Cholesterol Esterase and Cholesterol Oxidase Immobilized onto Arylamine Glass Beads

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Abstract Cholesterol esterase (CEase) from bovine pancreas and cholesterol oxidase (COD) from *Bravibacterium* recombinant type have been immobilized individually and co-immobilized onto arylamine glass (pore diameter 55nm) through the process of diazotization. CEase and COD retained 92.65% and 85.54% of the initial activity with conjugation yields of 7.2 mg/g and 8.3 mg/g support respectively when immobilized individually on arylamine glass beads, but retained 89.58% of the initial activity with a conjugation yield of 2.9 mg/g support when co-immobilized on the same support. The effects of pH, temperature, time of incubation, substrate concentration, serum inorganic salts & metabolites, thermal stability, storage stability in cold and reusability on the immobilized enzymes were studied and compared with those of free enzymes. The analytic use of both individually immobilized and co-immobilized enzymes in discrete analysis of total and free cholesterol in serum is demonstrated.

Key words cholesterol, cholesterol esterase, cholesterol oxidase, immobilization, arylamine glass, serum, cholesterol determination

Increased level of cholesterol is found in coronary artery disease, nephrosis, diabetes, myxoedema, obstructive jaundice and hypothyroidism; while decreased level of cholesterol is observed in cases of hypothyroidism, anemia, malabsorption and wasting syndromes[1]. Thus, cholesterol determination in serum is very important in clinical diagnosis. Various methods are available for cholesterol determination based on colorimetry[2~5] and by specific en-zymic methods[6~13]. However, colorimetric methods involve some complicated and time-consuming steps, while the enzymic methods require fresh costly enzyme in each assay. The immobilization of enzymes provides the preparation that combines high selectivity with an increase in stability and, furthermore, the enzyme can be reused and the method is rendered economical. An inorganic carrier, in general, is resistant to microbial attack, stable over a wide pH range, easy to manufacture, inexpen-sive and possesses a long working life span.

So far, cholesterol esterase (CEase) and cholesterol oxidase (COD) have been immobilized individually and also together onto alkylamine glass for the automated[14] and flow injection analysis[15] of total serum cholesterol. However, the immobilization of an enzyme on alkylamine glass through glutaraldehyde has the disadvantage of extensive self-polymerization nature of glutaraldehyde and protein cross-linking, therefore the support needs to be well washed prior to enzyme addition[16]. Further, the glutaraldehyde coupling involves Schiff's base formation, which has a drawback of the reversibility of the reaction at low pH[17]. Immobilization of an enzyme on arylamine glass through diazotization has no such problem, as it enables the inter-disposition of a spacer arm between the two reduced steric interactions and yields higher enzyme to carrier conjugation ratio than by other coupling procedures[16,17]. To the best of our knowledge, both CEase and COD have neither been immobilized/co-immobilized onto arylamine glass nor their kinetic properties have been studied after the immobilization. Hence, the present work was undertaken to immobilize CEase and COD individually and together onto arylamine glass and analyze the changes in their kinetic properties on doing so. The use of arylamine immobilized enzyme in determination of total and free serum cholesterol is demonstrated.

MATERIALS AND METHODS

Chemicals

Arylamine glass (pore diameter 55 nm) from Corning Glass Works, USA; CEase (EC 3.1.13, 0.308 units/mg) from bovine pancreas; peroxidase (EC 1.11.1.7, Rz = 1.0) from horseradish from SISCO Research Laboratory, Mumbai; 4-aminophenazone from Sigma Chemical Co., USA; Sephadex G-200 from M/s Pharmacia LKB Biotechnology, Sweden; and 25% glutaraldehyde from BDH Poole, England, were used. All other chemicals used were of AR

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grade.

