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(19)

(54) METHOD OF PURIFYING MICROBIAL ENZYME EXTRACTS

(71) We, EASTMAN KODAK COMPANY, a Company organized under the Laws of the State of New Jersey, United States of America, of 343 State Street, Rochester, New York 14650, United States of America do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a method for purifying microbial enzyme extracts.

The purification of enzymes from microbial extracts is complicated by the presence of nucleic acids which interfere with salt fractionation or chromatographic procedures that are commonly used in enzyme purification. Several methods have been developed to remove nucleic acids from crude cell extracts by precipitation, for example, complexing the negatively-charged acids with positively-charged materials such as protamine sulphate, streptomycin sulphate, or manganese chloride.

However, problems have been experienced with each of these methods. For example, protamine sulphate, a small protein containing arginine residues has been isolated only from the sperm of certain fish, and in application, this protein is difficult to dissolve in water. In addition, problems with reproducing results have been reported. Streptomycin sulphate an antibiotic containing the diguanido base streptidine is generally more consistent in use, but the extent of precipitation of nucleic acids is influenced by various electrolytes in the extract. Manganese chloride is inexpensive and convenient to use, but is less advantageous than the others in that addition of this salt frequently results in loss of enzyme activity.

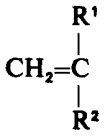
Nucleic acids, as well as proteins and any water-soluble, electrical charge-carrying polymers, are defined as polyelectrolytes by Katchalsky in "Poly-electrolytes", *Endeavor*, Vol. 12, page 90 (1953). He suggests that strong interactions occur between polyelectrolytes of opposite charge and that such interactions could result in mutual precipitation.

Various suggestions for precipitating impurities from enzyme solutions have been made in the prior art. For example, U.S. Patent No. 3,728,224 and British Patent 1,411,503 described the use of cationic surfactants such as quaternary ammonium compounds to precipitate impurities including nucleic acids and protein from enzyme solutions. The treatment of enzyme solutions produced by *Streptomyces rectus* with a polyoxyethylenealkylamine is described in Japanese Patent 73/33387. U.S. Patent 3,737,377 describes the use of polyacrylic acid to precipitate the enzyme lactase from solution in order to purify it.

A method for enriching or purifying protein solutions using precipitation by polyethylene-imines is described in British Patent 1,298,431. The precipitation of protein by this method using polyethylene-imines is dependent upon ion concentration. Furthermore, ethylene-imine itself is carcinogenic and polyethylene-imine may have harmful effects. Therefore polyethylene-imine is not considered to be a material of choice for commercial purification of enzymes.

According to the present invention there is provided a method of purifying a cell-free microbial enzyme extract by precipitating unwanted nucleic acids and protein, characterized in that the precipitation is caused by adding to the extract a

water-soluble, cationic polymer comprising units derived from a cationic monomer having the formula:



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wherein R^1 is a hydrogen atom or an alkyl group which may bear non-interfering substituents, and R^2 is a nitrogen-containing group capable of carrying a positive charge when the polymer is dissolved in an aqueous solution having a pH value not greater than the pKa of the polymer.

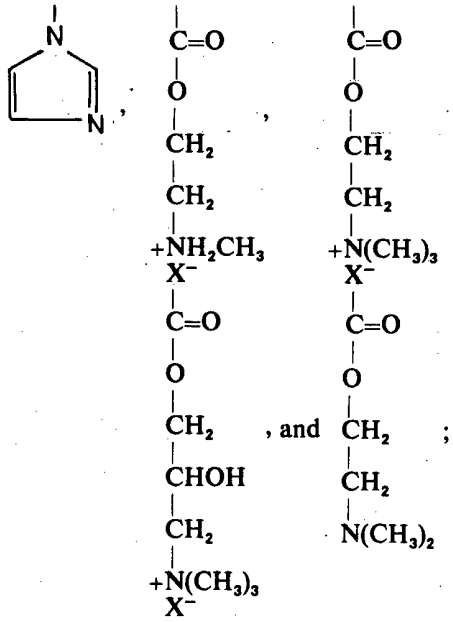
The cationic polymers employed herein tend to precipitate nucleic acid and unwanted protein molecules selectively from microbial enzyme extracts, leaving the desired enzyme in the extract. Among other advantages of the present method is that the effectiveness of complex formation and precipitation using these polymers is not strongly influenced by the pH nor, at least up to about the value of pKa of the polymer, by the protein concentration of the extract.

It will be understood that if the pH of the solution were greater (i.e. more alkaline) than the pKa of the polymer, it would have the effect of removing the required positive charge.

As used herein, the term "cationic" when applied to monomers and polymers described in this invention means that the monomer or polymer is capable of carrying a positive charge when dissolved in an aqueous solution having a pH value that is below the pKa of the monomer or polymer described.

R^1 is preferably an alkyl group of 1—4 carbon atoms. Any substituents thereon should be non-interfering that is, it should not interfere with the desired function of the resulting polymer.

Examples of groups which R^2 may represent include amine groups, and substituted amine groups particularly those containing a carbonyl or an oxycarbonyl group. Particularly useful groups R^2 include those of the following formula:



where X^- is an anion, for example, a halide ion, e.g. Cl^- or Br^-, or an organosulpho-containing anion, e.g. $CH_3OSO_3^-$.

