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ECO FRIENDLY SYNTHETIC ROUTES FOR THE REDUCTION OF CARBONYL AND THE SUBSTITUTED CARBONYL COMPOUNDS

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ABSTRACT : Incorporation of green methodology via biocatalytic and electrochemical steps using Baker's Yeast and electrons as reducing agent respectively have been employed as a novel and efficient route to furnish relevent chiral building blocks for fine chemicals and pharmaceuticals**.** Reduction of selected ketones such as 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone and ethyl-2-oxocyclopentanecarboxylate have been carried out by biotransformation (using whole cells of Baker's Yeast in their free as well as immobilized form in mixtures of glycerol and water) and via electrochemical method to the corresponding alcohols. Optimum conditions for electrochemical reduction like solvent, supporting electrolyte, reduction potential and pH were determined at glassy carbon electrode employing cyclic voltammetric technique. The effect of scan rate, pH were also studied. The electrochemical reduction was carried out at constant current using stainless steel (SS-316) electrodes. The products obtained were purified & then the results of both reduction routes (biocatalytic & electrochemical) were compared and then characterized by spectroscopic techniques.

Keywords: Biocatalysts (Baker's yeast), cyclic voltammetry (Glassy carbon electrode), constant current electrolysis (SS-316 electrodes), ketones& β- keto esters

INTRODUCTION

Biotransformation employing enzymes has advantages such as their ability to carry out a wide range of organic reactions at higher reaction rates over conventional chemical reactions in organic synthesis[1-2]. Biotransformations yield an enantiopure product and operate at relatively mild physical conditions of pH and temperature, which preserve the functional integrity of the biocatalysts and are advantageous when labile substrates or products are employed[3].

Among various kinds of biotransformation, reduction of carbonyl compounds using Baker's Yeast for producing corresponding optically active secondary alcohols is a convenient and useful synthetic route due to its eco friendly nature, low cost and easier handling[4-5]. A large number of optically active alcohols are involved as the potential chiral building blocks to synthesize industrially important chemicals such as pharmaceuticals, agrochemicals like herbicides, pesticides, insecticides, antioxidants and products used in flavour and fragrance industry [6-7]. Chiral juvenoids (Ethyl N-{2-[4-(2-hydroxy-1-cyclohexylmethyl)-phenoxy] ethyl} carbamate) can be synthesized using 2-substituted cyclohexanols [8].
Water is the first choice of solvent for baker's yeast reductions. Reduction of prochiral ketones with baker's yeast in water has several disadvantages like low solubility of the organic substrate, undesired side reactions and difficulties involved in isolation of the product. Therefore the enantioselective reduction of various prochiral ketones was also studied in different organic solvents such as toluene, hexane, but under these conditions cells are destroyed and has also severe environmental impact. Likewise the low suspension of yeast in organic medium as well as the negligible solubility of in these organic solvents leads to low reaction rates[9].

The electrochemical reactions serve as powerful methods for the synthesis of organic compounds because highly reactive species can be easily generated under mild conditions [10].
Electrochemical routes for organic synthesis provide a basis for environmentally friendly and sustainable methods for production. In electro synthesis, reactions can take place in a low-temperature environment, reducing the local consumption of energy, and reducing the risk of corrosion, material failure, and accidental release [11].
The electrochemical reduction is one of the greener approaches because it is pollution free as electrons may be regarded as one of the reagents and it reduces the use of at least one hazardous chemical reagent. Other advantages of this technique are specificity, selectivity and cost effectiveness[12-13]. In the field of electrochemistry, high selectivity has also been achieved via electro organic syntheses. To investigate kinetics and mechanisms of the reactions electrochemical techniques are also very powerful and useful and electro organic synthesis provide alternative synthetic route[14].
This paper reports novel biocatalyst mediated reduction of cyclic β-keto ester and substituted cyclohexanones using Baker's Yeast in a mixture of glycerol and water & electrochemical reduction of cyclic β-keto ester, substituted cyclohexanones under the conditions where most of the above stated limitations are taken care of.

