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Synthesis of sugars catalysed by microgel conjugated rabbit muscle aldolase in aqueous and aqueous-organic solvents II

Moustapha E. Sesse Koker*

Chemical Laboratory, University of Kent, Canterbury CT2 7NH, United Kingdom
Present Address: *Department of Chemistry, Fourah Bay College, University of Sierra Leone, Mount Aureol, Freetown, Sierra Leone*

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Abstract
Kinetic runs of microgel-aldolase mediated reactions between dihydroxyacetone phosphate cyclohexylamine salt and D-glyceraldehydes were made in CH_2CN, THF, DMF, DMSO and MeOH and aqueous-organic solution of these solvents at physiological temperature 37º. The same runs were effected between DHAP cyclohexylamine salt and non-natural aldehyde acceptors under similar conditions. Reaction was fastest in 1:3 DMSO/ H_2O, faster than in aqueous medium the environment in which this enzyme normally operates. Preparation of a non-natural sugar using this system is related.

Keywords: Microgel-aldolase, Kinetic physiological temperature, Natural acceptors, Non-natural acceptors.

INTRODUCTION

Work (see previous paper) on microgel-aldolase mediated reactions between DHAP and various aldehydes in aqueous, and aqueous-organic solvents at room temperature indicated that reaction took place in these solvents though it was generally slower in aqueous-organic solvent than in aqueous solvent. The cyclohexylamine salt of DHAP was employed in the hope of enhancing the reaction in organic solvents.

It was reasoned that a solubility of the DHAP salt in organic solvents aside from, and in addition to those employed in the preceding work, and carrying out reactions at physiological temperature (37º) may hold the prospect of improving reactions in organic solvents. To achieve this kinetic runs of microgel-aldolase mediated reactions between D-glyceraldhyde and DHAP salt in various solvents were carried out at 37º.

EXPERIMENTAL

Chemicals were purchased from Sigma-Aldrich and were both reagent and analytical grade. Dowex A Resins were obtained from British Drug House (BDH). Biochemicals including Aldolase (lyophilised) were purchased from Sigma-Aldrich Company. NMR spectra were recorded on either a JEOL PMX60 SI 60MHz, a JEOL JNM-PS-100MHz or JEOL JNM-GX270 FTNMR 270 MHz using tertramethylsilane (TMS) sodium-2,2-dimethyl-2-silapentane-5-sulphonate (DSS), or phosphate as internal standard. Fractogel

* Corresponding author. *E-mail*: mesessekoker@yahoo.co.uk *Tel*: +232-76693810
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was obtained from Merck Company. Optical rotations were measured with a Bellingham and Stanley Pepol "60" High Pressure Liquid Chromatography (HPLC) was performed on Philips Pye Unicam PU 410 instrument equipped with a refractometer Model 1109 Refracto Monitor III (range 10 x 10^6), a Philips PM 8251 single pen recorder (range 10mV) or a Philips Pye Unicam PV4810. Computing Integrator and a Dynamax Amino Column 4.6 x 250mm, particle size 8MM, pore size 60nm. The refractometer had a circulating water bath at room temperature connected to it.

Enzymatic assays and kinetic runs were performed using a Perkin Elmer 124 Double Beam Spectrophotometer of variable wavelength (to 850mm) connected to a power unit with Tungstern and Deuterium lamps, a thermostat bath at 25^o and a Servoscribe recorder at 10mV full scale deflection (FSD). Doubly distilled water was used in all enzymatic experiments. Moisture was excluded from, enzyme samples by storing them over silica in the refrigerator at appropriate temperatures.

Kinetic runs of the reaction between DHAP, salt and D-glyceraldehyde in water, organic solvents and aqueous-organic solvents catalysed by polymer bound aldolase at 37^o.

Several runs were made but the method of kinetic analysis was essentially the same.

A. H_2O: DHAP cyclohexylammonium salt (6 mg) and D-glyceraldehyde (5 mg) were dissolved in water, Polymer bound aldolase (0.5ml – 0.7ml) was added, the total volume of the reaction mixture being 2ml. This was placed in a water bath maintained at 37^o. 100 ml aliquots of this reaction mixture was withdrawn immediately after polymer bound enzyme was added, quenched with 10µl 7% $HClO_4$, and neutralized) pH 6.9) with 14µl M NaOH, 50µl of a filtered solution of this aliquot was determined for the amount of DHAP present, amount being in direct proportion to the change in absorbance (△Abs.) determined at 340nm. This is done for aliquots withdrawn 0.5 hr, 1 hr, 2 hr, 4 hr and 8 hr after start of the reaction.