Preparation of cholesteryl acetate solution

Cholesteryl acetate was used as a substrate for CEase to study its kinetic properties. 50 mg of cholesteryl acetate was dissolved in 1 mL of Triton X-100 by slow heating and stirring until the solution was clear. Sodium phosphate buffer (0.05 mol/L, pH 7.0) was added to get a concentration of 500 mg/dL. Solutions of different concentrations were prepared similarly.

Preparation of colour reagent

The colour reagent was prepared as described by Bais *et al*[18] and consisted of 50 mg 4-aminophenazone, 100 mg phenol and 1 mg peroxidase per 100 mL of 0.4mol/L sodium phosphate buffer (pH 7.0). It was stored in amber colored bottle at 4℃ and prepared fresh every week.

Assay of free CEase

Commercially available bovine pancreas CEase was dissolved in 0.02mol/L sodium phosphate buffer, pH7.0 (1.0mg/mL) and its assay was carried out as described by Allain *et al*[19] with modifications. The reaction mixture consisting 1.7 mL 0.05 mol/L sodium phosphate buffer (pH 7.0) containing 50mmol/L taurodeoxycholate, 0.1 mL COD (25 mu) and 0.1 mL CEase (30 mu) was pre-incubated at 37℃ for 2 min. The reaction was started by adding 0.1 mL cholesteryl acetate solution (500 mg/dL). After incubation at 37℃ for 5 min, 1.0 mL colour reagent was added and kept at 37℃ for 10 min to develop the colour. A_{520} was read in Spectronic-20 (Milton & Roy, USA) and the content of H_2O_2 generated in the reaction was calculated from the standard curve between H_2O_2 conc. and A_{520}. One unit of the enzyme is defined as the amount of enzyme required to generate 1μmol/L H_2O_2/(min/mL) under the standard assay conditions.

Preparation of COD

COD (EC 1.1.3.6) of *Brevibacterium* recombinant type was separated from the Reagent 1 of commercially available Enzo-kit (supplied by Miles India Ltd., Baroda) by gel filtration on Sephadex G-200 column. Reagent 1 of the kit containing powder of CEase, COD, peroxidase, chromogen and buffer was dissolved in a suitable quantity of potassium phosphate buffer (0.05 mol/L, pH 7.0) and loaded onto Sephadex G-200 column (2.5 cm × 30 cm) previously equilibrated with same buffer. The column was run using 0.02 mol/L potassium phosphate buffer (pH 7.2) at a flow rate of 0.5 mL/min. 2 mL fractions were collected and each fraction was monitored separately for COD activity. The active fractions were pooled and stored at -20℃ until use.

Preparation of cholesterol solution

201 mg cholesterol was dissolved in 10 mL isopropanol. The solution was made upto 100 mL by the addition of 4 mL/L solution of Triton X-100 in de-ionized water.

Assay of free COD

The assay of COD was carried out as described by Allain *et al*[19] with modification. The reaction mixture consisting of 1.8mL sodium phosphate buffer (0.05mol/L, pH7.0, containing 0.4% Triton X-100), 0.1mL cholesterol solution and 0.1mL COD solution were mixed well in a 15mL conical flask wrapped with black paper and incubated for 5min at 37℃. 1.0mL colour reagent was added and kept at 37℃ for 10min to develop the colour. A_{520} was measured and the content of H_2O_2 generated in the reaction was calculated from the standard curve of H_2O_2 concentration vs. A_{520}. One enzyme unit is defined as the amount of enzyme required to generate 1 μmol H_2O_2/min under standard assay conditions.