Examples of cationic monomers useful in preparing the polymers of this invention include N-methyl-2-aminoethyl methacrylate hydrochloride, 1-vinylimidazole, 2-(N,N,N-trimethylammonium)ethyl methacrylate chloride, 2-

(N,N-dimethylamino)ethyl methacrylate, 2-(N,N,N-trimethylammonium)ethyl methacrylate methosulphate, and 2-hydroxy-3-(N,N,N-trimethylammonium)-propyl methacrylate chloride.

In accordance with the present invention, microbial enzyme extracts are purified by adding a quantity of one of the above-described polymers to the extract or precipitate inpurities, i.e., nucleic acids and non-active protein. The amount to be added depends upon the charge density of the particular polymer and upon the amount of impurities in the extract. Conveniently, the amount of polymer added is based on the amount of protein in the extract.

A particular enzyme extract may be purified more effectively using a polymer having a low charge density or a high charge density. Generally, the polymers employed in this invention comprise at least 10 percent by weight, and preferably at least 20 percent by weight, of monomers having structure I in order to carry out effective purification without using unduly large quantities of polymer.

While the mechanism is not completely understood, it is believed that precipitation results from strong electrostatic attraction between oppositely charged groups on the molecules. Therefore, the effectiveness of a precipitant would be influenced by the charge density of the molecules. The extent of ionization of the charged groups also would then be important, since polymers which are more ionized are probably in an extended rather than a coiled configuration. Thus the polymers employed in the practice of this invention are generally useful over a range of pH values at least up to the value of the pKa for the cationic polymer. In general, a preferred pH range is 1—10. However, as is known, at high and low pH values, enzyme denaturation can take place. To avoid such denaturation, it is most preferred to employ the polymers in a pH range of 5—9.

Copolymerization of the cationic monomers described herein with non-charge-carrying monomers enables one to obtain polymers having a wide range of charge densities. One or more of the monomers capable of carrying a positive charge may be copolymerized with other monomers which, at least under the conditions of use, are not capable of carrying a charge. For example, various vinyl monomers known to those skilled in the art can be used. The only restrictions are that the non-charge carrying monomer should not interfere with the cationic nature of water-solubility of the polymer so as to make it ineffective in the practice of this invention. Particularly useful monomers for copolymerizing with the monomers of formula I are acrylamide and hydroxyalkyl acrylates, for example, 2-hydroxyethyl acrylate. Other equivalent monomers, however, would be equally suitable for copolymerization.

The cationic polymers employed herein have been found useful in purifying extracts from a variety of microorganisms. A partial list includes, *M. flavins, B. megaterium, P. testeroni,* and *S. faecalis.*

The amount of protein present in the enzyme extract may be conveniently estimated by a simple spectrophotometric technique described by Layne in Methods in Enzymology, Vol. 3, page 451 (1957) referred to hereinafter as the "280/260 spectrophotometric method". Generally, adding an amount of polymer in the range of from 0.01 to 0.5 mg per mg of protein in the extract, and preferably from 0.05 to 0.2 mg per mg of protein, has been found effectively to remove nucleic acid and non-active proteins from the extract. It should be noted that equivalent results can be obtained by determining the quantity of cationic polymer to be added to the extract based on any other parameter of the enzyme extract, such as, for example, the quantity of nucleic acids present.

After the cationic polymer has been added to the enzyme extract, the extract is incubated for a period of time with stirring in order to allow the polymer to complex with the nucleic acids and non-active proteins. Generally, the incubation is carried out at low temperatures, for example 0 to 4°C, in order to preserve enzyme activity. Other temperatures may be selected depending upon the particular enzyme extract being treated. The period of incubation is not critical as long as it is sufficient to allow complexing to take place. Generally, about 30 minutes has been found satisfactory. The extract is then centrifuged to remove the precipitated impurities.

In the Examples which are provided below to illustrate and clarify the invention further, unless otherwise noted, the following definitions apply.

1. *Polymers (Synthetic Polyelectrolytes)*

Polymer No.	Monomers	Monomer Weight Ratio	Weight % solids	Bulk Viscosity (cps)
1	E:C	20:80	10.5	705
2	E:C	40:60	10.7	99
3	E:C	40:60	10.6	375
4	E:C	60:40	10.7	554
4a	E:C	60:40	10.6	6150
5	E:C	80:20	11.0	1400
5a	E:C	80:20	11.0	4310
6	E:C	90:10	21.7	380
6a	E:C	90:10	24.0	4500
7	G:C	95:05	13.6	1400
8	G	100	10.0	high
9	H	100	20.6	74
10	F:C	90:10	9.7	40
11	A:D:C	10:80:10	12.3	93
12	A:B	40:60	10.4	25
13	EI	PEI—600*	33	5000

* Polyethylenimine, commercial product from Dow Chemical Company, Midland, Michigan

2. *Identification of Monomers*

Monomer	Name
A	Acrylamide
B	N-Methyl-2-aminoethyl methacrylate hydrochloride
C	2-Hydroxyethyl acrylate
D	1-Vinylimidazole
E	2-(N,N,N-Trimethylammonium)ethyl methacrylate chloride
F	2-(N,N-Dimethylamino)ethyl methacrylate
G	2-(N,N,N-Trimethylammonium)ethyl methacrylate methosulphate
H	2-Hydroxy-3-(N,N,N-Trimethylammonium)propyl methacrylate chloride
EI	Ethylene-imine

