilution: variation between two dilutions of the filtrate from one gel.

[b] CV = mean coefficient of variation
= mean $(\sigma/\bar{x})$, where σ is the standard deviation of %EM and $\bar{x}$ is the mean %EM
= slope of σ (%EM) versus %EM. (This is the slope of the line in Fig. 5.)

is much larger than can be accounted for by the errors in the method of measurement. This increment in CV is a reliable estimate of the reproducibility of polymerization. These results indicate that the only way to achieve a uniform, reproducible gel [e.g., σ (%EM) = 1%) is to achieve a high degree of polymerization (e.g., %EM = 1%, %PE = 99%]. [*Note:* Standard deviations given here are the square-root of the corresponding mean squares, and do *not* represent an analysis of "components of variance." Accordingly, these σ's provide markedly overconservative estimates of the variability between gels.]

2. Determination of Extractable Amide-Nitrogen as a Function of Initiator Concentration. Figure 6 depicts the %EM for 10%T, 2%C gels made in water (no buffer) using variable concentrations of RN (KP and TEMED are held constant at extremely low, ineffectual values). Under these conditions, there is a direct relation between RN concentration and %EM or its complement, the %PE.

3. Polymerization Efficiency in 4-Picoline Buffer (pH 7.42, 0°C) as a Function of KP, RN, and TEMED Concentrations. Table 4 lists the %EM for gels (phase GAMMA; system L, 10.2%T, 2%C) polymerized with variable catalyst concentrations. In this neutral buffer system, KP

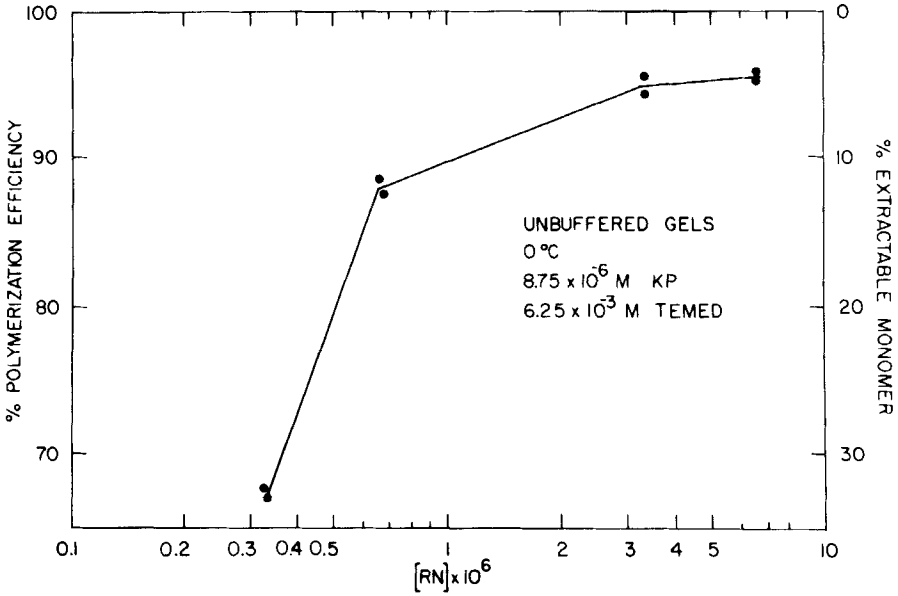

FIG. 6. %EM vs [RN], unbuffered gel.

and TEMED contribute to the polymerization efficiency in a compensatory fashion such that a decrease in one may be compensated for by an increase in the other. In contrast, decrease of RN concentration by a factor of 100 (from 7×10^{-5} to 7×10^{-7}) is without a major effect

TABLE 4

%EM as a Function of Catalyst Concentrations (System L, 10.2%T, 2%C)

[KP] × 10^3	[RN] × 10^6	[TEMED] × 10^3	%EM
0.525	65.6	62.5	3.1
10.5	65.6	.625	2.9
.70	65.6	12.5	2.5
1.09	65.6	6.25	2.3
3.5	65.6	3.12	2.5
0.263	6.56	12.5	8.6
0.875	6.56	3.125	7.1
5.25	0.656	62.5	2.1
10.5	0.656	12.5	3.5

TABLE 5A

%EM in Gels as a Function of Catalyst Concentrations (System 35, 10.2%T, 2%C)

[KP] × 10^4	[RN] × 10^5	[TEMED] × 10^3	n	%EM	[KP][RN] [TEMED] × 10^{12}
105	3.9	62.5	1	0.43	25800.0
105	6.56	6.25	1	0.59	4310.0
5.25	6.56	62.5	1	0.79	2150.0
5.25	3.9	28.0	4	1.95	579.0
5.25	6.56	14.0	4	1.48	482.0
5.25	6.56	14.0	4	1.48	482.0
5.25	6.56	28.0	1	1.55	965.0
5.25	6.56	6.25	2	1.96	2.5.0
5.25	4.59	6.25	1	1.98	151.0
5.25	6.56	7.0	4	2.16	241.0
5.25	3.9	14.0	3	2.06	289.0
5.25	1.3	62.5	6	2.08	431.0
5.25	5.25	6.25	1	2.51	172.0
5.25	3.9	6.25	1	2.96	129.0
5.25	1.3	42.0	1	3.1	289.0
5.25	1.3	25.0	1	3.47	172.0
5.25	6.56	3.5	3	3.50	121.0
5.25	3.28	6.25	1	3.58	108.0
5.25	3.9	7.0	3	3.76	145.0
26.25	0.13	62.5	4	3.80	215.0
5.25	1.3	28.0	1	3.9	193.0
5.25	6.56	2.1	3	4.00	72.4
26.25	1.3	25.0	4	4.81	860.0
5.25	2.6	6.25	1	4.98	86.0
5.25	3.9	3.5	3	5.60	72.4
26.25	6.56	1.56	5	6.44	269.0
105.0	6.56	0.31	5	6.66	215.0
26.25	1.3	6.25	1	7.01	215.0
5.25	1.3	14.0	1	7.10	96.5
52.5	1.3	6.25	1	8.62	430.0
1.05	1.3	6.25	1	8.65	8.6
10.50	1.3	6.25	1	9.05	86.0
5.25	1.3	7.0	1	9.20	48.2
0.0875	1.3	25.0	4	10.50	2.87
0.875	3.9	6.25	4	10.70	21.5
0.0875	3.9	25.0	4	11.6	8.6
105.0	3.9	1.56	1	11.7	650.0
5.25	3.9	1.56	4	12.7	32.3
5.25	1.3	6.25	10	15.7	43.1
0.0875	3.9	1.56	1	16.2	0.538

