j-Carrageenan as a carrier in affinity precipitation of yeast alcohol dehydrogenase

Kalyani Mondal, Ipsita Roy, and Munishwar N. Gupta*

Chemistry Department, Indian Institute of Technology, Delhi, Hauz Khas, New Delhi 110016, India

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Abstract

j-Carrageenan is a non-toxic polymer from seaweeds, which becomes reversibly insoluble upon the addition of Kþ. Its conjugate with the dye, Cibacron Blue 3GA, was used to purify alcohol dehydrogenase from crude yeast extract by affinity precipitation. Response surface methodology was used to optimize conditions for affinity precipitation of the enzyme with the polymer-dye conjugate. Recovery of 58% of the enzyme activity with 13.6-fold purification was obtained.

Keywords: Affinity precipitation; Alcohol dehydrogenase,; j-Carrageenan; Reversibly soluble-insoluble polymers; Protein purification

Affinity precipitation has emerged as a simple non-chromatographic approach for purification of proteins [1] and DNA [2]. The technique is based upon the use of polymers, which can be made soluble or insoluble in a reversible fashion with the help of a stimulus. The polymer can be linked to an affinity ligand and this smart macroaffinity ligand can be used to selectively co-precipitate along with the target protein by applying the appropriate stimulus. Mostly synthetic polymers like methylmethacrylates or poly(N-isopropylacrylamide) have been used for various applications [3-5]. Natural polymers with their inherent biodegradability constitute a "green" alternative and have also been successfully used, e.g., chitosan [6,7] and alginate [8].

Recently, we have described the use of j-carrageenan for affinity precipitation of pullulanase by exploiting the inherent affinity of j-carrageenan for pullulanase [9]. Carrageenan is a naturally occurring family of polysaccharides extracted from red seaweed. It consists of sulfated or non-sulfated galactose and 3,6-anhydro galactose units. j-Carrageenan has already been used for whole cell entrapment [10]. It is a non-toxic polymer and is also used in processed foods [11]. A useful feature of j-carrageenan as a smart polymer is that it becomes insoluble merely upon addition of $K^þ$ and no change in pH or temperature is required for reversible solubilization-insolubilization (as is the case of most of the smart polymers, which are pH-responsive or thermo-sensitive). Thus, the target protein/enzyme are not subjected to any potential denaturing conditions or structural stresses resulting from sudden changes in pH or temperature. The present work evaluates j-carrageenan as a general-purpose carrier of affinity ligands for use in affinity precipitation of proteins. The conjugate of j-carrageenan with the dye Cibacron Blue was used for purification of yeast alcohol dehydrogenase (ADH) using the known affinity of the dye for the enzyme [12]. It was decided to use response surface methodology to optimize conditions for affinity precipitation of the enzyme.

Materials and methods

Materials

j-Carrageenan (Catalog No. 22048) was purchased from Fluka Biochemika (St. Louis, MO, USA). Cyanogen bromide (CNBr), c-amino butyric acid (GABA), alcohol dehydrogenase (ADH) (semi-purified from yeast), and nicotinamide adenine dinucleotide ($NAD^þ$) were purchased from Sisco Research Laboratories,

Mumbai, India. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was obtained from Spectrochem, Mumbai, India. Cibacron Blue 3GA was purchased from Sigma Chemical (St. Louis, USA). All other chemicals used were of analytical grade.

Methods

Preparation of j-carrageenan solution

j-Carrageenan (0.5 g) was slowly added to 50 mL of 0.25 M of N,N-bis(2-hydroxyethyl)-2-aminoethane sulfonic acid (BES buffer), pH 7.0, with continuous stirring on a magnetic stirrer for 1 h to get a 1% (w/v) solution. The amount of j-carrageenan was estimated by the phenol-sulfuric acid assay [13].

Determination of alcohol dehydrogenase activity

Alcohol dehydrogenase activity was estimated according to the method described [14]. The assay mixture in a cuvette contained 0.032 M sodium pyrophosphate buffer (1.5mL), 2M ethanol (0.5mL), and 0.025M $NAD^{þ}$ (1 mL). The reaction, at room temperature, was started by the addition of 0.1 mL of appropriately diluted enzyme solution, and the generation of NADH was followed by measuring the initial rate of increase in absorbance at 340 nm. One unit of ADH activity was defined as the amount of enzyme which catalyzes the generation of 1 lmol of NADH per min under the conditions specified.

Protein estimation

Protein was estimated by the dye binding method [15] using bovine serum albumin as standard protein.