Immobilization of CEase and COD onto arylamine glass

CEase and COD were immobilized onto arylamine glass beads through diazotization[20]. To 200 mg glass beads in a 25mL flask kept in an ice bath, 2.0mL chilled 2 mol/L HCl was added and shaken immediately. Then, 50 mg solid sodium nitrite was added and the diazotization reaction was allowed to proceed for 30 min with occasional stirring. The excess of nitrous acid was decanted, followed by many washings with 0.1mol/L sodium phosphate buffer (pH 7.0) until the pH of washing was 7.0. The activated beads were divided equally into two parts and kept separately in two 15 mL flasks. One mL each of CEase solution and COD solution in 0.02mol/L sodium phosphate buffer, pH 7.0 was added to two different flasks and kept for 24 h at 4℃ for coupling with occasional shaking. The unbound enzyme from each flask was decanted and the glass beads were washed off with 0.1 mol/L sodium phosphate buffer (pH 7.0) until no activity was detected in the washing. The protein bound to glass beads was estimated by determining the loss of protein from the solution during enzyme immobilization using the Lowry method[21].

Co-immobilization of CEase and COD onto arylamine glass

The mixture of 1.0mL CEase and 1.0mL COD solution was co-immobilized onto 200 mg diazotized arylamine glass beads (diameter 50nm) as described for their individual immobilization. The protein and activity bound to glass beads were determined.

Assay of immobilized CEase and COD

The assay of individually immobilized CEase and COD was carried out as described for their respective native/free forms except that 100 mg arylamine glass beads bound to enzyme were taken in place of

free enzyme solution and the volume of reaction buffer was increased by 0.1mL. The reaction mixture was kept under continuous stirring during incubation. After development of the colour, the reaction mixture was withdrawn by Eppendorff pipette carefully avoiding the loss of glass beads and transferred to a cuvette. A_{520} was read.

Assay of co-immobilized CEase and COD

The assay of co-immobilized CEase and COD was carried out in the similar manner as for immobilized CEase except that co-immobilized enzymes were used in place of individually immobilized CEase, or COD.

Reuse and storage of the immobilized and co-immobilized enzymes

To reuse the immobilized/co-immobilized enzymes, 1mL of their respective reaction buffers was added to the reaction flask through its sidewall. The glass beads were shaken gently for 10 ~ 15s in this buffer and then allowed to settle down. The buffer was withdrawn from the reaction flask with the help of Eppendorff pipette avoiding the loss of beads. This process was repeated 3 ~ 4 times and the beads were used in the next assay. The glass beads were stored at 4℃ in distilled water when not in use.

Determination of total cholesterol in serum with immobilized or co-immobilized CEase and COD

Pre-treatment of serum. To 0.1mL serum was added 0.1mL Triton X-100, 0.1mL isopropanol and 0.2mL sodium phosphate buffer (0.05mol/L pH7.0). It was centrifuged at 3000r/min for 2 min. The supernatant was collected and used for determination of total cholesterol.

Determination of total cholesterol in serum. The assay of total cholesterol in serum was carried out as described for mixture of individually immobilized CEase and COD or co-immobilized CEase and COD except that cholesteryl acetate solution was replaced by 0.1mL pretreated serum. The value of total cholesterol in serum was calculated from standard curve between cholesteryl acetate concentrations ranging from 50 mg/dL to 500 mg/dL reaction mixture and A_{520}.

Determination of free cholesterol in serum by immobilized COD. The assay of free cholesterol in serum was done as described for immobilized COD except that cholesterol solution was replaced by 0.1mL pretreated serum. The value of free cholesterol in serum was extrapolated from standard curve between cholesterol concentration ranging from 4.0 mg/dL to 100 mg/dL and A_{520}.

RESULTS AND DISCUSSION

Individual immobilization and co-immobilization of CEase and COD

CEase from bovine pancreas and COD from *Brevibacterium* recombinant type have been immobilized individually onto arylamine glass beads through diazotization with a conjugation yield of 7.2 mg/g and 8.3 mg/g support respectively. The enzymes retained 92.65% and 85.54% of their initial activity on arylamine glass beads respectively (Table 1), which is higher than that reported on alkylamine glass beads for both the enzymes (81.35% for CEase and 61% for COD)[14]. This may be due to the different chemical reaction involved *i.e.* diazo coupling in immobilization on arylamine, since the diazo coupling minimizes the steric hindrance between groups and thus yields higher enzyme : conjugate ratio as well as higher enzyme activity.