(*continued*)

TABLE 5A (continued)

[KP] × 10^4	[RN] × 10^5	[TEMED] × 10^3	n	%EM	[KP][RN] [TEMED] × 10^{12}
26.25	3.9	0.31	5	17.0	32.3
105.0	1.3	1.56	1	17.2	215.0
105.0	1.3	6.25	6	19.2	860.0
0.875	6.56	0.31	5	20.0	1.79
105.0	1.3	0.31	1	27.8	43.1
0.0875	6.56	1.56	5	38.3	0.897
0	1.3	6.25	2	39.4	0
0.875	3.9	0.31	1	46.1	1.07

TABLE 5B

%EM in Nongelled Polymerization Mixtures as a Function of Catalyst Concentrations (System 35, 10.2%T, 2%C)

[KP] × 10^4	[RN] × 10^5	[TEMED] × 10^3	n	%EM	[KP][RN] [TEMED] × 10^{12}
5.25	1.3	6.25	1	85.25	43.1
105.0	0.13	1.56	1	91.0	21.5
26.25	0.13	6.25	1	83.5	21.5
5.25	0.656	6.25	2	99.5	21.5
5.25	0.13	25.0	1	96.0	17.2
5.25	1.3	1.56	4	97.42	10.8
26.25	1.3	0.31	1	100.5	10.8
0.875	1.3	6.25	1	91.3	7.18
5.25	0.13	6.25	5	94.88	4.31
5.25	1.3	0.31	3	95.43	2.15
0.0875	1.3	6.25	3	86.5	0.72
5.25	0.013	6.25	5	91.3	0.43
0.875	1.3	0.31	1	100.5	0.36
5.25	0.13	0.31	1	98.0	0.22
0.0875	1.3	1.56	2	95.8	0.18
0.875	0.13	1.56	1	97.0	0.18
0.0875	3.9	0.31	1	—	0.11
0.0875	0.13	6.25	1	94.0	0.07
0.0875	0.13	0.31	1	99.0	0.00
5.25	0	6.25	5	95.9	0
5.25	1.3	0	3	99.0	0

on polymerization efficiency. (RN is generally regarded to be relatively ineffective above neutrality.)

4. Polymerization Efficiency for 10.2%T Gels at pH 3.9 and 0°C (System 35) as a Function of KP, RN, and TEMED Concentrations. Nine concentrations of each of the three catalysts were used in many (129) but not all (729) combinations with each other. The values for %EM in gels of 10.2%T are shown in Fig. 7 and Table 5, together with the catalyst concentrations used. (Table 5A shows results for polymerization conditions that produced a solid gel; Table 5B shows results for solutions that, on gross inspection, appeared liquid and were expected to show 100 %EM.) If the concentrations of two catalysts are held constant, increase in the concentration of the third catalyst results in improved %PE. Examples for these are shown in Fig. 8 and Table 6.

In the buffer system used, the slope of %EM vs log [KP] (Fig. 7) appears to be smaller than the slope of %EM vs log [TEMED]. However, as a first approximation, we may assume that the three catalysts contribute in an equivalent fashion. Accordingly, the product of the three concentrations defining each set of polymerization conditions was calculated (Table 5). Plots of %EM vs [KP][RN][TEMED] are shown in Fig. 9 and 10. There is a general correlation between %EM and log [KP][RN][TEMED]. [Spearman's rank correlation coefficient = 0.85, for gelled mixtures (Table 5A).] Use of a log transformation of the %EM scale (Fig. 10) results in partial linearization and reduction of the nonuniformity of variance. Also, this (implicitly) gives more "weight" to

TABLE 6

%EM (Average) in Gels (System 35, 10.2%T, 2%C) Polymerized to 90% or More with 5.25×10^{-4} *M* KP and Variable RN and TEMED Concentrations

[TEMED] $\times 10^3$	[RN] $\times 10^5$		
	1.3	3.9	6.56
2.1			4.00
3.5		5.60	3.50
7.0	9.20	3.76	2.02
14.0	7.10	2.06	1.38
28.0	3.90	1.37	
42.0	3.10		