Preparation of dye-j carrageenan conjugate

Cibacron Blue 3GA dye was covalently linked to j-carrageenan as described [16]. j-Carrageenan solution (50 mL of 1%, w/v) was first activated with cyanogen bromide (CNBr). CNBr (10 g in 5mL of acetonitrile) was added dropwise to the polymer solution and stirred on a shaker for 2 min at 25 °C. To the activated polymer, c-amino butyric acid (GABA) was linked by addition of 10 g of GABA and stirring the solution for 18 h at 4 °C. Excess GABA was removed by precipitating the activated polymer with 0.1% (w/v) KCl and washing it thoroughly with 0.25 M BES buffer, pH 7.0, containing 0.1% (w/v) of KCl. The polymer was redissolved in 50 mL of 0.25 M BES buffer, pH 7.0, and 3 g of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 1 g of dye added. The solution was then stirred for 3 h at 25 °C whereafter the dye linked polymer was reprecipitated with 0.1% (w/v) KCl. The polymer was washed with 0.25 M BES buffer, pH 7.0, containing 0.1% (w/v) KCl until no dye appeared in the washings. The dye was estimated at 670 nm. The difference between the amount of dye added and that in the washings represented the amount of dye conjugated to the polymer.

Preparation of yeast homogenate

Saccharomyces cerevisiae cells were grown in YPD medium of pH 6.5, containing per liter: 10 g glucose, 5g yeast extract, and 5g of peptone [17]. The medium was maintained on agar slants by periodic transfer. Inoculum was prepared by inoculating a loopful of *S. cerevisiae* cells from the slant into 10mL of YPD medium followed by incubation at 28 °C with constant shaking at 120 rpm for 16 h. YPD broth (100 mL) was inoculated with 1 mL of 16 h broth culture and incubated at 28 °C, 120 rpm for 48 h. At the end of fermentation, biomass was harvested by centrifugation at 10,000 rpm, 4°C for 10 min. The biomass was washed twice and suspended in 0.02 M Tris-HCl buffer, pH 7.5, and cells were homogenized by sonication in 6 cycles of 15 s each. The release of protein and ADH activity were monitored after each cycle. No enzyme activity was found to be released after 6 cycles. The homogenate (after 6 cycles) was centrifuged (8000 g, 10 min) at 4 °C and the supernatant obtained was lyophilized and used as the source of ADH.

Design of experiments

Response surface methodology (RSM) is an efficient approach for obtaining optimum conditions (parameters) for a process when the latter depends upon two or more variable parameters [18,19]. It consists of four stages: (1) identification of important variable parameters. This presupposes some knowledge of the process, (2) various parameters are varied in the individual ranges. These again are decided by practical considerations, keeping in view the operation of the process, (3) performing the statistically designed experiments to obtain the "actual" values of the response and to compare these with the values "predicted" by the software. A good fit between the "actual" and "predicted" values gives a high correlation coefficient (R^2). These "actual" values are used to solve a multivariate equation to obtain the "predicted" values for the response curve, and (4) the solution of the multivariate equation is graphically represented as response surface curves, which are used to predict one optimum set of parameters.

Plackett-Burman design

Plackett-Burman experimental design is a first-order model, which assumes no interaction among the process parameters [20]. This design was used to evaluate the influence of various process parameters (polymer concentration, temperature, time and enzyme concentration) on the amount of j-carrageenan precipitated (Table 1).

Box-Behnken design

To describe the nature of the response surface in the experimental region, a Box-Behnken design [21] was used for the experimental plan. Tables 2a, 2b, and 2c represent the design matrix.

Table 1
Plackett-Burman experimental design for evaluation of factors affecting the precipitation of j-carrageenan, dye-carrageenan conjugate, ADH-dye-carrageenan complex

Variables	Range studied	Coefficient values obtained
j-Carrageenan		
Polymer conc. (%, w/v)	0.1-1.0	8.75
KCl conc. (%, w/v)	0.1-1.0	7.75
Temperature (°C)	10-40	)8.92
Time (min)	15-60	0.25
Dye-j-carrageenan		
Dye-polymer conc. (%, w/v)	0.1-1.0	3.67
KCl conc. (%, w/v)	0.1-1.0	2.50
Temperature (°C)	10-40	)2.83
Time (min)	15-60	0.17
Dye-j-carrageenan-ADH		
Dye-polymer conc. (%, w/v)	0.1-1.0	12.42
Enzyme (U)	2.0-20	1.75
KCl conc. (%, w/v)	0.1-1.0	)6.08
Temperature (°C)	10-40	2.75
Time (min)	15-60	3.92

The experiments were designed using the software, Design Expert Version 5.0 (Stat-Ease; Minneapolis, USA). All the experiments were carried out in triplicate and the average of amount of precipitation was taken as the response.