3. *Production of Enzyme Extract*

a. Cell Growth

A microorganism isolated in the laboratory was grown in a medium containing 2 percent L-aspartate, 0.4 percent potassium phosphate, and basal salts: 250 mg magnesium sulphate, 17 mg manganese sulphate, 28 mg ferrous sulphate, 6 mg sodium sulphate, 10 mg calcium chloride, and 0.6 mg zinc sulphate per litre of growth medium. The final pH of the growth medium was 7.0. Flasks containing sterile media were innoculated, then incubated at 30°C with mixing at 200 rpm for 24 hours. Cells were harvested by centrifugation at 13,700 × g for 20 minutes.

b. Cell-free Extract

Harvested cells were suspended in nine volumes of either 0.1 M potassium phosphate buffer, pH 7.0, containing 1.0 mM ethylenediamine tetraacetic acid and 1.0 mM dithiothreitol (DTT buffer) or in 0.15 M tris(hydroxymethyl)amino-methane chloride (Tris-Cl)buffer, pH 8.5. The suspension was cooled in a brine-ice bath, and cells were lysed by sonification. Cells and cell debris were removed by centrifugation at 27,000 × g for 15 minutes. The supernatant fraction was the cell-free extract.

4. *Polymer Treatment*

Aliquots of the extract were kept cool at 4°C and then polymer was added to the samples to give 0.2 mg polymer per mg protein, or as indicated; control samples had an equal volume of water added instead of polymer. Samples were incubated at 4°C and constantly stirred with a magnetic stirrer for 30 minutes. The extracts were then centrifuged at 34,800 × g for 20 minutes; the clear, supernatant fraction was saved for assay.

5. *Protein Assay*

Protein was determined using the spectrophotometric method described by Layne, Methods in Enzymology, Vol. 3, page 451 (1957).

6. *Nucleic Acid Assay*

The ratio of 280 to 260 mμ absorbance gives an indication of the protein to nucleic acid ratio described by Layne, supra. In these crude extracts, the ratio generally was 0.73, or approximately 0.09 mg nucleic acid per mg protein. Values determined by this technique are not rigorous, but are found to be closely related to the actual values and were used to follow changes in nucleic acid content of extracts following exposure to polymer.

7. *Enzyme Assay*

Aspartase, one of the major enzymes identified in the crude cell-free extracts, was selected to monitor enzyme activity in the examples using extracts from the laboratory isolated microorganism. Its activity was monitored by following the formation of fumarate at 240 nm (millimolar extinction coefficient was 2.53). Assay mixtures contained in 1.0 ml: 60.0 mM Tris-Cl buffer, pH 8.5, 1.8 mM magnesium sulfate, 0.09 mM ethylenediamine-tetraacetic acid, 50.0 mM L-aspartate, water and enzyme. Reactions were initiated by the addition of 0.01 unit of enzyme, and the reaction temperature was 30°C. A unit is defined as that amount of aspartase required to produce 1 μmole of fumarate per minute.

In the accompanying drawings some of which are referred to in the Examples below:

Fig. 1 is a graph illustrating the effect of polymer concentration on the removal of nucleic acid from enzyme extract for polymers described herein having various charge densities.

Fig. 2 is a graph illustrating the effectiveness of the polymers described herein in selectively removing non-active protein from an enzyme extract.

Fig. 3 is a graph illustrating the effectiveness of the polymers described herein having various charge densities in removing nucleic acid and non-active proteins from an enzyme extract.

Fig. 4 is a graph illustrating the effect of the size of the polymers described herein on the removal of nucleic acids and non-active protein from an enzyme extract.

Fig. 5 is a graph illustrating the effectiveness of removing nucleic acids from

enzyme extracts using two different buffers for various polymers described herein and prior art precipitants.

Fig. 6 is a graph illustrating the effect of pH on the effectiveness in removing nucleic acids and non-active proteins from an enzyme extract using a polymer described herein.

Fig. 7 is a graph illustrating the effect of protein concentration on the effectiveness of a polymer described herein in removing nucleic acids and non-active protein from an enzyme extract.

Fig. 8 is a graph comparing the effect of buffer concentration on nucleic acid removal and retention of enzyme activity for a polymer described herein and polyethyleneimine, PEI.

Fig. 9 is a graph illustrating the polydextran equivalent molecular weight distribution for certain polymers described herein.

Example 1

Effect of Polymer Concentration and Charge Density on the Precipitate

A series of copolymers containing increasing amounts of the cationic monomer 2-(N,N,N-trimethylammonium)ethyl methacrylate chloride (E) and proportionately decreasing amounts of 2-hydroxyethyl acrylate (C) were synthesized, and the effectiveness of each polyelectrolyte was tested. A comparison of the extent of removal of nucleic acids is presented in Fig. 1. More nucleic acid was precipitated as the amount of polymer added to the sample was increased; also, the effectiveness of the polyelectrolytes increased as the charged portion increased from 20 to 60 to 90 percent of the polymer. Protein in the extracts also was precipitated somewhat by these copolymers (Fig. 2), and this removal was polymer-concentration and charge-density dependent. However, no significant loss of enzyme activity occurred with exposure to these copolymers within the range of polyelectrolyte concentrations tested. A comparison of the removal of nucleic acid, protein and aspartase activity by an extended series of polymers of varying charge densities is presented in Fig. 3. All polymers were used at 0.2 mg per mg protein. None of the polymers tested precipitated enzyme, but nucleic acids were removed with maximum precipitation when the cationic monomer was 40 percent or greater of the copolymer. As noted above, more purification of enzyme, i.e., greater activity per mg protein, was obtained with the more charged precipitants.