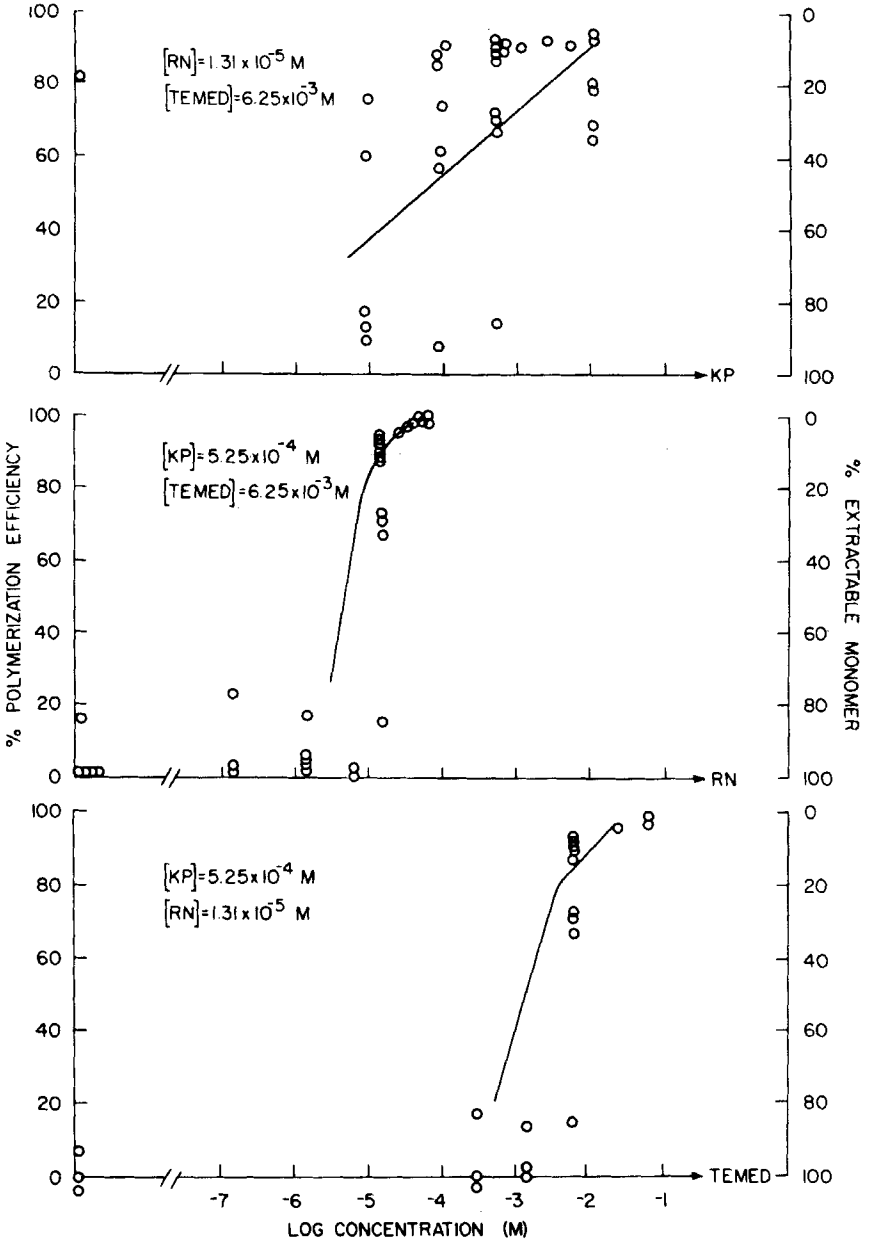

FIG. 7. %EM vs log [KP] (or [RN] or [TEMED]) when the concentrations of the two other catalyst concentrations are held constant at arbitrary levels.

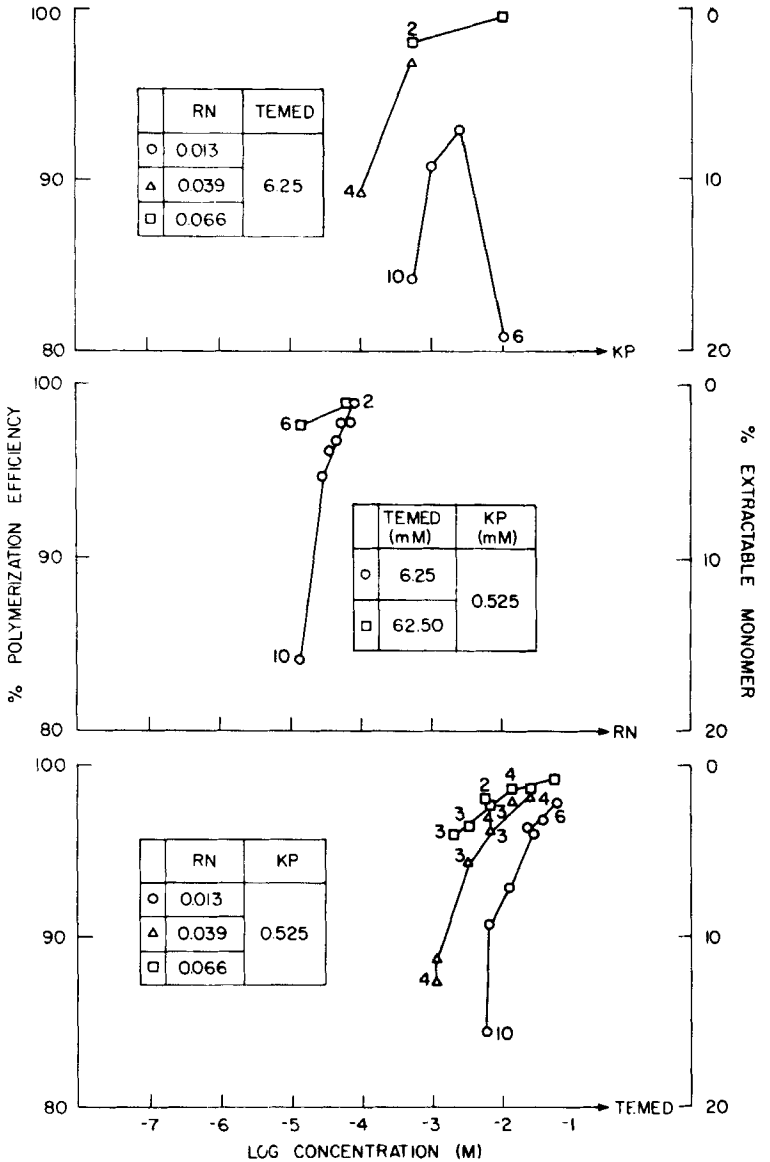

FIG. 8. Families of curves of the type shown in Fig. 7, when one catalyst is held constant at an arbitrary level and the other two catalyst concentrations are varied.