Affinity precipitation of ADH with dye-j carrageenan conjugate

Various aliquots of ADH (stock solution of 2 mg/mL in 0.025 M BES buffer, pH 7.5, containing 35.5 U/mL of enzyme activity) were added to 1 mL of dye-j-carrageenan solution. The final volume was made up to 3mL with 0.25 M BES buffer of pH 7.5. Affinity precipitation of ADH with dye-j-carrageenan conjugate was carried out under the conditions given in the Box-Behnken design for enzyme-dye-j-carrageenan complex (Table 2c). The enzyme bound dye-j-carrageenan was precipitated by adding 0.3 mL of 1.0% (w/v) KCl solution (final concentration of KCl in the solution was 0.1%, w/v). After 30 min of incubation at 25 °C, the precipitate was washed with 2mL of 0.25 M BES buffer, pH 7.5, containing 0.1% (w/v) KCl till no enzyme activity could be detected in the washings. The difference between the total enzyme activity loaded and the activity in the supernatant and washings represented the amount of enzyme bound. The enzyme bound polymer was dissolved in 3mL of 0.25 M BES buffer, pH 7.5, containing 0.5 M NaCl and kept at 25 °C for 18 h. Enzyme was then recovered by precipitating the polymer with 0.1% (w/v) KCl solution. Affinity precipitation of ADH from crude yeast homogenate was carried out under the optimized conditions for the purification of ADH from semi-purified commercial preparation.

Each set of the above experiments was carried out in duplicate and the difference in the values within a pair was found to vary within 5%.

Polyacrylamide gel electrophoresis

SDS-PAGE of the protein samples using 12% gel was performed according to Hames [22] using a Bangalore Genei electrophoresis unit and standard molecular weight markers (Bangalore Genei, Bangalore, India).

Results and discussion

The use of dyes as a robust and commercial affinity ligand in affinity chromatography has been quite

Table 2a
Box-Behnken experimental design for the effect of various process parameters on the amount of j-carrageenan precipitated

j-Carrageenan concentration (%, w/v)	KCl concentration (%, w/v)	Time (min)	Precipitation (%)	
			Actual value	Predicted value
0.10	0.55	15.0	49	46.6
1.00	0.55	15.0	76	76.1
0.55	0.10	15.0	78	79.0
0.55	1.00	15.0	65	66.2
0.10	0.10	37.5	31	32.4
1.00	0.10	37.5	78	76.9
0.10	1.00	37.5	42	43.1
1.00	1.00	37.5	52	50.6
0.55	0.55	37.5	33	37.4
0.55	0.55	37.5	40	37.4
0.55	0.55	37.5	35	37.4
0.55	0.55	37.5	38	37.4
0.55	0.55	37.5	41	37.4
0.10	0.55	60.0	50	49.9
1.00	0.55	60.0	70	72.4
0.55	0.10	60.0	75	73.7
0.55	1.00	60.0	72	71.0

Table 2b
Box-Behnken experimental design for the effect of various parameters on the amount of dye-j-carrageenan precipitated

Dye-polymer concentration (%, w/v)	KCl concentration (%, w/v)	Time (min)	Precipitation (%)	
			Actual value	Predicted value
0.10	0.10	37.5	35	36.4
1.00	0.10	37.5	85	84.4
0.10	1.00	37.5	47	47.6
1.00	1.00	37.5	55	53.6
0.10	0.55	15.0	51	49.6
1.00	0.55	15.0	77	77.6
0.10	0.55	60.0	51	50.4
1.00	0.55	60.0	75	76.4
0.55	0.10	15.0	78	78.0
0.55	1.00	15.0	65	65.7
0.55	0.10	60.0	76	75.2
0.55	1.00	60.0	68	68.0
0.55	0.55	37.5	40	41.2
0.55	0.55	37.5	40	41.2
0.55	0.55	37.5	40	41.2
0.55	0.55	37.5	43	41.2
0.55	0.55	37.5	43	41.2

Table 2c
Box-Behnken experimental design for the effect of various parameters on the amount of dye-j-carrageenan-ADH precipitated

Dye-polymer concentration (%, w/v)	Enzyme (U)	Time (min)	Temperature (°C)	Precipitation (%)	
				Actual value	Predicted value
0.10	2	37.5	25	55.0	51.6
1.00	2	37.5	25	85.0	83.0
0.10	20	37.5	25	51.0	51.4
1.00	20	37.5	25	98.0	99.8
0.55	11	15.0	10	77.0	78.4
0.55	11	60.0	40	81.0	81.0
0.10	11	37.5	10	52.0	51.7
1.00	11	37.5	10	98.0	99.8
0.10	11	37.5	40	55.0	56.2
1.00	11	37.5	40	89.0	90.1
0.55	2	15.0	25	69.0	70.2
0.55	20	15.0	25	83.5	81.7
0.55	2	60.0	25	72.0	74.6
0.55	20	60.0	25	80.0	79.6
0.10	11	15.0	25	51.0	52.1
1.00	11	15.0	25	93.0	92.8
0.10	11	60.0	25	53.0	54.0
1.00	11	60.0	25	93.5	93.2
0.55	2	37.5	10	75.0	75.0
0.55	20	37.5	10	82.0	81.8
0.55	2	37.5	40	70.0	71.5
0.55	20	37.5	40	81.0	81.8
0.55	11	37.5	25	82.0	79.8
0.55	11	37.5	25	82.0	79.8
0.55	11	37.5	25	78.0	79.8
0.55	11	37.5	25	78.0	79.8
0.55	11	37.5	25	79.0	79.8

extensive [23]. Cibacron blue in particular has been used as an affinity ligand for purification of a large number of enzymes [24,25] including yeast alcohol dehydrogenase [25]. In the case of NADþ-dependent dehydrogenases like yeast alcohol dehydrogenase, the dye is known to bind to the enzyme at the NADþ binding site [26]. Fig. 1 shows the amount of dye conjugated to j-carrageenan at various starting concentrations of the dye. The starting amount of dye beyond 5g/g of polymer decreased % conjugation. For further work, the polymer-dye