B. Organic solvents: The method is the same as in A only that water is replaced by organic solvent. Organic solvent employed were CH_3CN, THF, DMF, DMSO and CH_3OH.

C. Aqueous-organic solvents: Kinetic runs were made in:

(i) 3:1 H_2O-CH_3CN; 1:1 H_2O-CH_3CN; 1:3 H_2O-CH_3CN
(ii) 3:1 H_2O-THF; 1:1 H_2O-THF; 1:3 H_2O-THF
(iii) 3:1 H_2O-DMF; 1:1 H_2O-DMF; 1:3 H_2O-DMF
(iv) 3:1 H_2O-DMSO; 1:1 H_2O-DMSO; 1:3 H_2O-DMSO
(v) 3:1 H_2O-MeOH; 1:1 H_2O-MeOH; 1:3 H_2O-MeOH

The analysis for each system made in the same way as in A.

Reaction of DHAP salt and phenylacetaldehyde in 25% DMSO/H_2O catalysed by polymer bound aldolase .

DAHP cyclohexylammonium salt (355mg) containing 0.5 mmol DHAP and Phenylacetaldehyde 12µl, 1mmol) were dissolved in a mixture of DMSO/H_2O (2.5ml) and a solution of polymer bound aldolase (7.5ml, 2.5 units). The reaction, maintained at 37^o, stirred under nitrogen, was monitored by determining the amount (loss of) of DHAP with time. More phenylacetaldehyde was added at various times after the reaction was initiated, a total of 90µl being added, as well as polymer bound aldolase (3ml) and DMSO/H_2O mixture (0.7ml). The reaction was allowed to run for 30 hr after which it was quenched with 1ml 7% $HClO_4$, centrifuged to separate inactivated polymer bound aldolase to obtain product (17.5ml).

A portion of the product obtained (3ml) was treated with Dowex 50 W x 8 rain to pH 1.7, and the solution heated on a steam bath for 12 hr. A HPLC run of this hydrolysed product was carried with 25% H_2O/CH_3CN. A peak with retention time 5.5 mm was prominent in the HPLC. This was

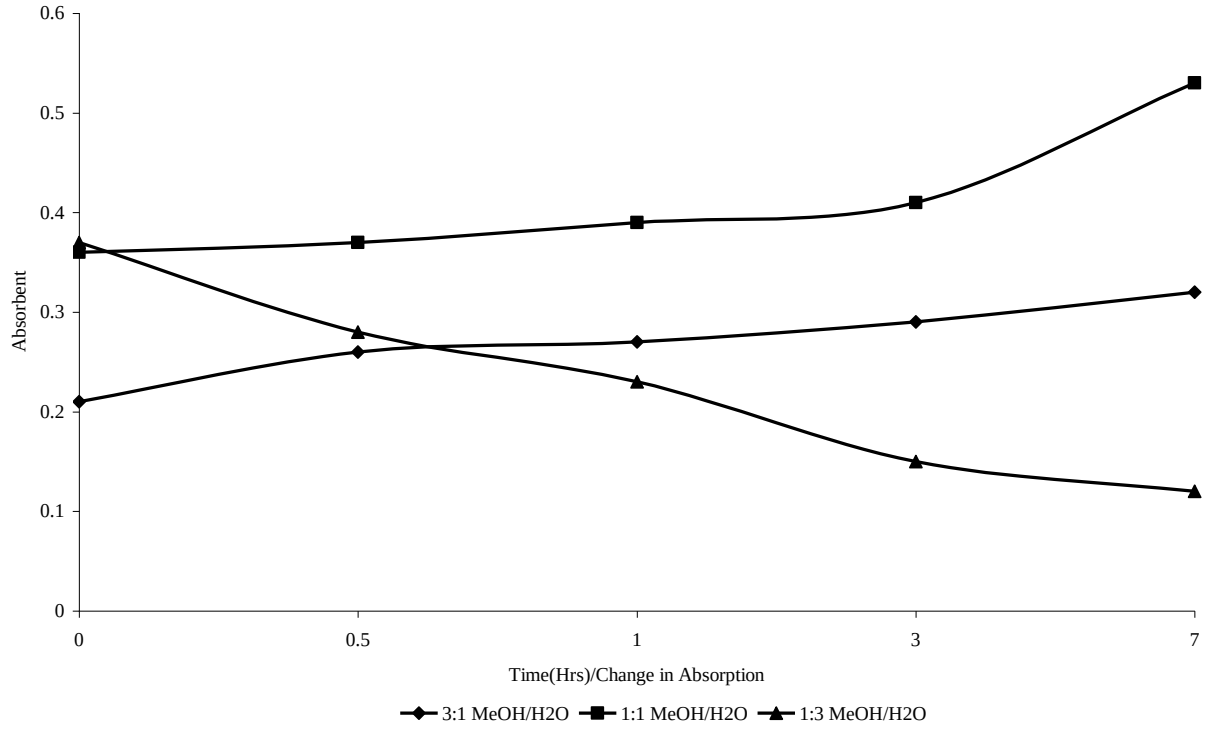
compared with the HPLC of the unhydrolysed product which showed a relatively weak peak of retention time 5.5 min.