MATERIAL AND METHODS

All the chemicals used in present investigation viz 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone, ethyl-2-oxocyclopentanecarboxylate, glycerol, absolute alcohol, methanol, sodium acetate ether etc. were of AR grade. The solvents and water were doubly distilled before use.^{1}H NMR spectra were recorded using Joel (Japan) 300MHZ spectrophotometer. FT-IR spectra were recorded from Nicolet (USA) FT-IR spectrophotometer.

General procedure for Bioreduction

In present investigation biocatalytic reduction of 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone, ethyl-2-oxocyclopentanecarboxylate, has been carried out by whole yeast cells in their free as well as immobilized form.

Reduction with free whole yeast cell

In a 500 ml flat bottom flask, a mixture of water and glycerol (50:50), 10 g fresh Baker's Yeast, 10 g sucrose were placed and the suspension was stirred for 30 minutes. Chosen carbonyl compound (2mM) dissolved in minimum amount of absolute alcohol was then poured into the suspension. The resulting mixture was magnetically stirred for appropriate time (Table 1). After completion of the reaction, the product was filtered using celite (filter aid powder), extraction was done with diethyl ether (30ml) and the procedure was repeated three times. The ether was first evaporated from ether extract and then dried over calcium chloride to yield the product which was then characterized by taking boiling point and spectral analysis viz. IR, NMR were carried out. (Table-1)

Reduction with Immobilized whole Yeast cell

For preparation of immobilized Baker's Yeast in 5% polyacrylamide gel the following solutions were prepared.

Solution A (10 ml): - 10gm Acryl amide and 2.5gm N, N-methylene bis acrylamide in 100ml doubled distilled water.

Table: 1 Free Baker's Yeast Results

Product Name	Reaction Time (in hrs)	Boiling Point (0C)	Free Baker's Yield (%)	IR Data (cm-1)	NMR(δ-Value)
2-methyl cyclohexanol	72	165	74	3355(OH), 2930(CH-str), 1480&1360(CH-ben), 1150(C-O))	2.1(OH), 3.5-3.7(-CH-OH), 1.05-1.07(-CH3), 1.64(-CHCH3), 1.47(para-CH2)
3-methyl cyclohexanol	72	163	81	3360(OH), 2940(CH-str), 1465&1380(CH-ben), 1130(C-O)	2.3(OH), 3.6-4.41(-CH-OH), 1.33-1.37(-CH2), 1.11-1.15(-CH3)
4-methyl cyclohexanol	72	172	78	3365(OH), 2970(CH-str), 1470&1380(CH-ben), 1145(C-O)	2.4(OH), 3.2-3.4(-CH-OH), 0.96-0.97(-CH3)
Ethyl-2-hydroxy cyclopentanecarboxylate	96	129	82	3440(OH), 2990(CH- str), 1740(C=O str in esters), 1460&1370(CH-ben, 1200(C-O str in ester)	2.0(OH),1.3(CH3), 1.6(orthoCH2), 1.77(metaCH2), 2.5(CHC=O), 4.1-4.2(-CH2-O-)

Solution B (5 ml): - 5.98gm Trihydroxy methyl amino methane, 0.46ml N, N, N', N"-tetramethyl ethylenediamine and 48ml 1N Hcl solution to 100ml solution.

Solution C (5 ml): - 560mg Ammonium per sulphate in 100ml doubled distilled water.

Solution D (20 ml): - 34.2 gm Sucrose in 100ml doubled distilled water

The above solutions were then mixed in the following sequence solution A, B, D, Baker's Yeast (5 g) and solution C.

The resulting solution was then deareated & allowed to polymerize for nearly 1hr. The resulting gel was cut into small pieces. Then the method adopted for immobilized Baker's Yeast mediated reduction was similar as follow in case of free Baker's Yeast mediated reduction. The resulting final products obtained were characterized by boiling point measurement and spectral analysis viz. IR, NMR were carried out (Table-2).