Example 2

Effect of Polymer Size on the Precipitants

As indicated previously, although the mechanism of precipitation is not understood, it was theorized that removal of contaminants by this type of precipitation requires that the polyelectrolytes combine, probably by molecular pairing, and most likely, the paired molecules are randomly twisted together rather than in regular spirals. The size of precipitants, therefore, could influence the extent and effectiveness of this complex formation. Polymer size relative to other polymers can be estimated by a comparison of sample viscosities, since viscosity increases with the length of the molecule. To investigate this possibility, two samples of polymer 4 with viscosities of 554 and 6150 centipoises (cps) were tested. No significant modification in the pattern of enzyme activity or nucleic acid precipitation was found when increasing concentrations of either of these copolymers were added to crude extracts (see Fig. 4). The amount of protein precipitated also was similar with both polymers.

Example 3

Effect of Other Polymers on Precipitation

The general usefulness of the class of cationic polymers used in the present invention for removing nucleic acids and generally "cleaning-up" crude bacterial extracts, is demonstrated by testing the following spectrum of polymers employed in the practice of this invention as precipitants. The precipitants included copolymers containing secondary (B), tertiary (D,F) and quaternary (E,G) amines. The polymers were tested in two buffers, 0.15 M Tris-Cl, pH 8.5, and DTT buffer, and the results are presented in Figs. 5A and B, respectively. PEI, protamine sulphate (referred to in the Figures as PSO_4, owing to space limitations) and manganese chloride are also included as precipitants for comparison.

Some removal of aspartase activity was detected in either buffer with the concentration of polymer used (0.2 mg per mg protein). However, enzyme levels

were lower in DTT-buffered samples than in similar Tris-buffered samples, and more enzyme activity was lost from samples containing either buffer when a polymer containing 100 percent of the quaternary ammonium salt (G) was used as compared to extracts exposed to other polymers. The patterns of enzyme activity and nucleic acid precipitation by these synthetic polymers compared very well with the patterns found when protamine sulphate was the precipitant.

Manganese chloride was not very effective in precipitating nucleic acid from the extract containing Tris buffer, while it removed all of the aspartase activity from solution. The precipitated fraction of the samples was not examined for activity. Enzyme activity also was lost from the DTT-buffered sample with manganese chloride addition but not as completely as from the previous sample. It is known that manganese chloride addition to cell extracts results in large losses of enzyme activity, and as reported, this effect is a major problem in using manganese as a precipitant.

Example 4

Effect of pH on Precipitation

The pH of crude extract was varied over the pH range 7.5 to 9.2, and patterns of precipitation of nucleic acids and proteins, and loss of aspartase activity were determined (Fig. 6). Polymer 12, at 0.2 mg per mg protein, was the polymer used in this example. No significant loss of enzyme activity was found at the pH values tested. Some modification in the extent of removal of nucleic acids and proteins was indicated. This could mean that at the higher pH some of the secondary amines on polymer 12 have been deprotonated, and therefore, the charge density of the polymer has decreased. This would lower the tendency of the polyelectrolyte to complex with nucleic acids and proteins. Other polymers, however, especially those containing quaternary amines, are not similarly affected by elevated pH values, and thus the data with polymer 12 were interpreted to show no pH effect for the polyelectrolyte technique.

Example 5

Effect of Protein Concentration on Precipitation

It is well known that the effectiveness of protamine sulphate as a precipitant can be negatively influenced by increases in protein concentration of samples. This was not the case when polymer 12 was tested with extracts where protein concentration was varied from 0.6 to 26.4 mg per ml (see Fig. 7). Nucleic acid content remained 0.09 mg per mg protein for all untreated samples. Polymer 12 was added to each sample at a constant ratio to the protein (0.2 mg polymer per mg protein). No decrease in effectiveness of this polymer to precipitate nucleic acids was observed with changes in protein concentration, while approximately 80 percent of the nucleic acids was removed. No loss of aspartase activity was found following exposure to the polymer in any of the samples. A slight decrease in the removal of protein was observed at the higher protein levels,

Example 6

Effect of Buffer Concentration on Precipitation

British Patent 1,298,431 reports that buffer concentration influences the ability of PEI, a synthetic polyelectrolyte, to precipitate nucleic acids and proteins. At high salt concentrations (0.05 M phosphate buffer, pH 7.0), nucleic acids were precipitated selectively, while at lower salt concentrations (0.01 M phosphate buffer, pH 7.0), proteins were precipitated also. Such a trend was demonstrated, but not as conclusively, with data presented in Fig. 8. This moderate response, not totally precipitating the nucleic acid, probably was due to the low level of polymer used here (0.1 mg per mg of protein). As with other synthetic cationic polymers tested, PEI also had a polymer concentration dependence for maximum nucleic acid or protein precipitation. In addition, a comparison of the effectiveness of polymer 12 at two buffer concentrations is presented in the Fig. 8. Unlike PEI, polymer 12 did not decrease aspartase activity in the extract at the lower buffer concentration. Nucleic acid precipitation, however, was more efficient with polymer 12 at 0.1 M Tris-Cl than it was at 0.01 M buffer.