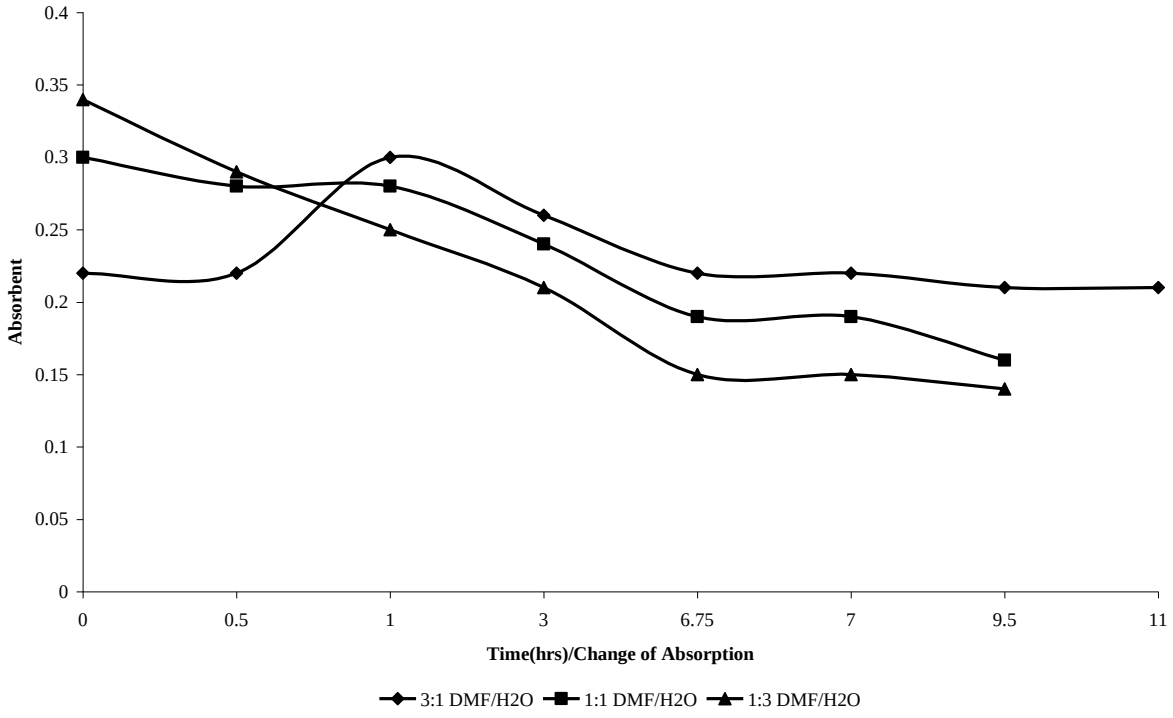
RESULTS

The solubility of DHAP cyclohexylammonium salt was carried out in MeOH, DMF, DMSO, THF and MeCN. Kinetic runs of the reaction between this DHAP salt and D-glyceraldehyde catalysed by Microgel-Aldolase were carried out at 37^o in aqueous-organic solvents in proportion of 3:1, 1:1 and 1:3 organic solvent-water for each of the solvents listed above. The rate of reaction was determined by the disappearance of DHAP salt.