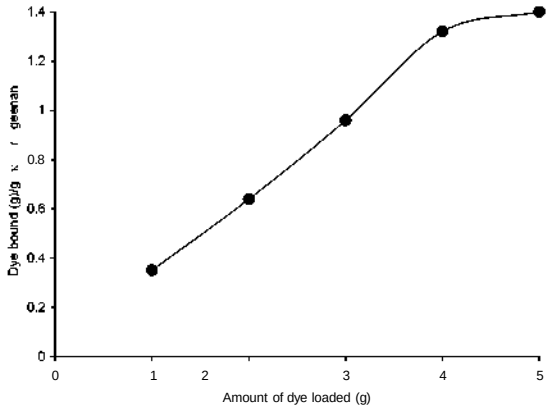

Fig. 1. Conjugation of different concentrations of dye to j-carrageenan. Varying amounts of dye were covalently coupled to 100 mL of 1% (w/v) j-carrageenan solution (containing 1 g of polymer). The conjugation was carried out as given in Materials and methods. The difference between the initial amount of dye added and that in the washings represented the amount of dye coupled to the polymer.

conjugate prepared with 2 g of dye/g of polymer as the starting dye concentration was used.

Response surface methodology was used to optimize the precipitation of the polymer, polymer-dye conjugate, and yeast alcohol dehydrogenase in the presence of the polymer-dye conjugate.

To identify key process variables which affect the precipitation behavior of j-carrageenan, dye-j-carrageenan conjugate and dye-j-carrageenan-ADH complex, a Plackett-Burman design was first used. The process variables, the ranges taken, and the coefficients of the variables obtained in the analysis are given in Table 1. For j-carrageenan, temperature of precipitation showed a negative coefficient, hence, it was concluded that temperature did not affect the precipitation behavior of the polymer. It had been observed earlier that the amount of j-carrageenan precipitated at 37 °C is marginally higher than at 25 °C [9]. Hence, for the Box-Behnken design for the polymer (Table 2a), the temperature of precipitation was kept constant at 37 °C.

For dye-j-carrageenan conjugate, Plackett-Burman design gave a similar result; hence, the temperature was once again kept constant at 37 °C for the Box-Behnken design (Table 2b).

In the case of enzyme-dye-j-carrageenan complex, however, a negative coefficient was obtained for KCl concentration; hence in the Box-Behnken design (Table 2c), the KCl concentration was kept constant at 0.1% (w/v), the minimum of the range taken. Analysis of variance (ANOVA) of the regression results is given in Table 3. A high R^2 value of 0.986, 0.995, and 0.987 for j-carrageenan, dye-j-carrageenan, and ADH-dye-j-carrageenan, respectively, showed a good fit between the actual and the predicted values of the models. Besides, the computed Fmodel values were much greater than the tabular *F* values at 1% confidence level for all the three systems, indicating that the treatment was highly significant. The models also showed statistically insignificant lack of fit, as was evident from the low Flack of fit values. The models were hence found to be adequate for prediction of the optimum result within the range of variables employed. The predicted values and their experimental verifications are given in Table 4. They were found to be in good correlation. Figs. 2-4 give the experimental results in the form of surface profiles.

For j-carrageenan, increasing polymer concentration at low concentrations of KCl (at a constant time of 60min) significantly enhanced the precipitation of the polymer (Fig. 2A). Increasing the time of precipitation with increase in j-carrageenan concentration also increased the amount of precipitation (Fig. 2B). At a high j-carrageenan concentration (1%, w/v), varying time

Table 3
Analysis of variance (ANOVA) of the calculated model for j-carrageenan, dye-j-carrageenan, and dye-j-carrageenan-ADH

	Amount of precipitation in		
	j-Carrageenan	Dye-polymer	Dye-polymer with enzyme
Model			
Sum of squares	4848	4393	5372
Degree of freedom	9	9	14
Mean squares	539	488	384
•fmodel	55.5	162	77.8
Probability	0.0001	0.0001	0.0001
Residual			
Sum of squares	68	21	69
Degree of freedom	7	7	14
Mean squares	9.7	3.0	4.9
F-ratio	—	—	—
Probability	—	—	—
Flack of fit	0.67	1.27	1.24
Correlation coefficient (R^2Þ	0.986	0.995	0.987

Table 4
Experimental verification of the effect of optimized conditions on the amount of j-carrageenan precipitated