Example 7

Molecular Weight Distribution of Some Polymer Samples

The molecular weight distributions of some polymer samples have been determined by aqueous exclusion chromatography. The analytical procedure

consisted of diluting 0.1 ml of the polymer dope, as received, with 10 ml of eluant (0.1 M aqueous NaCl at pH 2.5). The solution was chromatographed on a series of five columns packed with CPG-10 porous glass of porosities 2000, 1400, 370, 175 and 75 Å. The packings had previously been derivatized with the silane reagent γ-aminopropyltriethoxy silane. The eluate was monitored with a Waters R403 differential refractometer and a Varian (trade mark) 635 spectrophotometer at $\lambda = 220$ nm.

Polydextran equivalent number average and weight average molecular weights for the samples are given in Table I below.

TABLE I

Polymer	Mn	Mw	Mw/Mn	Inherent Viscosity, η (.1M NaCl)
6	659,000	1,350,000	2.05	1.17
6a	588,000	2,050,000	3.49	1.90
9	195,000	609,000	3.11	.59

These results appear consistent with the viscosity data discussed earlier.

Computer-drawn curves showing the polydextran equivalent molecular weight distributions for the polymers are shown in Fig. 9. They indicate that all of the polymer molecules of the No. 6 and 6a polymer samples and that about 90 percent of the polymer molecules of polymer No. 9 are essentially larger than a polydextran molecule of molecular weight 100,000.

Example 8

Effect of Several Polymers on a Variety of Microorganisms

A variety of microorganisms, listed in Table II, was grown in nutrient broth for 24—48 hours at 30°C. Cells were harvested by centrifuging (27,000 × g for 15 minutes), suspended in approximately 9 volumes of 10 mM Tris-Cl buffer, pH 7.5 (*S. faecalis* cells were suspended in 10 mM sodium acetate, pH 5.0), and sonically disrupted. Residual cells and debris were removed by centrifuging as above, and the supernatant fraction was used in the studies. Protein and nucleic acid content of each fraction were estimated by the 280/260 spectrophotometric method. Enzyme activity was measured by standard spectrophotometric techniques, and is expressed as units per ml enzyme sample, where 1 unit converts 1 μmole of substrate per min at 37°C. Following centrifugation, the cell-free extract was supplemented with 0.1 mg of indicated polymer per mg protein. The solution was mixed for 20 minutes at 4°C then centrifuged (27,000 × g for 15 minutes) to precipitate the complexed material. The clear supernatant was assayed for enzyme, protein, and nucleic acid content as described above. The results are presented in Table II.

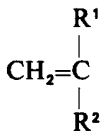
TABLE II

Source	Polymer	Amount Retained, %		
		Enzyme Activity	Nucleic Acid	Protein
		Catalase		
M. flavus	**	100	100	100
	12	103	50	68
	4	103	50	74
	6	4	25	16
		Catalase		
B. megaterium	**	100	100	100
	12	95	28	65
	4	105	32	85
	6	37	48	52
		Catalase		
P. testeroni	**	100	100	100
	12	96	15	42
	4	34	16	23
	6	73	67	87
		Lactate Oxidase		
S. faecalis	**	100	100	100
	12	115	31	73
	4	125	23	61
	6	123	17	27
		a-glycerophosphate Oxidase		
S. faecalis	**	100	100	100
	12	108	31	73
	4	108	23	61
	6	95	17	27

** Control – no polymer

WHAT WE CLAIM IS:—

1. A method of purifying a cell-free microbial enzyme extract by precipitating unwanted nucleic acids and protein, characterized in that the precipitation is caused by adding to the extract a water-soluble, cationic polymer comprising units derived from a cationic monomer having the formula



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wherein R^1 is a hydrogen atom or an alkyl group which may bear non-interfering substituents, and R^2 is a nitrogen-containing group capable of carrying a positive charge when the polymer is dissolved in an aqueous solution having a pH value not greater than the pKa of the polymer.

2. A method as claimed in Claim 1 characterized in that the cationic polymer is produced from a mixture of monomers of which the cationic monomer comprises at least 10 percent by weight.

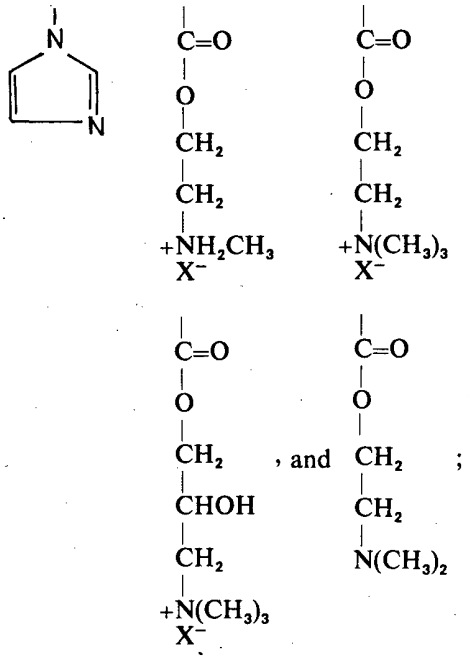
3. A method as claimed in Claim 1 or 2 characterized in that the cationic polymer further comprises units derived from a vinyl monomer incapable of carrying a charge under the conditions of use.

4. A method as claimed in Claim 3 characterized in that the vinyl monomer is acrylamide or a hydroxyalkyl acrylate.

5. A method as claimed in any of Claims 1—4 characterized in that the cationic polymer is added to the extract in an amount of from 0.01 to 0.5 mg per mg of protein in the extract.