A typical run involved dissolving equimolar amounts of DHAP salt and D-glyceraldehyde in appropriate solvent system followed by microgel-aldolase addition, the total volume of the reaction mixture noted then placed in a water bath maintained at 37^o. 100µl aliquots of the reaction mixture was withdrawn immediately after microgel-aldolase was added, quenched with 7% $HClO_4$ and the mixture neutralized with IM NaOH 50µl of a filtered solution of this aliquot was determined for the amount of DHAP salt present, the amount being in direct proportion to the change in absorbance (△Abs) determined at 340nm. This was done for aliquots withdrawn at 0.5 hr, 2 hr, 4 hr and 8 hr after the start of the reaction. Graphs of kinetic runs for each solvent system are presented in figures 1 to 5. It general the reaction was found to be fastest in DMSO-H_2O systems followed by MeOH-H_2O, DMF-H_2O, THF-H_2O and MeCN-H_2O. The kinetic run in 1:3 DMSO-H_2O was found to the fastest in the entire solvent systems employed in all the runs. Reaction in this system was complete in less than one hour. (Fig. 6).

A kinetic analysis of the spontaneous decomposition of DHAP salt in 1:3 DMSO-H_2O at 37^o indicated no decomposition two hours after the runs were started (Fig. 7). A similar run of DHAP salt in 1:3 MDF-H_2O was made (Fig. 7).