Table: 2 Immobilized Baker's Yeast Results

Product Name	Reaction Time (in hrs)	Boiling Point (0C)	Immobilized Baker's Yeast Yield (%)	IR Data (cm-1)	NMR(δ-Value)
2-methyl cyclohexanol	72	165	79	3360(OH), 2940(CH-str), 1480&1375(CH-ben), 1120(C-O str)	2.1(OH), 3.6(-CH-OH), 1.07(-CH3), 1.64(-CH-CH3), 1.46(para-CH2)
3-methyl cyclohexanol	72	163	88	3355(OH), 2950(CH-str), 1460&1380(CH-ben), 1130(C-O str)	2.1(OH), 3.5(-CH-OH), 1.66(-CH-CH3), 1.53(Ortho-CH2), 0.93(-CH3)
4-methyl cyclohexanol	72	172	84	3360(OH), 2975(CH-str), 1470&1385(CH-ben), 1140(C-O str)	2.0(OH), 3.6(-CH-OH), 1.1(-CH3), 1.63(-CH-CH3)
Ethyl-2-hydroxy cyclopentanecarboxylate	96	129	88	3450(OH), 2990(CH- str), 1730(C=O str in esters), 1460&1370(CH-ben, 1210(C-O str in ester)	2.0(OH), 1.2(CH3), 1.78(metaCH2), 2.5(CHC=O), 4.1(-CH2-O-)

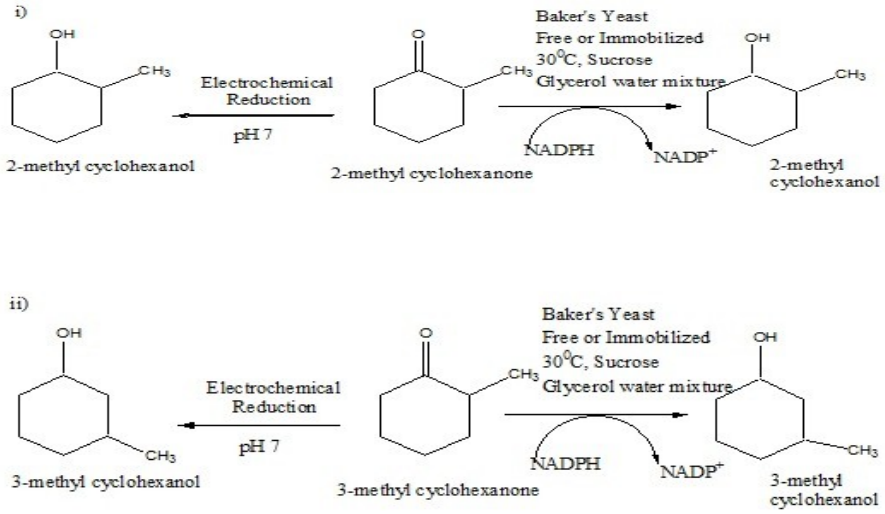
General procedure for Electrochemical Reduction

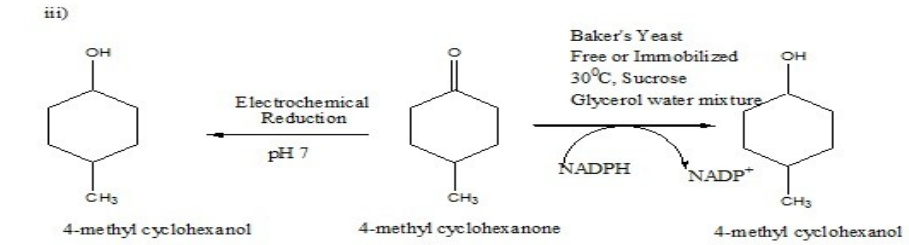
Cyclic voltammograms of selected compounds were recorded on a Computer based ECDA-001, supplied by Con-Serv Enterprises, Mumbai (INDIA) at different pH and scan rates in aqueous methanol using potassium chloride as supporting electrolyte at glassy carbon electrode. For this purpose potassium chloride (1ml, 1.0M), 5ml of BR buffer of desired pH, were taken in the electrochemical cell and methanol was added to this mixture so as to make up the final solution volume to 10 ml. After purging this solution with nitrogen for 10 minutes to remove the dissolved oxygen, the blank cyclic voltammogram was recorded. 0.01M reactant was added to blank solution then initial potential, final potential, scan rate and current sensitivity were provided and the resulting current was measured as a function of applied potential. In cyclic voltammetric cell assembly three electrodes viz. Glassy Carbon electrode (working), Ag/AgCl electrode (reference) and platinum wire electrode (counter) were used.