Variables	Range studied	Optimization predicted	Precipitation (%)	
			Predicted	Observed
j-Carrageenan				
Polymer conc. (%, w/v)	0.1-1.0	1.0	95.3	93
KCl conc. (%, w/v)	0.1-1.0	0.1		
Time (min)	15-60	60		
Dye-j-carrageenan				
Dye-polymer conc. (%, w/v)	0.1-1.0	1.0	98.3	95
KCl conc. (%, w/v)	0.1-1.0	0.1		
Time (min)	15-60	57.75		
Dye-j-carrageenan-ADH				
Dye-polymer conc. (%, w/v)	0.1-1.0	1.0	99.8	99
Enzyme (U)	2.0-20.0	20		
Temperature (°C)	10-40	25		
Time (min)	15-60	37.50		

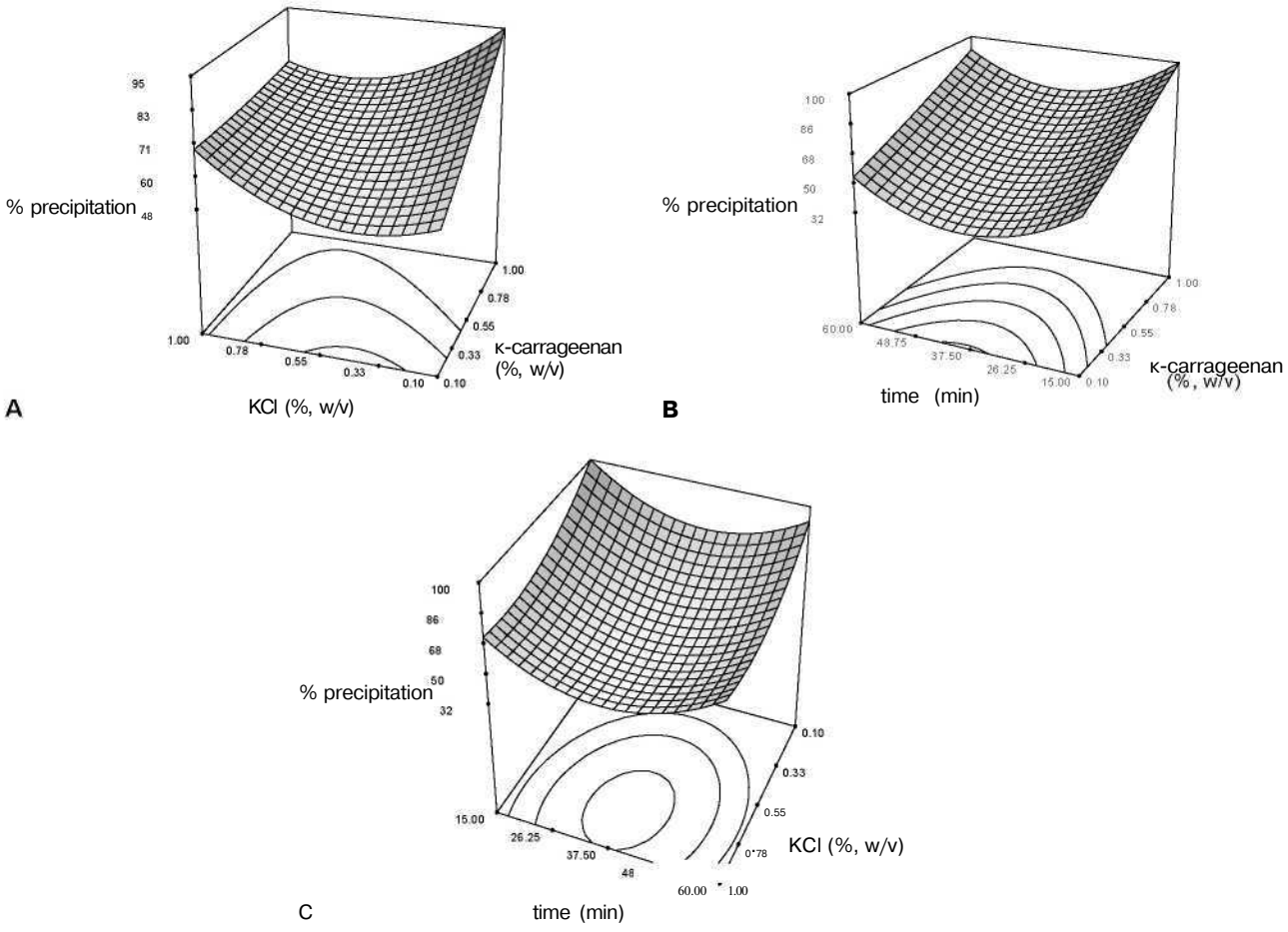

Fig. 2. (A) Effect of variation of j-carrageenan and KCl concentration on the amount of j-carrageenan precipitated at a constant time of 60 min. (B) Effect of variation of j-carrageenan concentration and time on the amount of j-carrageenan precipitated at a constant KCl concentration of 0.1% (w/v). (C) Effect of variation of KCl concentration and time on the amount of j-carrageenan precipitated at a constant j-carrageenan concentration of 1.0% (w/v).

and KCl concentration affected the precipitation to a lesser extent. The patterns of the response profiles are similar for dye-carrageenan conjugate (Figs. 3A-C).