6. A method as claimed in Claim 5 characterized in that the polymer is added in an amount of from 0.05 to 0.2 mg per mg of protein in the extract.

7. A method as claimed in any of Claims 1—6 characterized in that the group R^2 is a group of one of the following formulae:



, and

;

where X^- is an anion.

8. A method as claimed in any of Claims 1—7 characterized in that said cationic polymer contains units derived from at least one of the following cationic monomers: N-methyl-2-aminoethyl methacrylate hydrochloride, 1-vinylimidazole, 2-(N,N,N-trimethylammonium)ethyl methacrylate chloride, 2-(N,N-dimethylamino)ethyl methacrylate, 2-(N,N,N-triethylammonium)ethyl methacrylate metho-

sulphate or 2-hydroxy-3-(N,N,N-trimethylammonium)propyl methacrylate chloride.

9. A method according to Claim 1 substantially as described herein and with reference to the Examples.

10. A purified microbial enzyme extract produced by the method of any of Claims 1—9.

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Agents for the Applicants

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Sheet 1

FIG. 1

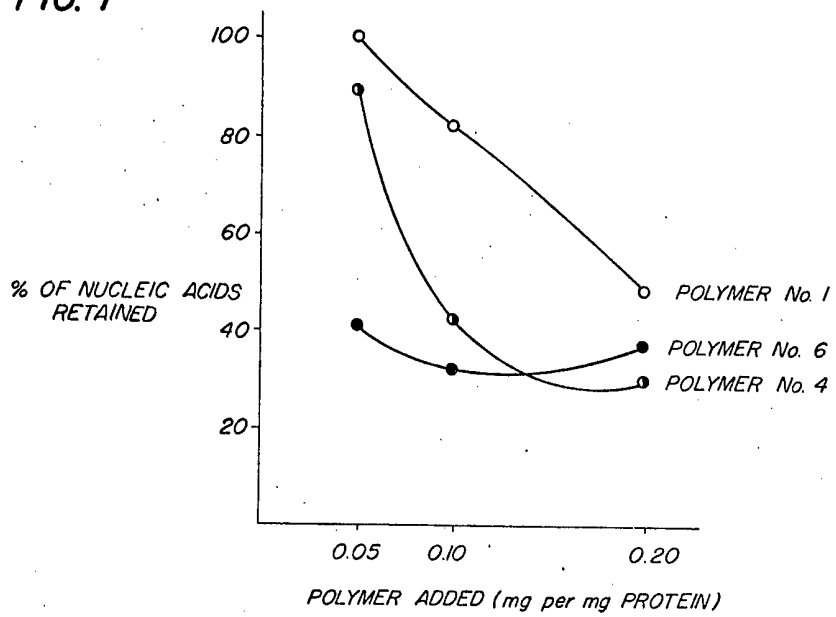


FIG. 2

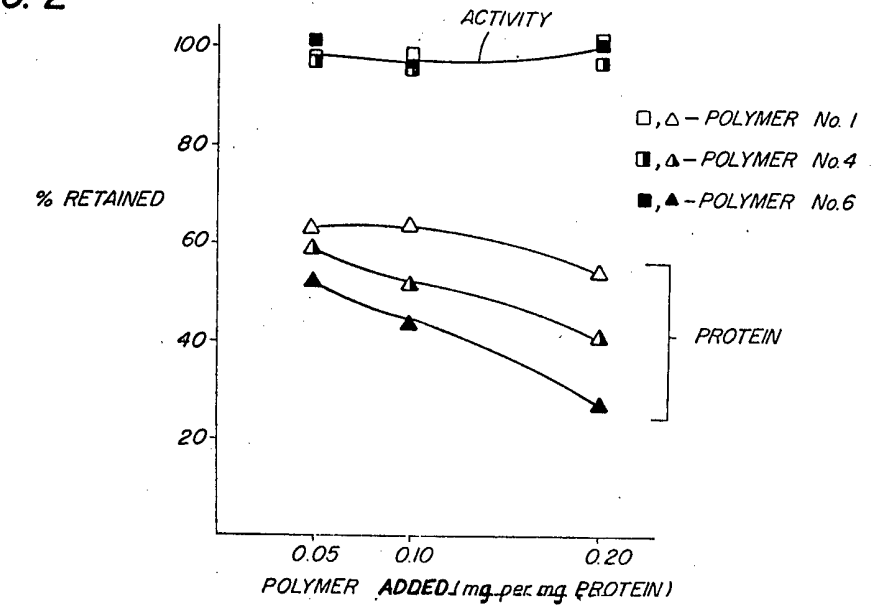


FIG. 3

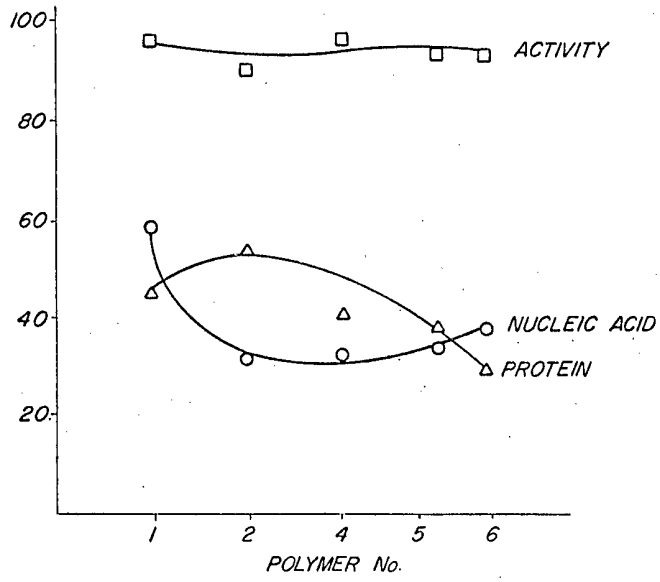

100
80
60
40
20
% RETAINED
ACTIVITY
NUCLEIC ACID
PROTEIN
1
2
4
5
6
POLYMER No.