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7
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Time(hrs)/Change of Absorption
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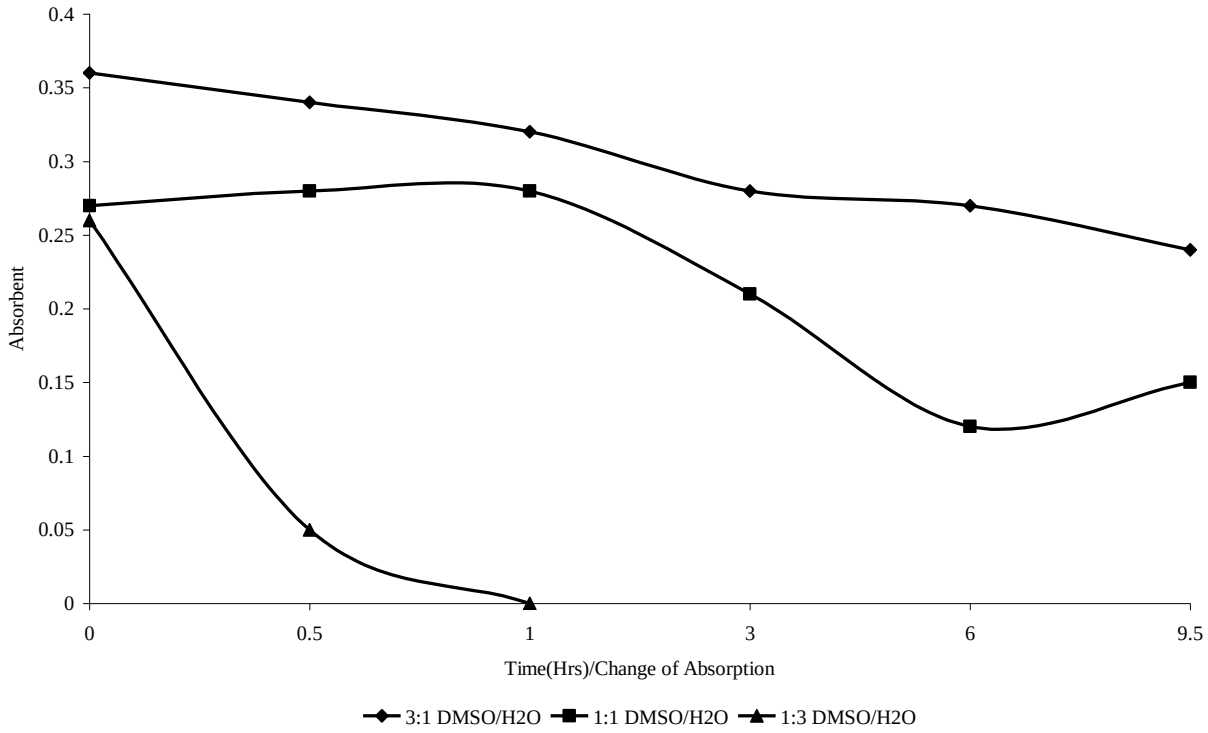
1:1 DMF/H2O