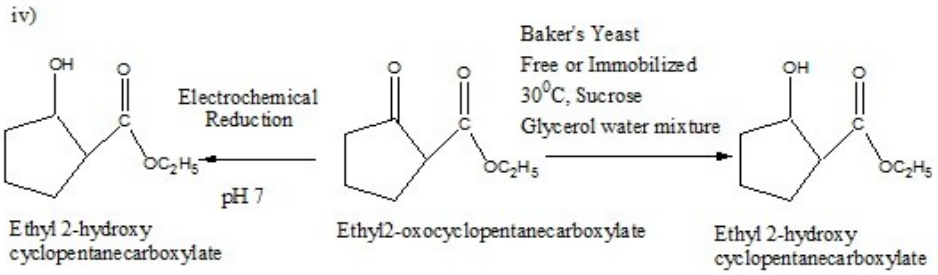
The appropriate optimum conditions for electoreduction under galvanostatic condition were set viz nature of solvent, pH required for electrolysis by carrying out cyclic voltammetry experiments. The conventional H-type cell with two limbs separated by G-4 disc was used for constant current electrolysis. The stainless steel electrodes (type 316) of size 4cmx6cm were used as cathode as well as anode. Electrolysis solution (1M sodium acetate) was filled in both the limbs. The reactant (2mM) was dissolved in minimum amount of methanol and poured in cathodic chamber and the pH of cathodic solution was adjusted at neutral (pH 7.0). After the completion of reaction, extraction was done with diethyl ether (30ml) and the procedure was repeated three times. The ether extract was dried over calcium chloride and then characterized by combined application of boiling point measurement, chromatographic and spectral techniques. (Table-3)

The products of electrochemical and biocatalytic reduction of 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone and ethyl-2-oxocyclopentanecarboxylate have found to show its importance in manufacturing of utility products of various fields like pharmaceutical, textile, food soap and perfume industry (Table-5).

Electrochemical and biocatalytic reduction of 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone and ethyl-2-oxocyclopentanecarboxylate can be depicted as follows.







RESULTS AND DISCUSSION

Biocatalytic Reduction

In asymmetric reduction either whole cells or purified enzymes can be used, the whole cell method is easier, cheaper, and does not require addition of expensive cofactor like NAD^+,NADH etc. Though water is the most suitable and natural solvent for biocatalysis from the viability and activity point of view, an alternative green solvent is glycerol. It has the advantage with respect to substrate solubility and product separation. Therefore performing the asymmetric reduction in a mixture of water and glycerol has advantages of both the solvents while carrying out the reduction using either free or immobilized whole cells. The reduction carried out using whole cells of immobilized Baker's yeast gave high yield as compared to free whole cells.

In asymmetric reduction of carbonyl compounds using whole cells of Baker's Yeast as biocatalysts two enzyme systems are involved. One of them is the enzyme catalyzing the asymmetric reduction and other is the cofactor regeneration system, which supplies NADPH from $NADP^+$ through the oxidation of the energy source such as carbohydrates. *S. cerevisiae* cells has an extra cellular invertase (β-D-fructosidase), that hydrolyzes sucrose into glucose and fructose, which are transported into the cell by hexose transporters and metabolized through glycolysis. Addition of sucrose to the reaction mixture increases the bioreduction. It is due to enhanced regeneration of the co-factor in baker's yeast in the presence of glucose that uses as electron donor.

Immobilization enhances the operational stability of FBY and isolation of the products becomes easier. The immobilized cells can be easily removed from the reaction medium and can be reused repeatedly. Under these conditions, the product formation rates are usually high.