FIG. 4

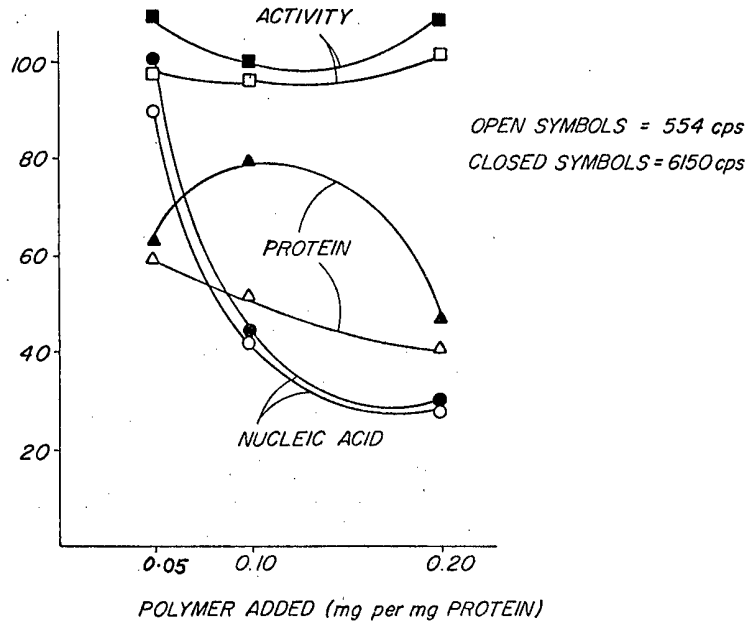

100
80
60
40
20
% RETAINED
ACTIVITY
PROTEIN
NUCLEIC ACID
OPEN SYMBOLS = 554 cps
CLOSED SYMBOLS = 6150 cps
0·05
0.10
0.20
POLYMER ADDED (mg per mg PROTEIN)

FIG. 5

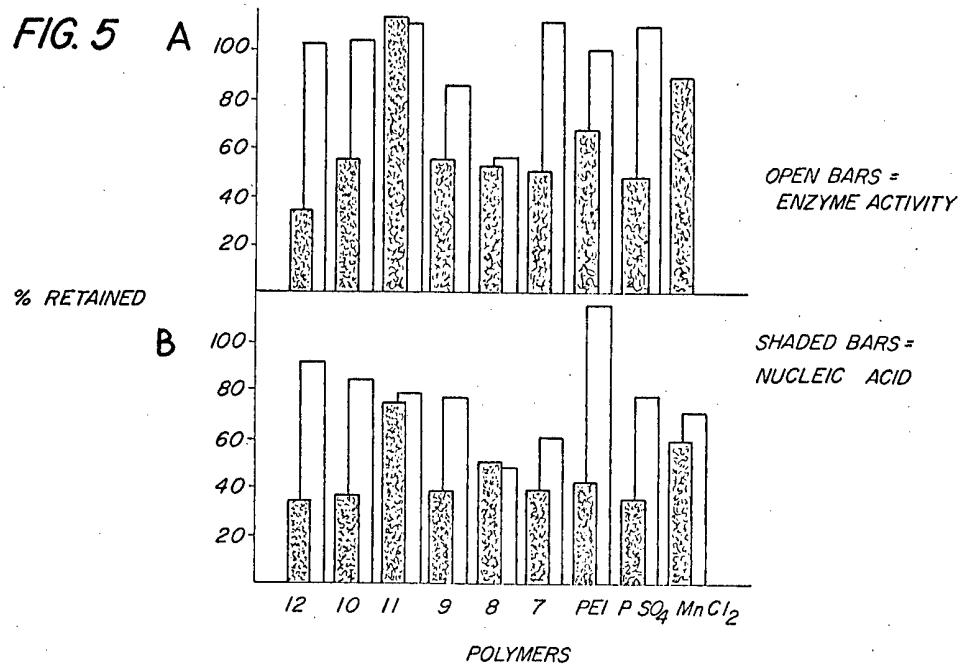

A
B
100
80
60
40
20
% RETAINED
OPEN BARS = ENZYME ACTIVITY
SHADED BARS = NUCLEIC ACID
12 10 11 9 8 7 PEI P SO4 MnCl2
POLYMERS