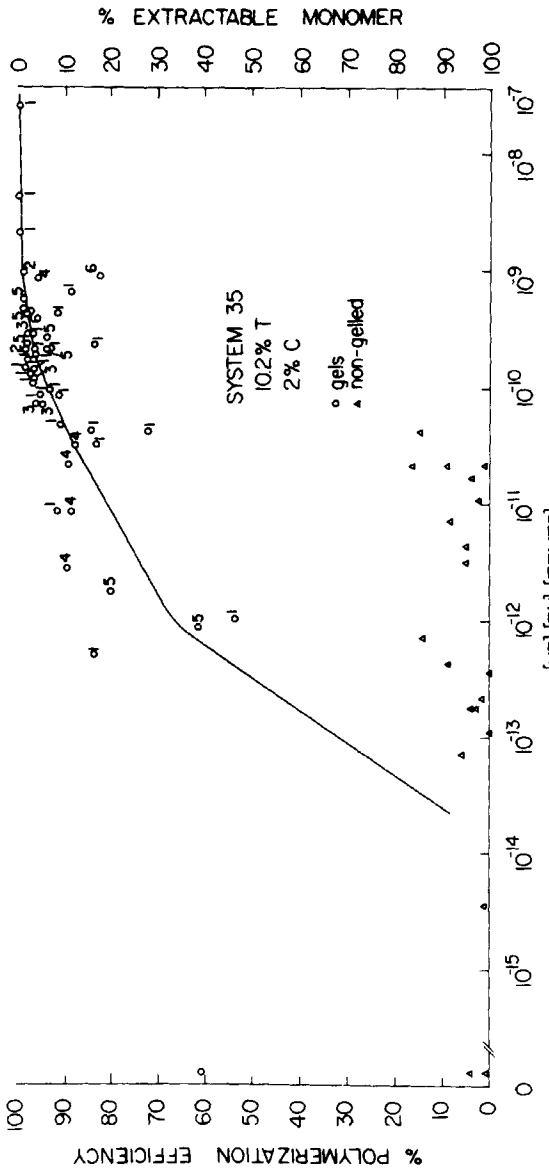

FIG. 9. Plot of %EM vs log ([KP][RN][TEMED]). Buffer system 35.

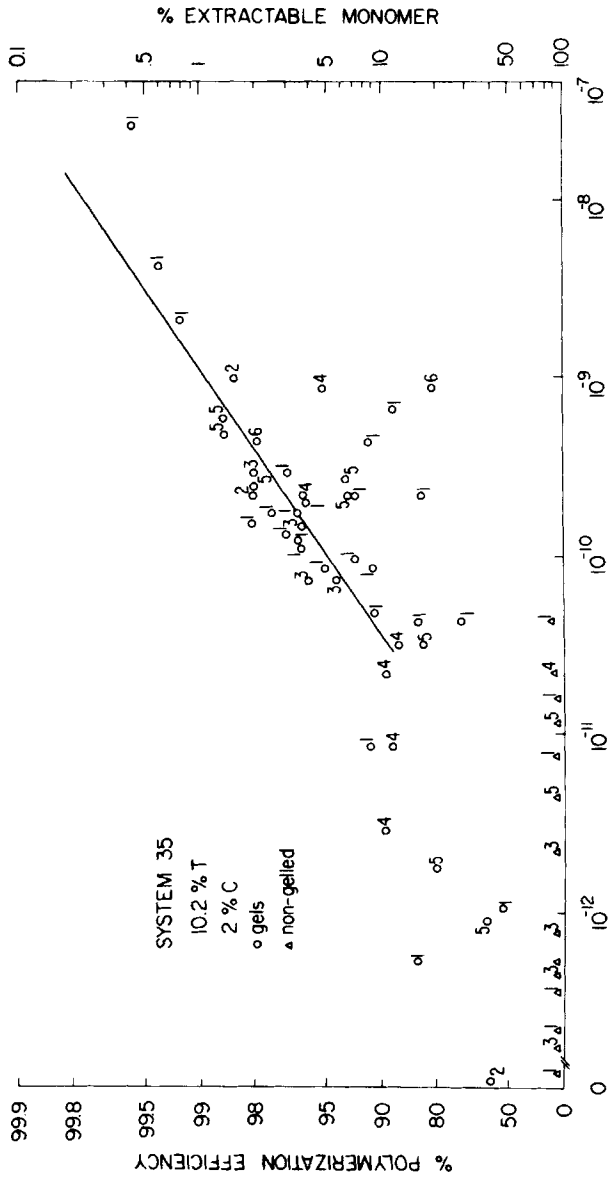

FIG. 10. Plot of log (%EM) vs log ([KP][RN][TEMED]). Buffer system 35.

the low values of %EM (i.e., "good" gels), and less weight to the poorly polymerized gels. [In view of the relationship between σ (%EM) and %EM, a change from 1 to 10% EM is as significant as a change from 10 to 50%. This is emphasized by the log transform (Fig. 10).]

Several attempts were made to develop statistical models to describe these data. One empirical model, for purposes of curve fitting, was

$$\%\text{EM} = \frac{k}{[\text{KP}]^a[\text{RN}]^b[\text{TEMED}]^c}$$

The parameters of this model were estimated by multiple regression after log transforms of both sides. For the gelled mixtures only (Table 5A), $a = 0.2$, $b = 0.8$, $c = 0.6$. This was in accord with the findings of Fig. 7: KP appears less effectual than the other two catalysts. The above model did not "converge," i.e., no satisfactory estimates of the parameters (k, a, b, c) were obtained when data from both gelled and nongelled mixtures (Tables 5A and 5B) were combined. The above model is unsatisfactory except over a limited range, since %EM would increase without limit as the catalyst concentrations approach zero.