Table: 3 Electrochemical Reduction Results

Product Name	Reaction Time (in hrs)	Boiling Point (^{0}C)	Electrochemical Yield (%)	IR Data (cm^{-1})	NMR(δ-Value)
2-methyl cyclohexanol	6	165	77	3350(OH), 2945(CH-str), 1480&1370(CH-ben), 1090(C-O str)	2.1(OH), 3.5(-C**H**-OH), 1.07(-CH_3), 1.64(-C**H**-CH_3), 1.47(para-CH_2)
3-methyl cyclohexanol	6	163	81	3355(OH), 2950(CH-str), 1470&1380(CH-ben), 1130(C-O str)	2.5(OH), 3.3-3.4(-C**H**-OH),
4-methyl cyclohexanol	6	172	86	3360(OH), 2975(CH-str), 1470&1380(CH-ben), 1140(C-O str)	2.1(OH), 3.2-3.4(-C**H**-OH), 0.97(-CH_3)
Ethyl-2-hydroxy cyclopentanecarboxylate	5	129	89	3440(OH), 2990(CH-str), 1740(C=O str in esters), 1460&1370(CH-ben), 1200(C-O str in ester)	2.0(OH), 1.2(CH_3), 1.79(metaCH_2), 2.5(C**H**C=O), 4.1(-CH_2-O-)

Electrochemical behavior

Electrochemical reduction of 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone and ethyl 2-oxocyclopentanecarboxylate and has been carried out using constant current electrolysis employing Cyclic voltammtric technique. In all cases single irreversible peak was observed due to the reduction of >c=o moiety to the corresponding secondary alcohol. Parameters evaluated from cyclic voltammograms are given in Table 4.

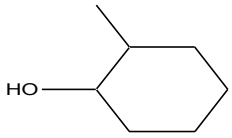
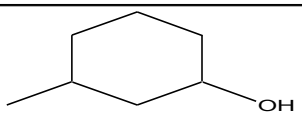
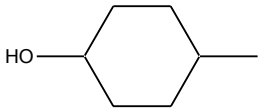
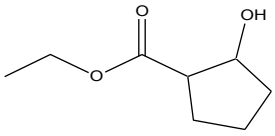
Table: 4 Voltammetric data evaluated from cyclic voltammograms at pH 7.0

	ν (mV/s)	Epc(mV)	Ipc(μA)	Ip/√ν
2-methylcyclohexanone	100	-578	159	15.9
	200	-609	232	16.57
	300	-626	299	17.58
	400	-631	359	17.95
	500	-636	478	21.72
3-methylcyclohexanone	100	-503	141	14.1
	200	-516	203	14.5
	300	-532	250	14.7
	400	-550	323	16.15
	500	-556	363	16.5
4-methylcyclohexanone	100	-515	150	15
	200	-526	218	15.3
	300	-534	269	15.8
	400	-548	321	16.05
	500	-559	355	16.13
Ethyl-2-oxocyclopentanecarboxylate	100	-508	168	16.8
	200	-519	242	17.2
	300	-529	313	18.4
	400	-545	394	19.7
	500	-566	469	21.38

Effect of scan rate

The effect of scan rate on the peak potential and peak current was studied in methanol at different pH viz pH 5.0, pH 7.0 and pH 9.0 using Britton Robinson buffer. In all these cases on increasing scan rate peak potential is shifted towards more negative values indicating irreversible nature of electrochemical process. The dependence of the voltammetric peak current (Ip) of the wave on the square root of scan rate ($v^{1/2}$) is linear (graph 1) with correlation coefficients close to unity at all the pH [15]. Under these conditions the current process was diffusion controlled. For example cyclic voltammograms of 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone and ethyl- 2-oxocyclopentanecarboxylate at various scan rates are shown as Figure (1-4).

Table: 5 Field of application of products

S.No.	Name of the product	structure	uses
1.	2-methylcyclohexanol	 2-methylcyclohexanol	In manufacturing of camphor, perfumery bases, Herbicides, Pesticides and Insecticides, In the process of photo sensitive lithographic printing Plates.
2.	3-methylcyclohexanol	 3-methylcyclohexanol	In flavor industry
3.	4-methylcyclohexanol	 4methylcyclohexanol	In manufacture of Cellulose Esters and Ethers, Antioxidant in Soaps and Detergents, De-greasing agent in artificial Silk industries & Fragrances.
4.	Ethyl-2-hydroxy cyclopentanecarboxylate	 Ethyl-2-hydroxy cyclopentanecarboxylate	In Pharmaceutical industry