CEase and COD in mixture were also co-immobilized on arylamine glass through diazotization. A conjugation yield of 2.9 mg protein/g, support was obtained with 89.58% retention of initial specific activity, which is higher than that reported on alkylamine glass beads (61%)[14]. The activity of co-immobilized enzymes was 42.5 nmol H_2O_2/min (Table 1).

Kinetic properties of immobilized and co-immobilized CEase and COD

The comparative data on kinetic parameters of free and arylamine conjugated enzymes is given in Table 2. The following kinetic properties were studied.

pH optima. Both arylamine bound CEase and COD showed an optimum pH of 7.5, which is higher than that of their free forms (pH 7.0) (Fig. 1 & 2). This shift in pH optima may be attributed to the formation of a diazo bond between the enzyme and activated support in the ortho position to the -OH group of the tyrosine residue of enzyme.

The co-immobilized CEase and COD showed pH optimum at 7.0 which is higher than that of the mixture of free enzymes (pH 6.5) (Fig. 3).

Table 1 Immobilization and co-immobilization of cholesterol esterase (CEase) and cholesterol oxidase (COD) onto arylamine glass (pore diameter 55 nm)

Enzyme	Glass beads used/mg	Enzyme added to 100 mg glass beads/mg	Enzyme coupled to 100 mg glass beads/mg	Conjugation yield	% enzyme coupled	Total activity bound/(μmol H_2O_2/min)	Retention of specific activity/%
CEase	100	0.96	0.72	7.2	75	0.215	92.65
COD	100	1.65	0.83	8.3	50	92	85.54
CEase + COD	200	0.72	0.29	2.9	77.77	42.5	89.58

One enzyme unit is defined as the amount of enzyme required to generate 1μmol H_2O_2 per min under the standard assay conditions.

Table 2 Kinetic properties of free, individually immobilized and co-immobilized cholesterol esterase (CEase) and cholesterol oxidase (COD) onto arylamine glass beads (pore diameter = 55nm)

Parameter	CEase		COD		CEase + COD	
	Free	Immobilized	Free	Immobilized	Free	Co-immobilized
Optimum pH	7.0	7.5	7.0	7.5	6.5	7.0
Temperature for maximum activity/℃	40	40	45	40	30	35
*E*a/(kCal/mol)	5.9	2.74	2.28	7.62	11.44	11.89
Thermal stability at 75℃ for 30 min/(% activity retained)	18	43	26	55	26	48
Time for linearity/min	6	8	8	6	6	8
K_m/(mmol/L)	6.41	1.33	7.33	6.05	4.6	6.4
V_{max}/(μmol/min/mg)	0.02	0.02	0.066	0.022	10	13.33
Storage in distilled water at 4℃ for 3 months during regular use	30% loss	17% loss	45% loss	25% loss	38% loss	18% loss

To determine the optimum pH following (0.05mol/L) were used: for pH 5.0 to 6.0, sodium succinate buffer; for pH 6.5 to 8.0, sodium phosphate buffer; and for pH 8.5 and 9.0, glycine-NaOH buffer. *E*a was calculated from Arrhenious plot. *K*m and *V*max were determined from Lineweaver-Burk plot.

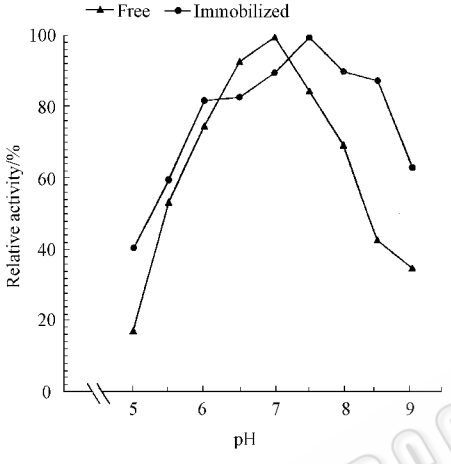

Fig.1 Effect of pH on the activity of free and arylamine glass immobilized cholesterol esterase