Several other models were tested, e.g.,

$$\%\text{EM} = 100 - a[\text{KP}] - b[\text{RN}] - c[\text{TEMED}]$$

$$\frac{100}{\%\text{EM}} = \frac{k}{a[\text{KP}] + b[\text{RN}] + c[\text{TEMED}]}$$

However, once again, the regression analysis failed to converge. Thus,

TABLE 7

%EM as a Function of Gel Concentration in Gels (System 35, 2%C) Polymerized by Several Combinations of Catalyst Concentrations

[KP] × 10^4	[RN] × 10^5	[TEMED] × 10^3	5.0%T, 2%C	10.2%T, 2%C	15.0%T, 2%C
5.25	6.56	62.5	2.4	0.8	0.5
5.25	3.90	25.0	3.8	1.5	1.0
0.875	3.90	62.5	4.9	1.6	1.2
26.00	3.90	0.31	46.0	17.0	11.0
0.875	6.56	0.31	95.0	19.6	23.0
0.0875	6.56	1.56	27.0	45.8	33.0

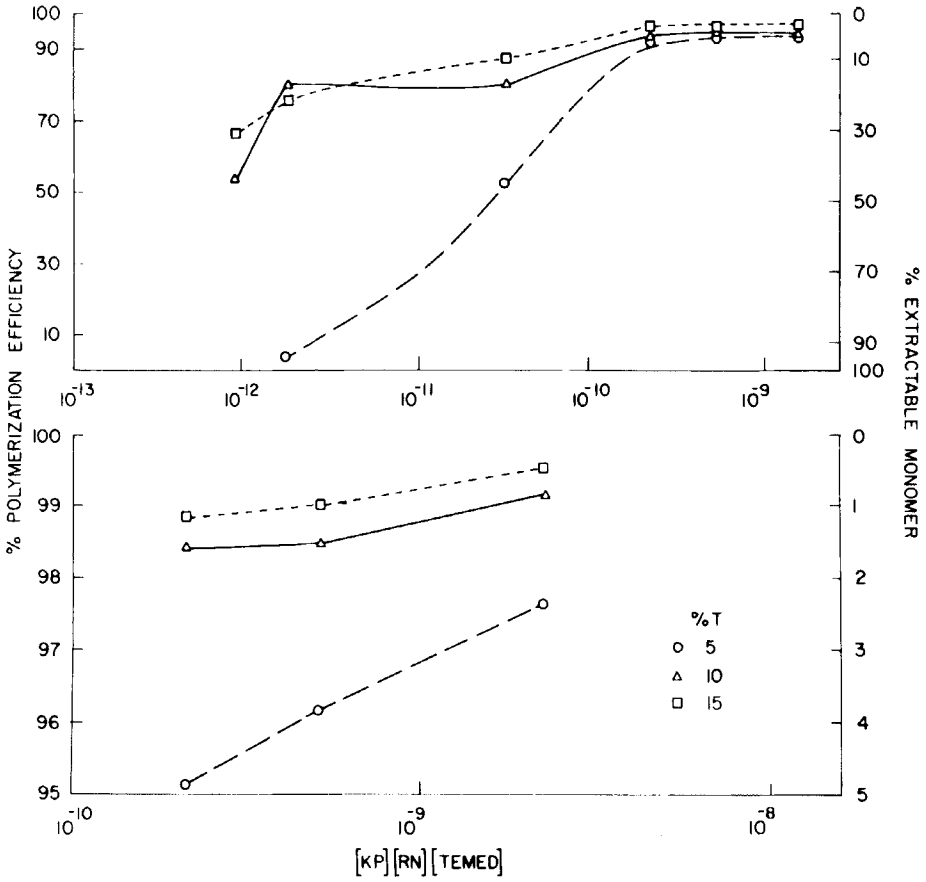

Fig. 11. Effect of gel concentration on the relationships shown in Fig. 9 and 10.

it appears that the precision of the data does not justify further attempts to develop a mathematical or chemical model to relate %EM to the catalyst concentrations. The problem is further complicated by the apparent dichotomy between the behavior of gelled and nongelled mixtures (Figs. 9 and 10, Tables 5A and 5B).

5. The Effect of Gel Concentration on Polymerization Efficiency (Table 7 and Fig. 11). The efficiency of any one of several arbitrarily selected sets of catalyst concentrations increases with increasing gel concentration. Figure 11 presents these data in a manner analogous to Fig. 9 and 10.

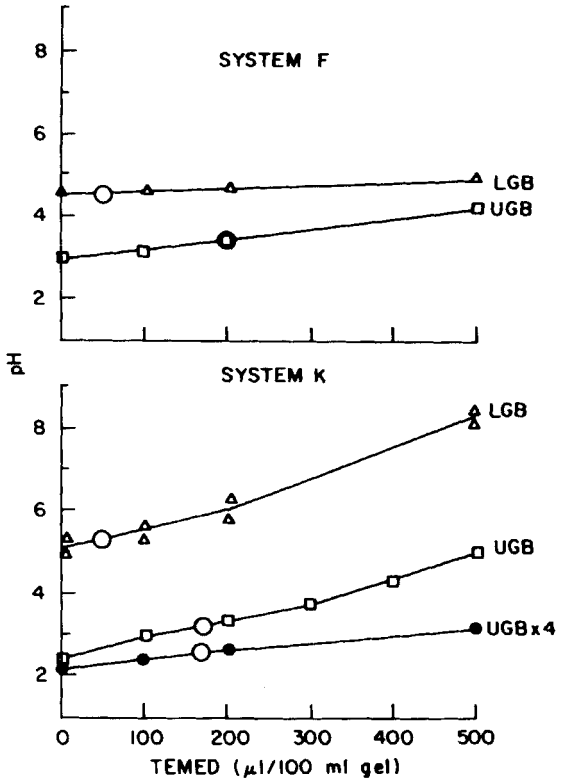

FIG. 12. pH vs [TEMED] for UGB and LGB for buffer systems F and K.