For enzyme-dye-polymer complex, increasing enzyme and dye-j-carrageenan concentration [at a constant time (37.5 min) and temperature (25 °Q]

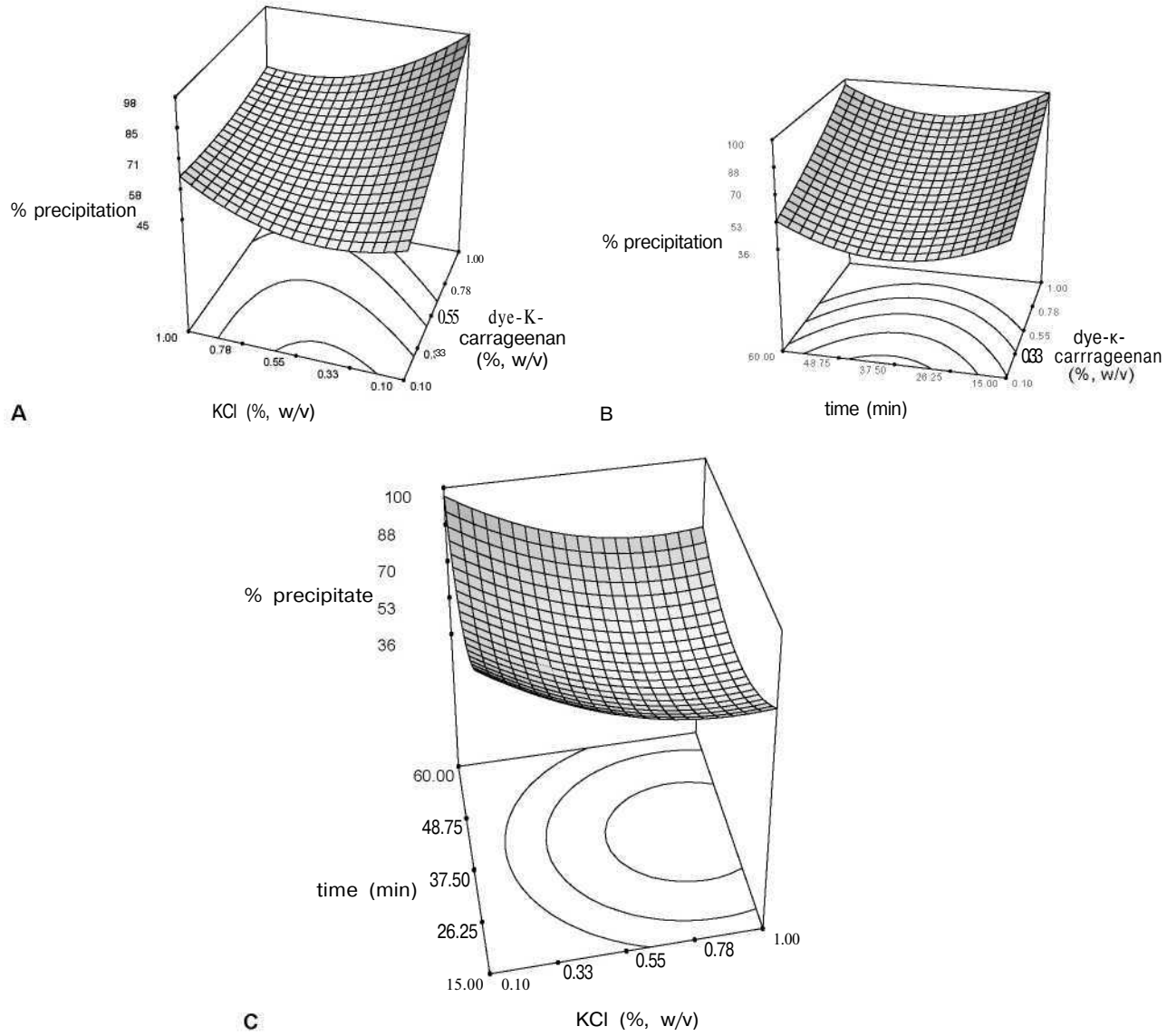

Fig. 3. (A) Effect of variation of dye-j-carrageenan and KCl concentration on the amount of j-carrageenan precipitated at a constant time of 57.75 min. (B) Effect of variation of dye-j-carrageenan concentration and time on the amount of j-carrageenan precipitated at a constant KCl concentration of 0.1% (w/v). (C) Effect of variation of KCl concentration and time on the amount of j-carrageenan precipitated at a constant j-carrageenan concentration of 1.0% (w/v).

significantly increased the amount of dye-polymer precipitated (Fig. 4A). This was also true for the variation in dye-j-carrageenan concentration and time (Fig. 4B). However, variation of enzyme amount and time at a high constant concentration of dye-polymer (1%, w/v) did not result in significant difference in the extent of precipitation of dye-j-carrageenan (Fig. 4C). This is similar to the result observed earlier with pectinase [27]. It was found that after a certain concentration of j-carrageenan, increasing the concentration of polymer, in the presence of pectinase, did not increase the amount of polymer precipitated. For example, in the case of precipitation of pullulanase and pectinase with j-carrageenan, the amount of polymer precipitated, in the presence of the enzymes, was 91%, whereas in the absence of the enzymes, only 76% polymer could be precipitated [9]. These results concerning precipitation behavior of j-carrageenan, dye-j-carrageenan, and enzyme-dye-j-carrageenan indicate that all the three cases differ. Thus, at least with this system, one cannot extrapolate the results on the optimized conditions for precipitation of the polymer to affinity precipitation of the target enzyme.