Standard assay conditions were used except for the pH of the reaction buffer which was varied from 5.0 to 9.0 as indicated. The following buffers were used each at final concentration of 0.05mol/L: Sodium succinate for pH 5.0 to 6.0; Sodium phosphate for pH 6.5 to 7.5; and Glycine-NaOH for pH 8.0~9.0

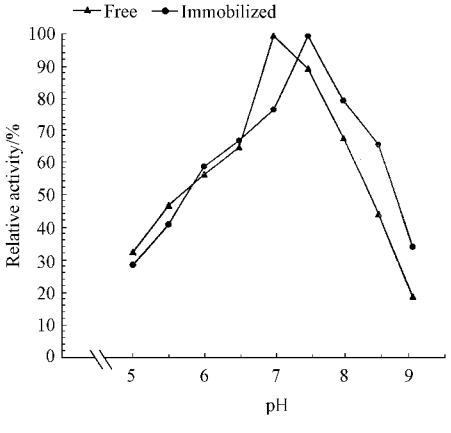

Fig.2 Effect of pH on the activity of free and arylamine glass immobilized cholesterol oxidase

Standard assay conditions were used except for the pH of the reaction buffer which was varied from 5.0 to 9.0 as indicated. The following buffers were used each at final concentration of 0.05mol/L: Sodium succinate for pH 5.0 to 6.0; Sodium phosphate for pH 6.5 to 7.5; and Glycine-NaOH for pH 8.0~9.0

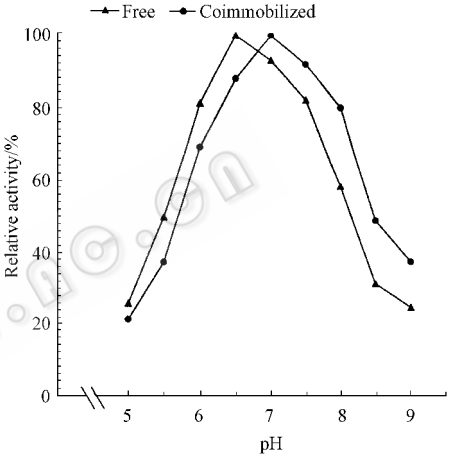

Fig.3 Effect of pH on the activity of free and arylamine glass co-immobilized cholesterol esterase and cholesterol oxidase

Standard assay conditions were used except for the pH of the reaction buffer which was varied from 5.0 to 9.0 as indicated. The following buffers were used each at final concentration of 0.05mol/L: Sodium succinate for pH 5.0 to 6.0; Sodium phosphate for pH 6.5 to 7.5; and Glycine-NaOH for pH 8.0~9.0

pH stability. Arylamine glass bound CEase and COD were kept overnight in the different buffers of pH ranging from pH 5.0~9.0 by using the following buffers, each at 0.05mol/L final concentration: Sodium succinate for pH 5.0~6.0, sodium phosphate for pH 6.5~7.5 and glycine-NaOH for pH 8.0~9.0. Next morning, the buffer was withdrawn by using the Eppendorff pipette and the activity of immobilized enzyme was assayed. The individually immobilized CEase and COD showed fairly good activity over a broad pH range of 6.0 to 8.5 and 6.5 to 9.0 respectively, while the co-immobilized CEase and COD were stable in the pH range of 6.5~8.0, which was higher by pH 0.5 compared to their free forms in each case.