The finding of an inverse relation between catalyst concentrations and %T agrees with previous experience in most buffer systems. For many years, as an empirical rule in this laboratory, the TEMED concentration has been varied in inverse proportion of %T, while KP and RN were held constant.

6. Effect of TEMED Concentration on pH of the Gel Buffer, pH of the gel, and R_f. Figure 12 shows the effect of TEMED concentration on the pH of two representative gel buffers (systems F and K). Addition of conventional amounts of TEMED to either the upper gel buffer or the lower gel buffer raises the pH of these buffers significantly. Accordingly, one can expect the mobility of the PI–LAMBDA boundary (*1*) and the R_f values for proteins to depend on the amount of TEMED used.

Figure 13 and Table 8 show that increasing the TEMED concen-

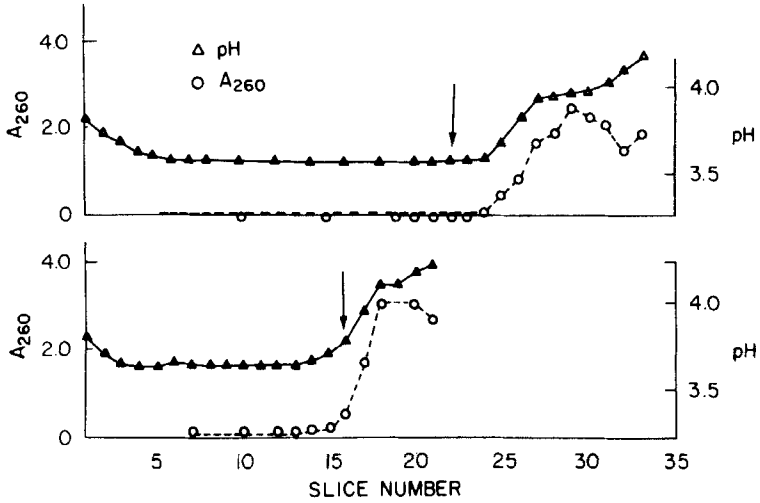

FIG. 13. The pH profile within the gel after electrophoresis for a single [TEMED], showing effect on stacking of Methyl Green. (Top) buffer system 35; (Bottom) buffer system 35.11.

TABLE 8

R_f of Methyl Green and Brilliant Green as a Function of TEMED Concentration (Systems 35 and 35.11, 10.2%T, 2%C)

No.	[TEMED] (μl/100 ml)	pH (9)	RM(1, 9)	R_f Methyl Green	R_f Brilliant Green	System
Ideal	0	3.50[a]	0.359	—	—	35
A	224	3.56	0.255	0.86	0.50	
B	1000	—	—	—	0.60	
Ideal	0	3.67[a]	0.301	—	—	35.11
C	112	3.62	0.320	0.90	0.45	
D	224	3.58	0.335	0.95	0.50	
E	448	3.69	0.290	1	0.60	
F	1000	3.93	0.215	1	0.74	

[a] From Table 2 and Jovin *et al.* (7).

[b] RM (1, 9) was predicted on the basis of pH (9) in each case.

tration can result in stacking of the tracking dyes, Methyl Green and Brilliant Green, in the moving boundary between pyridinium and β-alaninium (systems 35 and 35.11).

Figure 14 shows the effect of TEMED concentration on the mobility (R_f) for several proteins and dyes in separation gels of 10.2%T, 2%C, system 35.11. A contrasting case is depicted in Fig. 15: for system B, the buffering capacity of the separation gel is sufficient to prevent a rise of pH when TEMED concentration is increased from 3.27 to 6.54 mM. Consequently, the velocity of the moving boundary remains constant, and bromphenol blue remains in the stack.

DISCUSSION

Method for Determination of Polymerization Efficiency

Polymerization efficiency has not been previously determined for aqueous, cross-linked PA under conditions generally used in PAGE. The above methodology for the measurement of "methanol-extractable monomer" from PA is still very crude. The %EM obtained by this

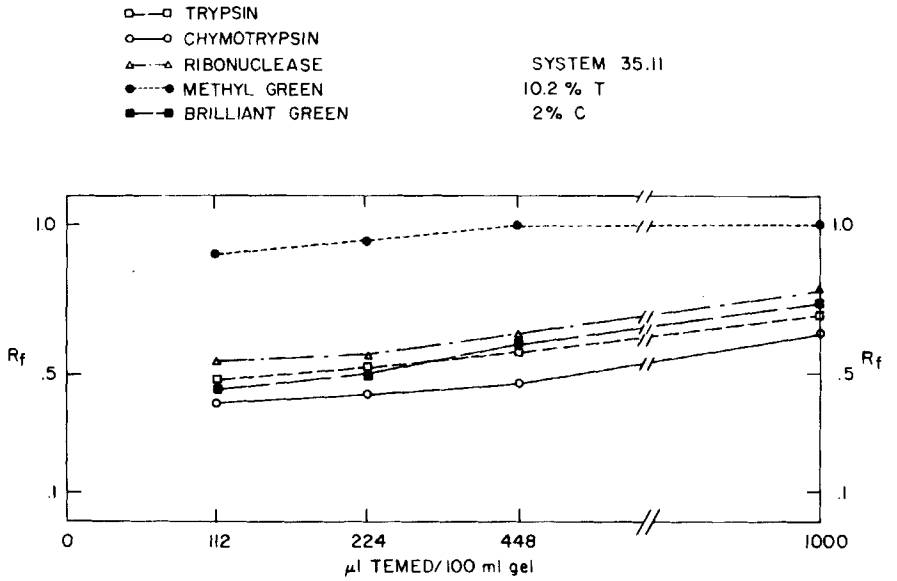

FIG. 14. Plot of R_f (system 35.11) for five proteins or dyes vs TEMED.