1:3 DMF/H2O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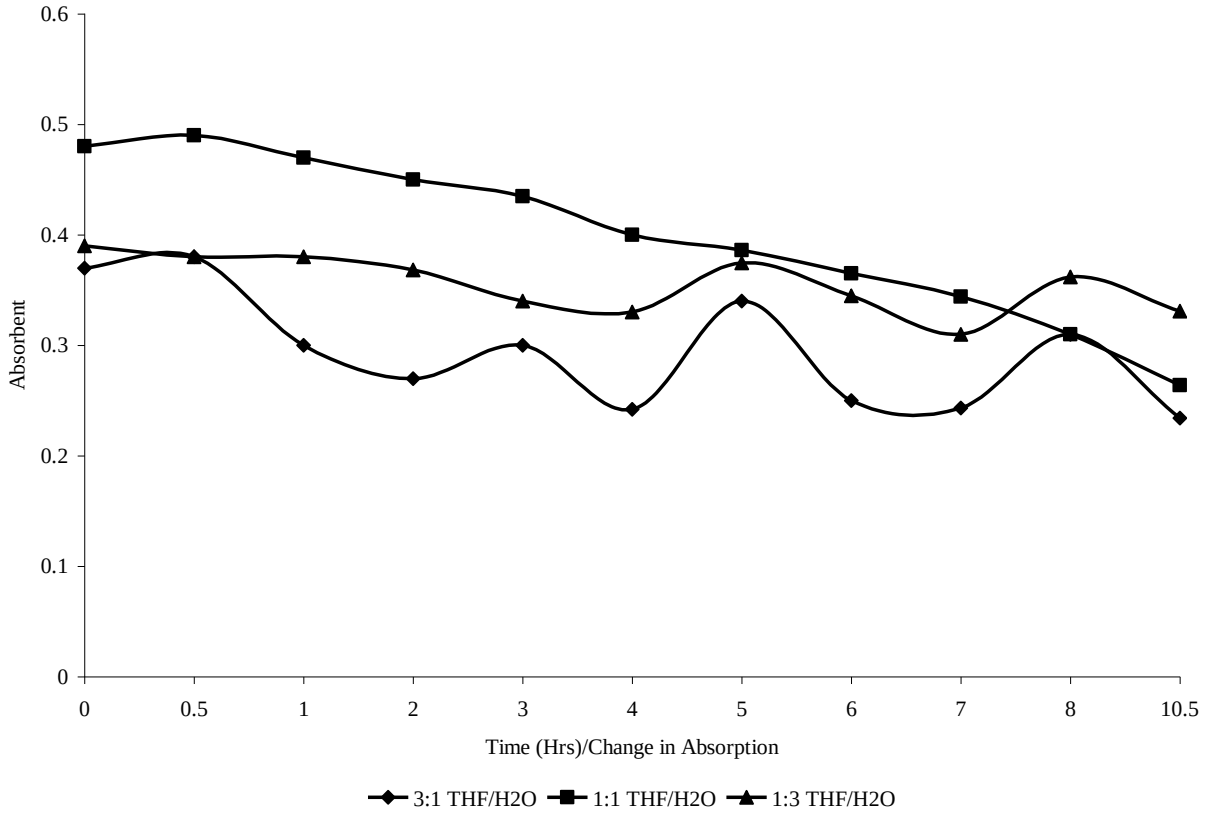
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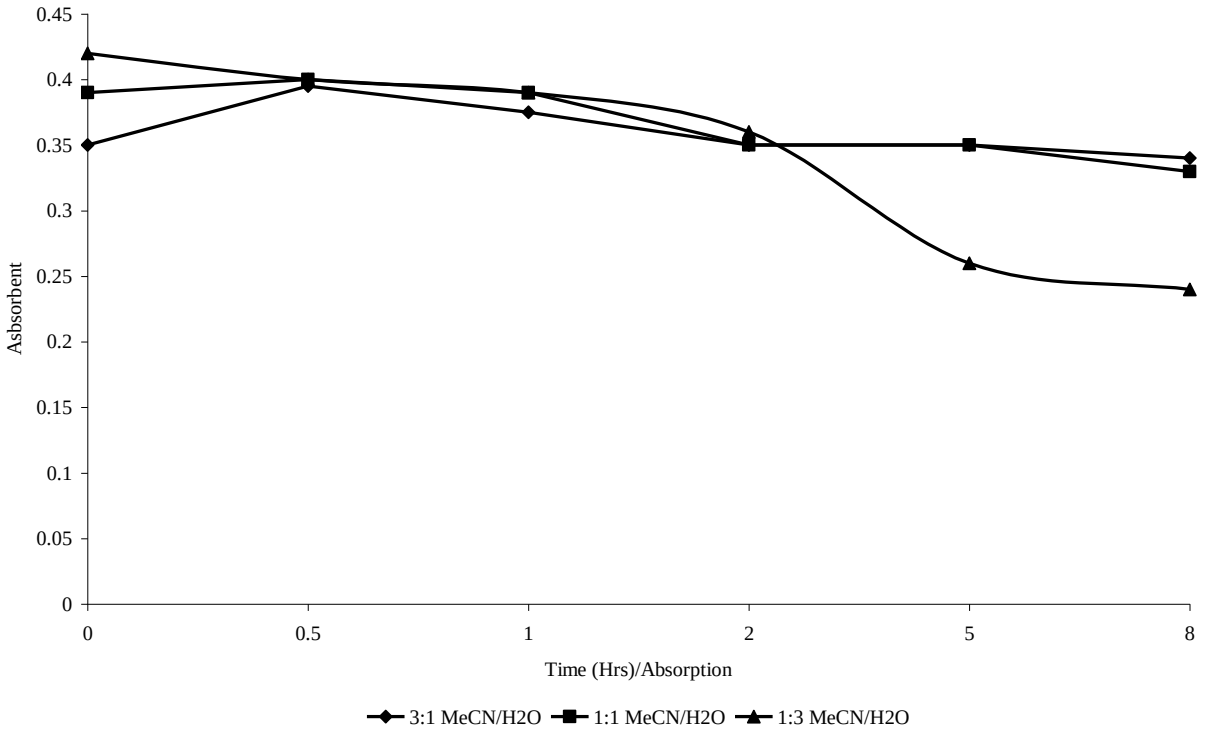
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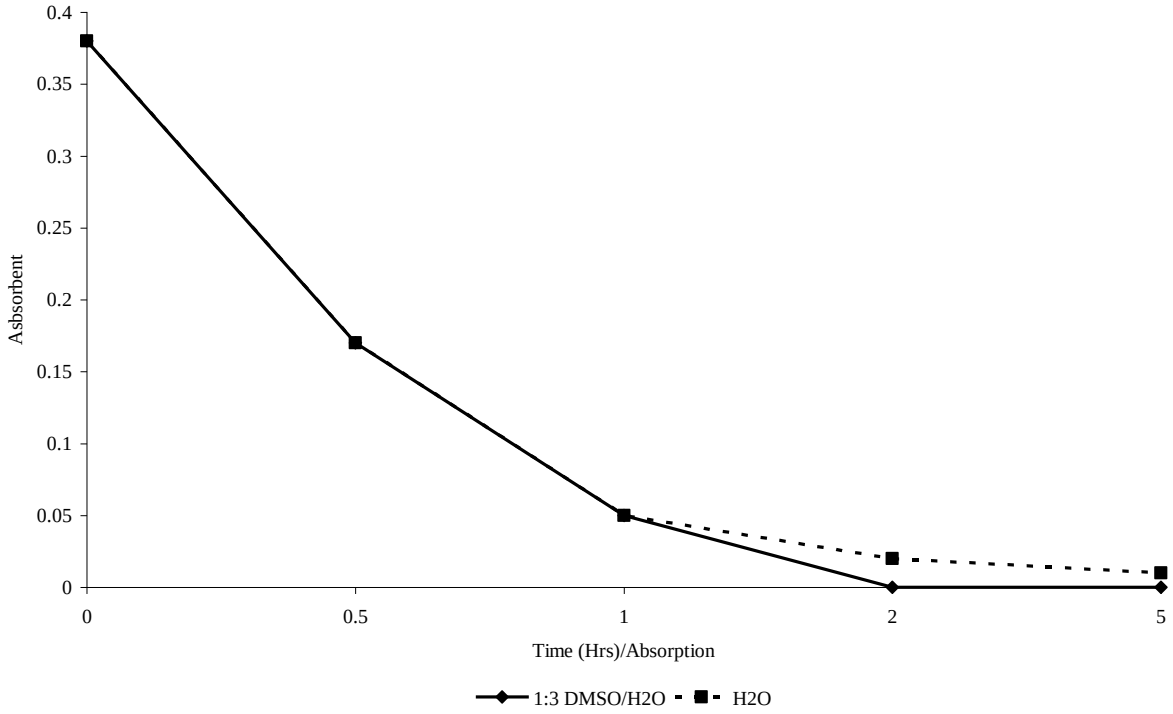