Optimum temperature and thermal stability. The incubation temperature for maximum activity of enzyme was unchanged for CEase (40℃), decreased from 45℃ to 40℃ for COD but increased from 30℃ to 35℃ for mixture of CEase and COD after immobilization. Earlier Karube *et al*.[22] reported the maximum activity of Octyl-aga-

rose bound COD at 43℃. The Energy of activation (Ea) was decreased for CEase, but increased for COD and remained unaltered for mixture of CEase and COD after immobilization (Table 2). Ea represents the minimum energy required for the collision between the molecules to be effective and determines the influence of temperature on reaction velocity. The kinetic energy of an enzyme molecule becomes low due to restriction of conformational movement after immobilization. The higher Ea value upon conjugation to alkylamine glass could be attributed to the introduction of a strain in the enzyme, unfavorable electron flow between reactants and polymer and unfavorable change of polarity or ionic strength in the vicinity of the active site[23]. The thermal stability of both CEase and COD and also their mixture was increased after immobilization (Table 2). The increased thermal stability of the immobilized enzyme probably results from the prevention of conformational inactivation of the enzyme and steric shielding that minimize attack by the reactive solutes[23]. Tabata *et al*[14] also found an increase in thermal stability of alkylamine glass bound enzyme after immobilization.

Time course study. The time for linearity of the H_2O_2 formation was increased from 6 to 8 min for arylamine bound CEase and mixture of CEase and COD but decreased from 8 min to 6 min for COD after immobilization onto arylamine glass.

Table 3 Effect of metal salts on free, individually immobilized and co-immobilized cholesterol esterase (CEase) and cholesterol oxidase (COD) onto arylamine glass beads (pore diameter = 55nm). Data are given in terms of relative activity (%)

Metal salt	CEase		COD		CEase + COD	
	Free	Immobilized	Free	Immobilized	Free	Co-immobilized
None	100	100	100	100	100	100
NaCl	84	86	120	132	92	103
KCl	100	100	122	54	108	100
$MgCl_2$	82	120	111	107	85	114
$MnCl_2$	73	110	118	116	98	130
$CaCl_2.2H_2O$	80	104	100	130	95	100
$ZnSO_4$	150	143	60	93	125	150
$FeSO_4$	102	102	55	63	80	82
K_2SO_4	88	76	107	102	102	100
$MgSO_4.7H_2O$	110	100	108	83	100	135
$CuSO_4$	350	150	107	100	100	100
$CaSO_4$	79	95	96	114	100	100
NaI	102	100	93	100	89	95

Standard assay conditions were used except for the addition of metal salt, which was added in the reaction mixture at final concentration of 1 mmol/L.

Effect of substrate concentration. There was a hyperbolic relationship between substrate concentration [S] and the initial activity [v] of reaction for both individually immobilized and co-immobilized CEase and COD similar to that of their free forms using cholesteryl acetate for CEase and CEase plus COD and cholesterol for COD as substrate. The substrate concentration required for saturation of enzyme was 8 mmol/L for immobilized CEase, similar to its free form (Fig. 4), 9.0 mmol/L for immobilized COD, higher than its free form (Fig. 5) and 11.0 mmol/L for co-immobilized CEase and COD which was also higher than the mixture of their free forms (Fig. 6).

K_m and V_{max}. The K_m value as calculated from Lineweaver Burk plot was decreased for individually immobilized CEase and COD but increased for co-immobilized CEase and COD compared to their free form. V_{max} was unaltered in case of CEase but decreased in case of COD and increased in case of mixture of CEase and COD after immobilization (Table 2). When an enzyme is covalently bound to a solid support, the kinetic pattern of the reaction changes considerably leading to changes in K_m and/or V_{max}. The changes in the kinetics of the immobilized enzyme, are controlled by four factors viz. change in enzyme conformation after immobilization, steric effects, microenvironmental effects and bulk & diffusional effects[24].