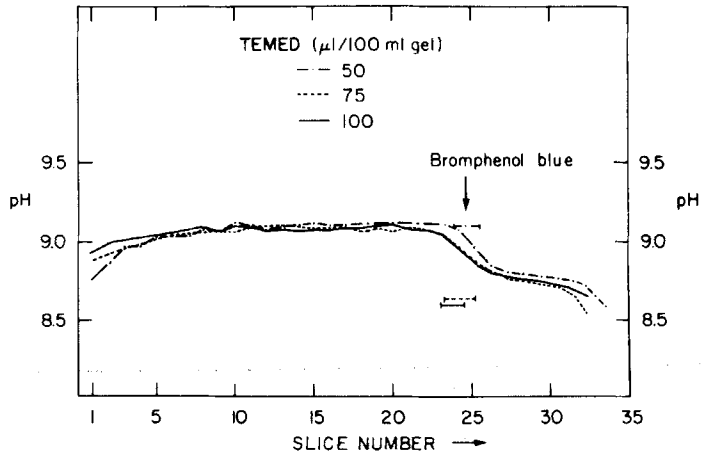

FIG. 15. Stacking of bromphenol blue is unaffected by TEMED concentrations studied in system B.

method is a minimal value, since there is likely to be additional monomer (or low-molecular-weight oligomer) remaining unextracted from the polymer. Also, the present method has several disadvantages: (1) it is a laborious multistep procedure; (2) it is necessary to "age" the dilutions of the filtrate from the ion-exchange step; (3) although it is applicable to quantitation of polymer, an additional step (acid hydrolysis) is required; and (4) it does not characterize the gel in terms of percent cross-linking. Nonetheless, the present procedure is the only one available and can be readily set up in any laboratory.

Application of Measurement of Polymerization Efficiency to Estimate Reproducibility of Pore Size in PA Gels

Until now only indirect estimates of pore size variability were available, based on the variance of relative mobilities (R_f) of proteins in electrophoresis (*3*, *12*, *13*). The variability in %PE for gels made on different days was found to be significantly greater than variation between replicate gels made on the same day. The variance due to methodological sources and error within experiments was small by comparison. This correlates with the finding that between-experiment variance in R_f is approximately 4 times larger than within-experiment variance (*13*). The direct proportionality between σ (%EM) and %EM implies

that high %PE is necessary for satisfactory reproducibility. This must be considered when one attempts to compare and integrate PAGE data from different experiments or laboratories.

Effects of TEMED on Gel pH

The present results indicate that in the "difficult" buffer system used here, complete polymerization cannot be achieved unless appreciable amounts of TEMED are used, which may increase the pH of the gel (Figs. 12–14). In some cases (Fig. 15) the buffer value (BV) of the gel may be sufficient to prevent an appreciable rise in pH in the gel. Increase in gel pH affects multiphasic buffer systems by changing the stacking limits in a particular gel. Since under the effect of TEMED, the stacking limits in the particular multiphasic buffer systems are changed (see Table 8), both the relative and absolute mobilities of the species of interest (Fig. 14) may also change. [*Note:* We are using an approximation in calculating the RM(1, 9) expected on the basis of pH(PI): perturbation of the pH by TEMED will not have exactly the same effect as changing the concentration of the two buffer constituents in the gel as made (GAMMA phase). However, more exact calculations are not possible for a ternary system at the present time.]

Inspection of Fig. 14 indicates that R_f values should be extrapolated to zero TEMED concentrations, although the slope of R_f vs [TEMED] curve is quite small in the 112–224 μl/100 ml gel region. Experimentally, the TEMED concentration cannot be reduced much further, since the gel would be incompletely polymerized, the "pore size" would increase, and the mobility of proteins (if not the dyes as well) would increase. The absence of a biphasic R_f vs [TEMED] curve could, in fact, be taken as evidence that polymerization is complete or at least reproducible. This test should be most sensitive when a large protein with high K_R is used.

One approach, to minimize the deleterious effects of TEMED or KP, is the use of pre-electrophoresis. Although this procedure will reverse the effect of TEMED on pH of electrophoresis (not polymerization), it does not eliminate the effects of these ionic catalysts on the ionic strength of the gel. Also, it appears that pre-electrophoresis may introduce more new problems than it can solve (*15*). An alternative approach, to remove catalysts, is to equilibrate the gel with buffer by diffusion (*15*, *16*). This appears to be the best method to "purify" gels (*15*). However, this results in marked swelling of the gel—the effective

gel concentration must then be calculated from the nominal values by use of water-regain data (*16*). Thus, the gel concentration is not under complete control of the investigator. Also, this method is time-consuming, is not readily applicable to multiphasic buffer systems, and wall adherence is lost, so that most of the available present-day apparatus and procedures of PAGE are inapplicable.

Application to Selection of Optimal Catalyst Concentrations

Previous work in this laboratory, based on an observation by T. M. Jovin, had shown that, at neutral or acid pH, use of combinations of all three catalysts resulted in gels with superior mechanical properties and avoided the need to employ excessive concentrations of one of the catalysts, as had been used by many workers (e.g., Ref. *14*). Most of the present studies were done under very unfavorable polymerization conditions, i.e., the combination of acid pH 3.9 and a temperature of 0°C.