Earlier work has shown that in affinity precipitation, the concentration of the polymer/polymer-affinity ligand conjugate at the time of binding influences the ease of dissociation (and hence the recovery) of the enzyme from its complex with the polymer-affinity ligand conjugate [8,28]. Generally, low polymer concentration favors the enzyme activity recovery. Table 5 shows that in

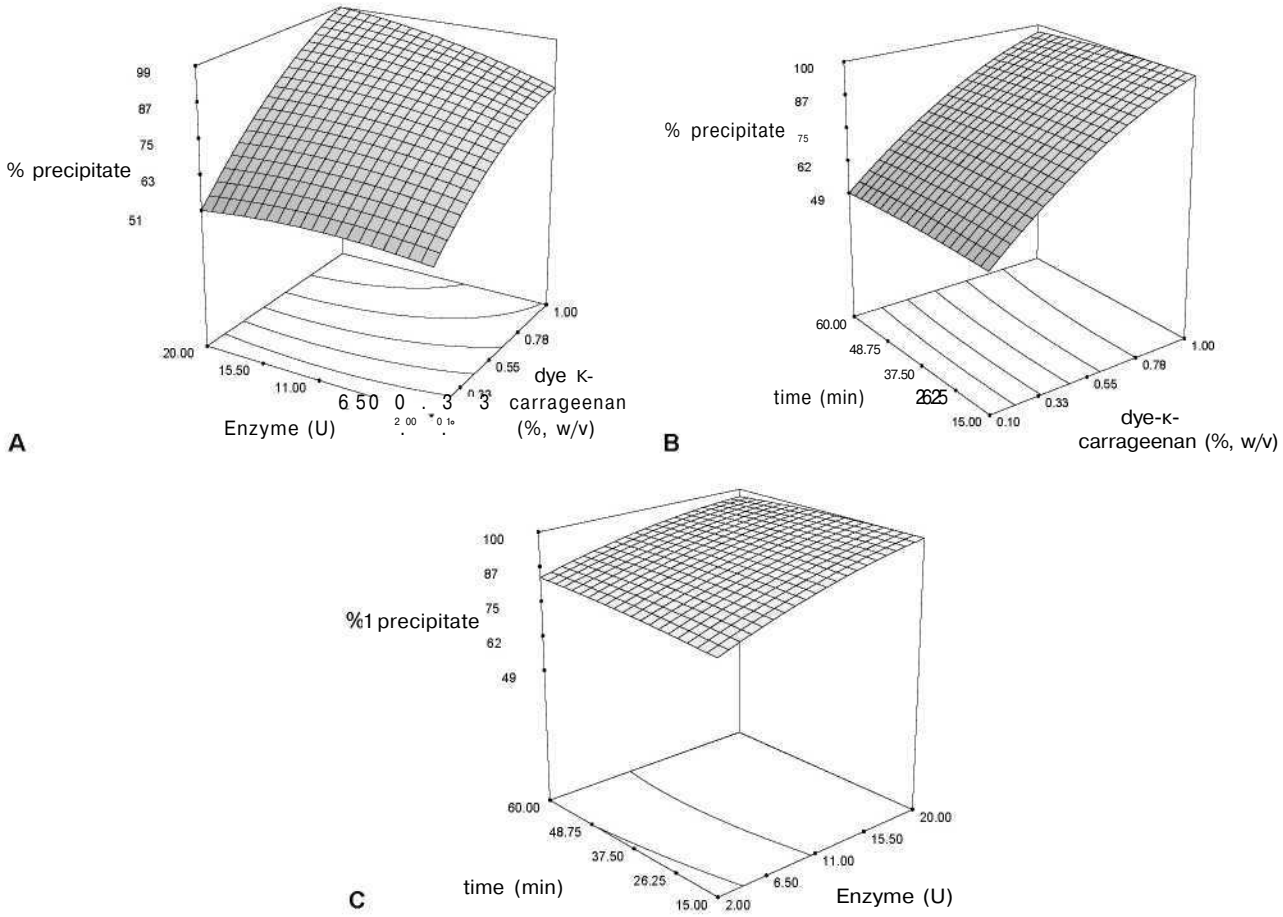

Fig. 4. (A) Effect of variation of dye-j-carrageenan and enzyme units on the amount of j-carrageenan precipitated at a constant time of 37.50 min and temperature. (B) Effect of variation of dye-j-carrageenan concentration and time on the amount of j-carrageenan precipitated at a constant KCl concentration of 0.1% (w/v). (C) Effect of variation of enzyme units and time on the amount of j-carrageenan precipitated at a constant j-carrageenan concentration of 1.0% (w/v).