Effect of metal salts and metabolites. To study the effect of various inorganic salts and metabolites found in serum on immobilized and co-immobilized CEase and COD, the following compounds were added individually in the reaction mixture at a final concentration of 1.0 mmol/L: NaCl, KCl, $MgCl_2$, $MnCl_2$, $CaCl_2\cdot 2H_2O$, $ZnSO_4$, $FeSO_4$, K_2SO_4, $MgSO_4\cdot 7H_2O$, $CuSO_4$, $CaSO_4$, NaI, pyruvate, glucose, citrate, ascorbate, haemoglobin, albumin, sodium bicarbonate, urea, uric acid, creatinine, glutathione, acetone and citrulline. Among these compounds, ascorbate strongly inhibited the colour reaction catalyzed by peroxidase in the assays of both individually immobilized and co-immobilized CEase and COD. Immobilization of enzyme on arylamine glass protected it from inhibition by $MnCl_2$, $CaCl_2\cdot 2H_2O$, $CaSO_4$ and glutathione in case of CEase and by $ZnSO_4$ and $FeSO_4$ in case of COD. There was a stimulation of immobilized CEase by $MgCl_2$, sodium bicarbonate, creatinine and albumin and immobilized COD by NaCl and co-immobilized CEase and COD by $ZnSO_4$. Arylamine conjugated COD was inhibited by KCl, unlike its free form which was stimulated. Rest of the metal salts had practically no effect (Table 3). The arylamine conjugated COD showed lesser inhibition by pyruvate, ascorbate & glutathione (indicating the resistance of these enzymes after immobilization) compared to that of free enzyme. However, immobilized COD was inhibited by urea unlike its free form, which was markedly stimulated. Rest metabolites had no effect (Table 3).

Table 4 Effect of metabolites on free and individually immobilized cholesterol esterase (CEase) and cholesterol oxidase (COD) onto arylamine glass beads (pore diameter = 55nm). Data are given in terms of relative activity (%)

Metabolite	CEase		COD		CEase + COD	
	Free	Immobilized	Free	Immobilized	Free	Co-immobilized
None	100	100	100	100	100	100
Pyruvate	85	83	14	68	60	92
Glucose	100	100	114	128	94	100
Citrate	100	90	92	85	110	100
Ascorbate	ND*	25	24	53	ND*	15
Haemoglobin	93	95	95	92	100	100
Albumin	115	140	114	135	83	100
Sodium bicarbonate	130	182	128	108	89	100
Urea	92	100	131	85	120	100
Uric acid	100	108	104	138	84	102
Creatinine	120	122	100	90	109	105
Glutathione	80	102	57	82	74	100
Acetone	100	84	92	90	110	100
Citrulline	95	98	137	105	114	100

Standard assay conditions were used except for the addition of metabolite, which was added in the reaction mixture at final concentration of 1 mmol/L. (* ND = Not detected)

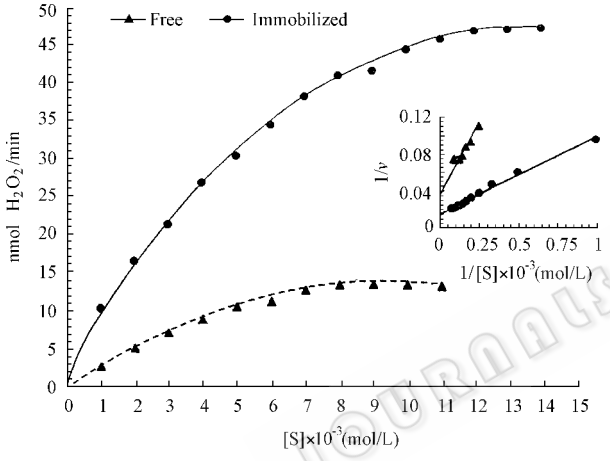

Fig.4 Effect of cholesteryl acetate concentration on the activity of free and arylamine glass immobilized cholesterol esterase