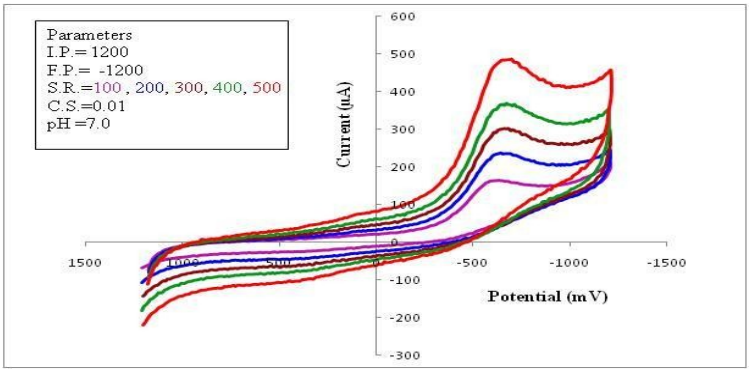


Fig.1 Cyclic voltammogram of 2-methylcyclohexanone at various scan rate

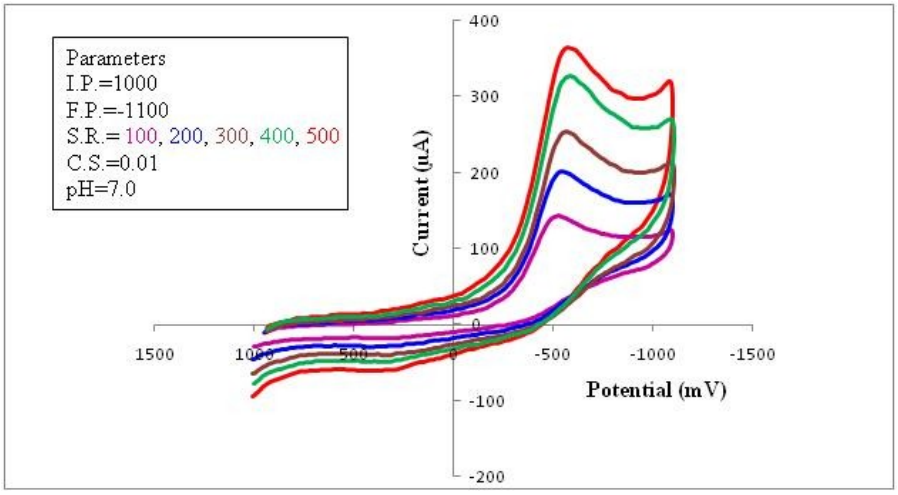


Fig.2 Cyclic voltammogram of 3-methylcyclohexanone at various scan rate

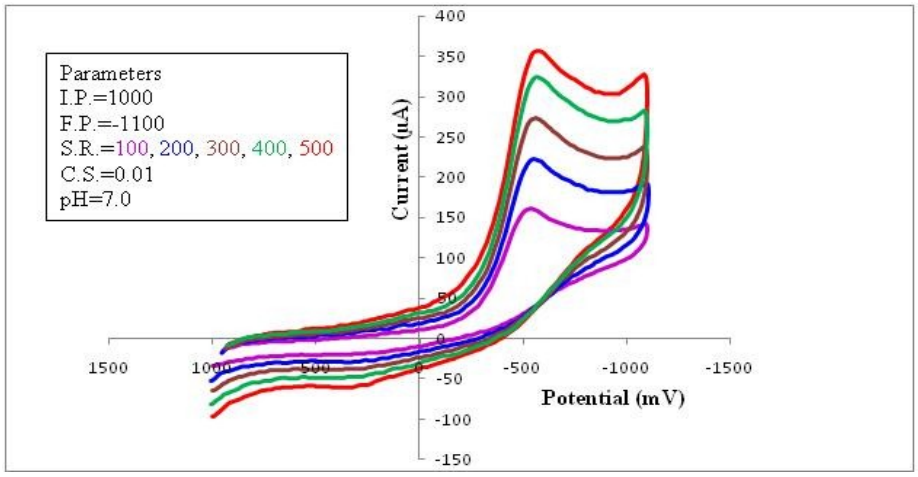


Fig.3 Cyclic voltammogram of 4-methylcyclohexanone at various scan rate

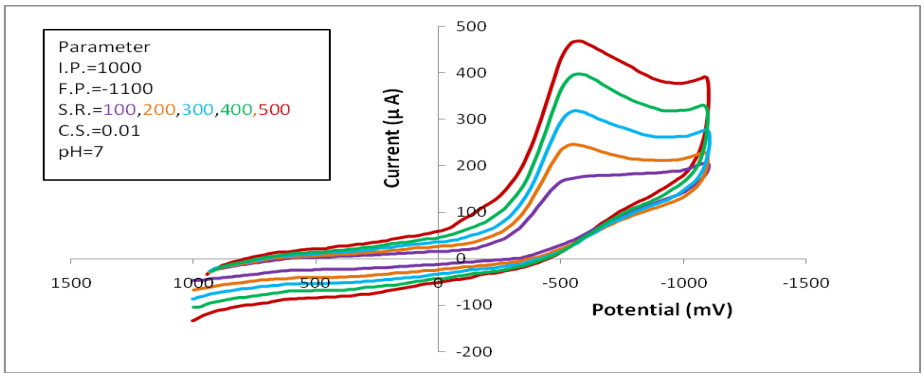


Fig.4 Cyclic voltammogram of ethyl- 2-oxocyclopentanecarboxylate at various scan rates

Effect of pH

In all these cases cyclic voltammogram at pH 7.0 shows clear reduction peak. The electrochemical reduction was performed at pH 7.0. For example cyclic voltammograms of 2-methylcyclohexanone, 3-methycyclohexanone, 4-methylcyclohexanone and ethyl-2-oxocyclopentanecarboxylate at different pH are shown as figures (5-8).