Table 5
Recovery of ADH (from semi-purified commercial source) at different polymer concentrations

Polymer conc. (%, w/v)	Activity		Recovery (%)	
	Load (U)	Bound (%)	0.5 M NaCl	1 M NaCl
0.3	11	94	36	22
0.5	11	100	62	39
1	20	100	55	8

Alcohol dehydrogenase was bound to dye-j-carrageenan as described in Materials and methods. Bound enzyme activity was recovered as described in Materials and methods using different concentrations of NaCl. The activity of the enzyme initially added was taken as 100%.

Table 6
Purification of ADH (from a semi-purified source) by affinity precipitation with dye-jcarrageenan conjugate

Steps	Activity (U)	Protein (mg)	Specific activity (U/mg)	Yield (%)	Purification fold
Crude	11	0.5	22	100	1
Supernatant + washing; of precipitate	0.0	0.2	0	—	—
Elution					
0.5 M NaCl in 0.25 M BES buffer, pH 7.5, at 25°C, 18h	6.8	0.07	97	62	4.4

The concentration of polymer used for affinity precipitation was kept constant at 0.5%. The experimental details are as described in Materials and methods.

Table 7
Purification of ADH (from crude yeast extract) by affinity precipitation with dye-j-carrageenan conjugate

Steps	Activity (U)	Protein (mg)	Specific activity (U/mg)	Yield (%)	Purification fold
Crude	11	2.3	4.7	100	1
Supernatant + washing of precipitate	0.0	1.5	-	—	—
Elution					
0.5 M NaCl in 0.25 M BES buffer, pH 7.5, at 25°C, 18h	6.4	0.1	64	58	13.6

Affinity precipitation of ADH was carried out under the optimized conditions obtained with semi-purified commercial preparation.

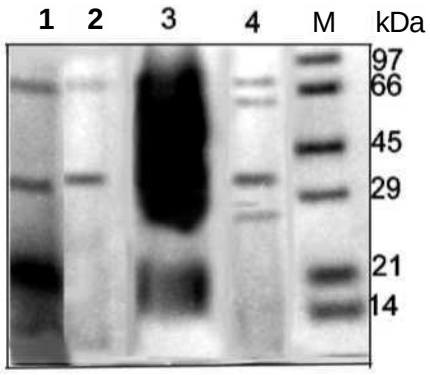

Fig. 5. SDS-PAGE of purified ADH. Lane M: marker proteins; lane 1: crude ADH (from commercial source); lane 2: purified ADH (from commercial source); lane 3: crude ADH (yeast homogenate); and lane 4: purified ADH (from yeast homogenate). The amount of protein loaded in each lane was 20 μg. The gel was stained with Coomassie blue for 45min and then destained with a solution of 40% (v/v) methanol and 10% (v/v) acetic acid.

the case of enzyme-j-carrageenan-dye conjugate, there is an optimum concentration for maximum recovery of the enzyme activity. Thus, 0.5% (w/v) dye-polymer concentration was used at the time of binding the enzyme.

Table 6 shows the purification of ADH from a semi-purified preparation of the enzyme. 62% activity recovery and 4.4-fold purification were obtained. SDS-PAGE (Fig. 5) showed significant purification in this one-step purification process. This prompted us to attempt purification of ADH from yeast extract. Table 7 shows that even with the crude extract as starting material, 58% yield and 13.6-fold purification were obtained. These values are comparable with the reported values of 66% recovery and 20-fold purification of the enzyme [12]. Again SDS-PAGE (Fig. 5) reflects considerable purification of the enzyme. In fact, the SDS-PAGE (lane 4) shows fewer bands than those reported by Guoquiang et al. [12] for their purified preparation and less contaminants than the enzyme purified from commercial yeast alcohol dehydrogenase preparation (lane 2). It may also be mentioned that: (a) as reported by Guoquiang et al. [12], yeast cell contains several enzymes which are known to bind to Cibacron blue so it is expected that the enzyme preparation obtained by affinity precipitation with dye-j-carrageenan will contain some impurities, (b) the protocol followed by Guoquiang et al. [12] employed polyethyleneimine pretreatment before the affinity precipitation. In our work, we did not find any pretreatment (other than simple centrifugation) step necessary, and (c) affinity precipitation, as a single plate separation process, is expected to give less resolution as compared to a chromatographic process. The main bands in SDS-PAGE (lanes 2 and 4) correspond to the reported molecular weight of yeast ADH (35 kDa) [29].

As a ‘‘proof of the concept,’’ this work shows that J-carrageenan can be used as a reversible soluble-insoluble carrier for designing macroaffinity ligands for affinity precipitation of proteins.

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