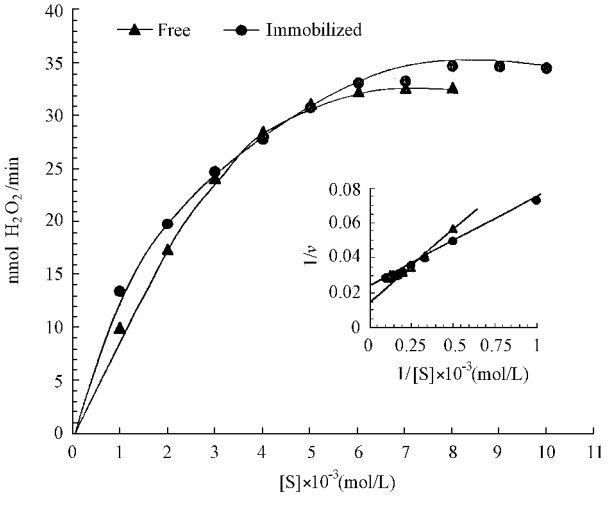

Fig.5 Effect of cholesterol concentration on the activity of free and arylamine glass immobilized cholesterol oxidase

Storage stability. The storage stability of CEase and COD at 4℃ in distilled water for over a period of 3 months was increased after their

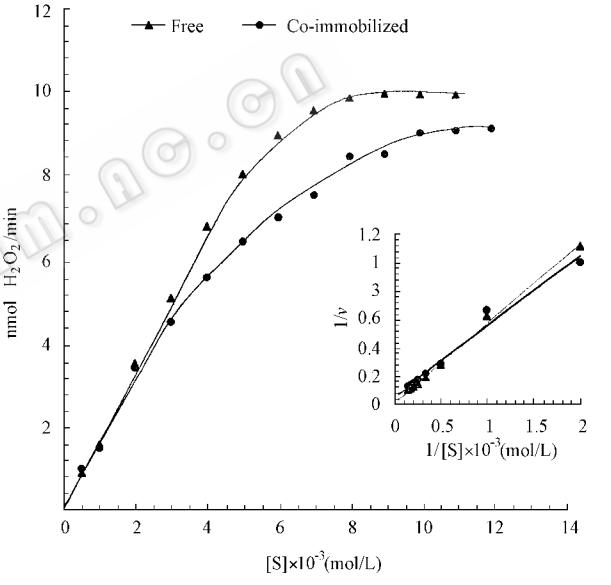

Fig.6 Effect of cholesteryl acetate concentration on the activity of free and arylamine glass co-immobilized cholesterol esterase and cholesterol oxidase

individual immobilization and co-immobilization. The individually immobilized CEase, COD and co-immobilized CEase and COD lost only 17%, 25% and 18% of their initial activities, during their regular use for over a period of 3 months compared to 30%, 45% and 38% loss of initial activity of their free form respectively, under the similar conditions.

Determination of total cholesterol in serum with immobilized and co-immobilized CEase and COD

The total cholesterol in serum obtained from apparently healthy male and female adults was determined employing the mixture of immobilized CEase and COD and found to be in the range 187 ~ 287 mg/dL (n=50). These values were comparable to those obtained by

other methods[10]. The total cholesterol was also determined in the sera of patients of hypercholesteroleamia by the same method and found to be in the range 312 ~ 612 mg/dL($n = 40$).

Determination of free cholesterol in serum with immobilized COD

The free cholesterol in serum samples obtained from apparently healthy individuals was measured by employing arylamine conjugated COD and horseradish peroxidase and found to be in the range 21.7 to 53.07 mg/dL ($n = 40$). The mean values of free cholesterol obtained in the present study were comparable with those obtained by standard chemical method based on Leibermann-Burchard reaction[25,26] (range 23.2 ~ 54.9 mg/dL) and by commercial enzymic colourimetric method[27] employing free COD and peroxidase (range 20 ~ 55.5 mg/dL).

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以芳香胺玻璃为载体的固定化的胆固醇脂酶和胆固醇氧化酶

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关键词　胆固醇，胆固醇脂酶，胆固醇氧化酶，固定化，芳香胺玻璃，血清，胆固醇测定

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