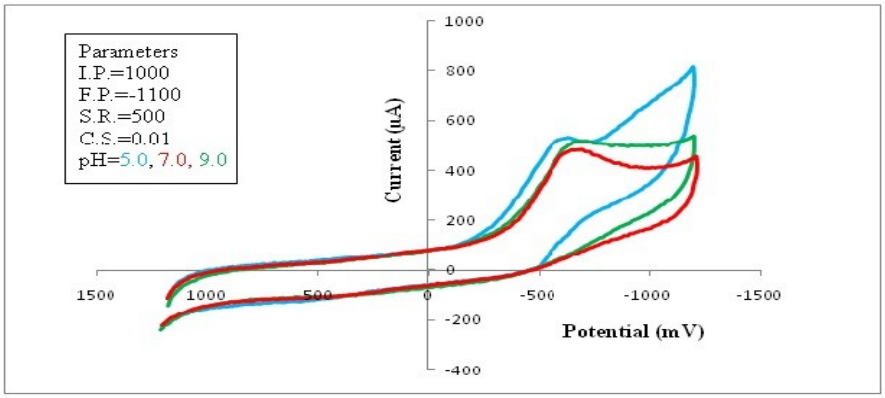


Fig.5 Cyclic voltammogram of 2-methylcyclohexanone at different pH

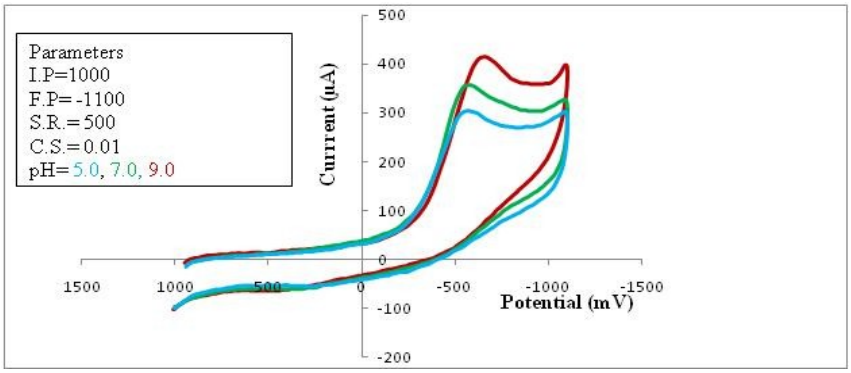


Fig.6 Cyclic voltammogram of 3-methylcyclohexanone at different pH

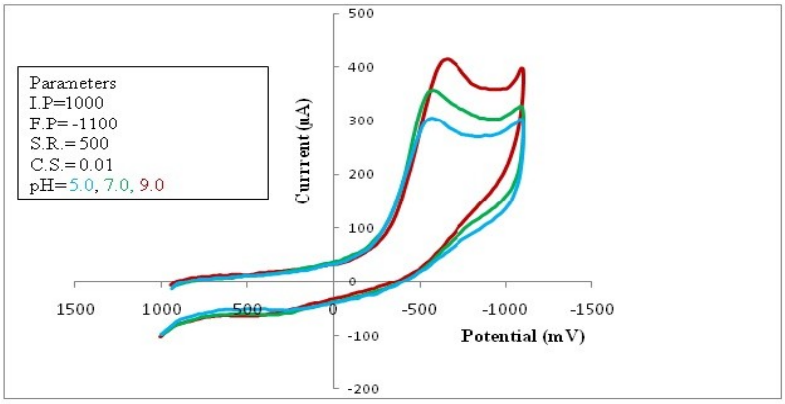


Fig.7 Cyclic voltammogram of 4-methylcyclohexanone at different pH

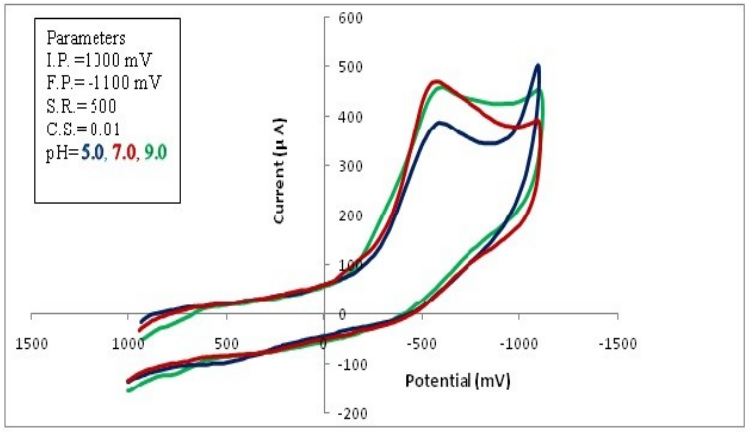
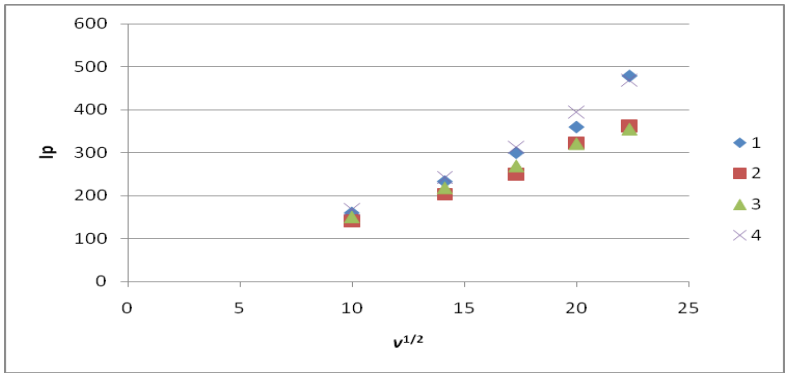


Fig.8 Cyclic voltammogram of ethyl-2-oxocyclopentanecarboxylate at different pH



Graph 1 Variation of the cathodic peak currents (Ip) with $v^{1/2}$ for of 1(2-methylcyclohexanone), 2(3-methylcyclohexanone), 3(4-methylcyclohexanone) and 4 (Ethyl-2-oxocyclopentanecarboxylate) at pH 7.0

CONCLUSION

Biocatalytic and electrochemical processes involve clean and green methodology over conventional chemical methods. Reagents used for carrying out reduction through biocatalytic procedure via Baker's Yeast and in electrochemical procedure with the use of electrons as reducing agent are more effective, safe, economical, environmental friendly, easy to handle and having no toxic effects. High efficiency of these processes make them attractive alternatives to existing methods in asymmetric catalysis for obtaining highly functionalized chiral alcohols in enantiomerically pure form.

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