FIG. 6

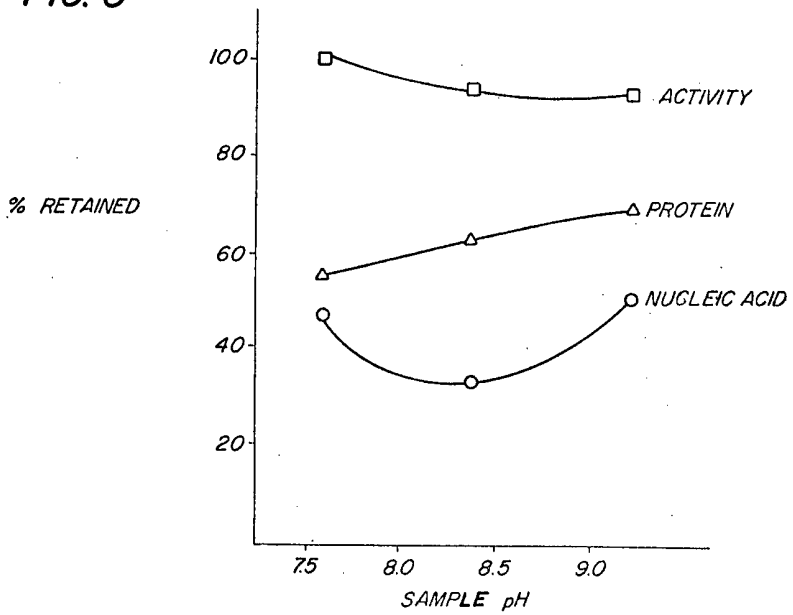

100
80
60
40
20
% RETAINED
ACTIVITY
PROTEIN
NUCLEIC ACID
7.5 8.0 8.5 9.0
SAMPLE pH

FIG. 7

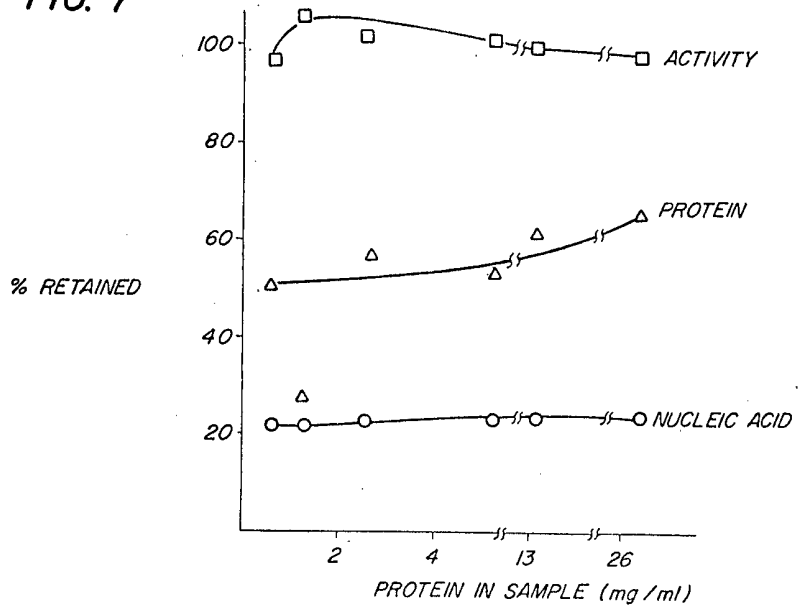
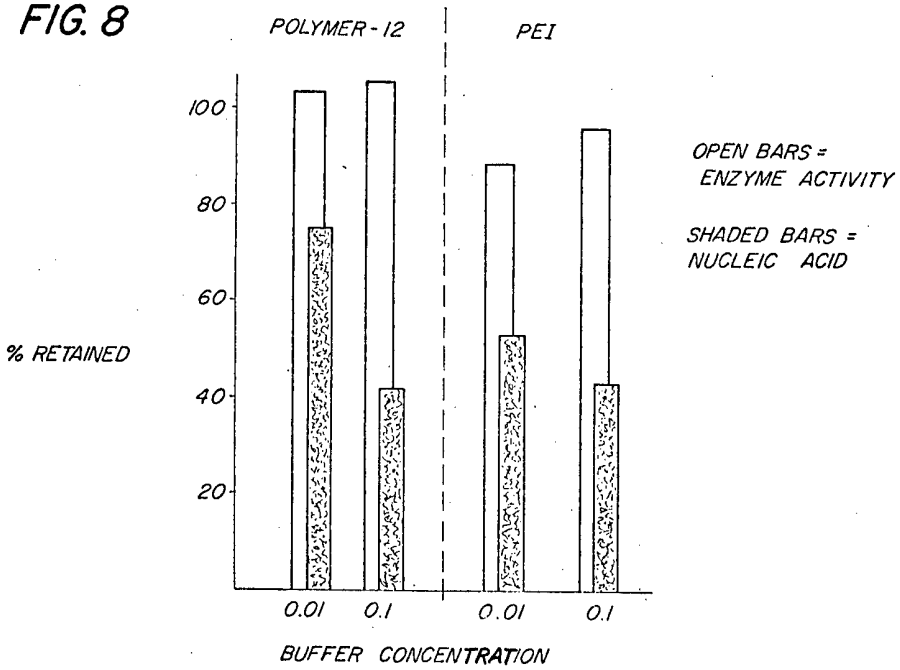

100
80
60
40
20
% RETAINED
ACTIVITY
PROTEIN
NUCLEIC ACID
2
4
13
26
PROTEIN IN SAMPLE (mg/ml)

FIG. 8


POLYMER-12
PEI
100
80
60
40
20
% RETAINED
OPEN BARS = ENZYME ACTIVITY
SHADED BARS = NUCLEIC ACID
0.01
0.1
0.01
0.1
BUFFER CONCENTRATION

1590310 COMPLETE SPECIFICATION
5 SHEETS This drawing is a reproduction of the Original on a reduced scale
Sheet 5

FIG. 9