On the basis of this study and of our experience with many buffer systems, with a wide range of %T, %C, pH, ionic strength, and buffer constituents, as well as temperatures of 0 and of 25°C, the practitioner of PAGE can be assured that polymerization of a cross-linked acrylamide gel can be effected under all conditions. A general stretegy for polymerization may be formulated as follows:

1. Select starting concentrations of 0.01 *M* KP, 0.0015 *M* RN, and 0.005 *M* TEMED for a 10%T gel at 0°C; RN may be ommitted for pH > 8.
2. Pyrex glass should be used for the polymerization vessel (*1*). Controlled deaeration is necessary for reproducible results. At pH values less than 4, Parafilm should be used to seal the lower end of the tube; at higher pH, rubber stoppers may be used.
3. If polymerization does not occur within 10 min, increase all three catalyst concentrations by a factor of 2.
4. Once polymerization is achieved, reduce the concentration of one reagent in steps of $\frac{1}{2}$, until a minimal concentration is found that results in polymerization within 10 min (refractive index end point) and a hard, straight surface.
5. Using a gel made with these concentrations, determine %EM or %PE. If %PE is less than 98%, increase one of the catalyst concentrations by a factor of 2.

6. For polymerization at various gel concentrations, adjust the concentration of TEMED in inverse proportion to gel concentration.

7. Check the effect of TEMED on pH of gel buffers, the gel as polymerized (slices), and the gel after pre-electrophoresis to remove TEMED and KP. If the TEMED perturbs the pH, several avenues are available: (1) by decreasing TEMED with a reciprocal change in [KP][RN], (2) by pre-electrophoresis of the lower gel against LGB; (3) by pre-electrophoresis of the combined stacking and separation gels using UGB in the upper buffer reservoir; and (4) by increasing of the concentration (and BV) of the gel buffer.

8. Choose between KP and TEMED. In anionic migration the KP almost always migrates ahead of the protein, and may even migrate ahead of the stack [depending on RM(2, 2) or RM(1, 9)]. Conversely, in cationic migration, the protein will never be in contact with the TEMED, which can be tolerated at very high concentrations without danger of excessive reaction with the protein.

9. Check the effect of all catalysts on R_f of the molecule of interest.

10. The time of reappearance of a refractive interface between the gel and the overlying solvent is a readily observed parameter: catalyst concentration should be adjusted so that this occurs after 5 to 15 min.

Note that these steps have to repeated for each new set of polymerization conditions (pH, ionic strength, temperature, partial pressure of argon in the polymerization mixture, or change in millimeters of Hg of evacuation of the polymerization mixture, etc.).

As a general rule, polymerization at 25°C requires only half the catalyst concentrations ([KP][RN][TEMED]) required at 0° for the corresponding buffer system.

Polymerization in a Universal Solvent

Since thousands of buffer systems are available for PAGE (*7*), one might contemplate that thousands of different polymerization conditions must be developed, tested, and optimized. Evidently, it would be desirable to establish standard polymerization conditions, e.g., for gels in 0.015 *M* NaCl, pH 7, at 0 or 25°C. After polymerization, the desired buffer could be introduced, either by pre-electrophoresis or by diffusion (*16*). However, the drawbacks of the two methods, as outlined above, are serious obstacles to this approach (15).

Alternative Catalysts

The choice of the three catalysts (KP, RN, TEMED) is arbitrary, although these are the most popularly used today. Rational selections of catalysts as applied to the polymerization of linear PA (*6*) have not been applied to cross-linked PA to date. Numerous catalyst systems are available (*17*) of which only very few have been used (*1*). The catalyst, dimethylaminopropionitrile (DMNAP) has been studied systematically by Kingsbury and Masters (*18*). They investigated the effect of DMNAP on gelling time and on the mobility of bromphenol blue and of carbonic anhydrase. In addition to the effect of polymerization rate on average chain length, suggested by Kingsbury and Masters, it is likely that DMNAP alters mobilities by alteration of pH and ionic strength, as observed for TEMED in the present study. Preliminary studies with peroxide–Fe^{2+}–ascorbic acid (*19*) in this laboratory suggested that it was more difficult to control than the KP–RN–TEMED system used in the present study. It is evident, that %PE will provide a useful tool for the evaluation of catalysts and should be measured for each new catalyst system.

Projected Studies

The present study of the polymerization reaction is not a kinetic study—only the final state of the gel is measured. In principle the temperature within the gel, index of refraction, absorbance, or light scattering, can be used to monitor the rate of the polymerization reaction continuously. This would allow one to evaluate available catalysts and catalyst efficiency in terms of the reaction rate constants. .

Detailed, systematic studies of the effects of pH, ionic strength, temperature, and gel concentration on the requirements for catalysts remain to be performed. The necessary methodology is now available. This should make it possible to refine the above guidelines and to reduce progressively the amount of experimentation necessary to polymerize in a new buffer system.

Projected studies of percent polymerization and average chain length: Use of ethylene diacrylate (*20*) and *N*,*N*′-diallyltartardiamide (*21*) as cross-linking agents allows for the polymerization of soluble cross-linked PA. This should make it possible to measure true percent polymerization and average chain length on these gels, after cleavage of the cross-links (by alkali or periodate, respectively), using conven-

tional polymer chemical methodology (*6*), e.g., viscometry, osmometry, and light scattering.

Alternative Gels

The problems involved in achieving complete and reproducible polymerization of acrylamide represent a major weakness of PAGE. Despite these problems, the control of pore size for PA is still superior to other media, such as starch, agar, and agarose. However, stable hydrophilic polymer gels should be screened for use in electrophoresis. Preliminary data are available (*22*) on linear polyethylene oxide, transformed into cross-linked gels by exposure to a γ-ray source. This offers the prospect of "factory-made" gels. The problems of introduction of suitable buffer (discussed above) remain. These polyethylene oxide gels also present as yet unresolved problems of polymerization rate, air-bubble formation, surface properties, and pore size reproducibility.

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