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Time (Hrs)/Absorption
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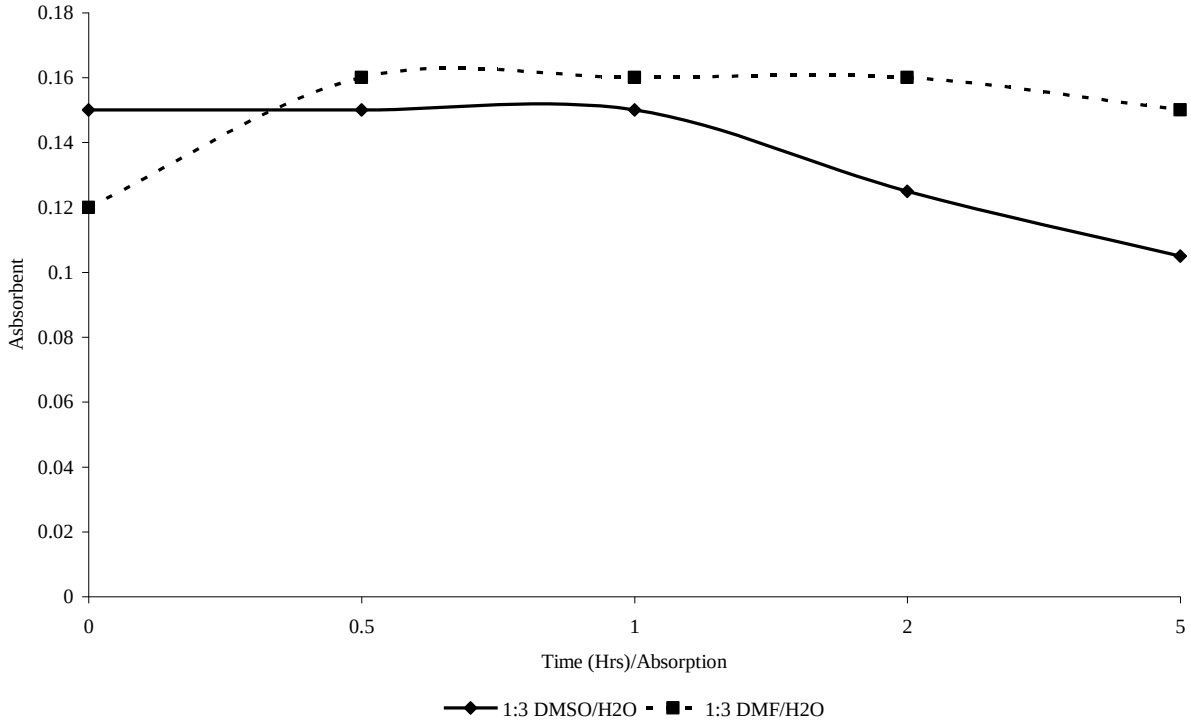
H2O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Time (Hrs)/Absorption
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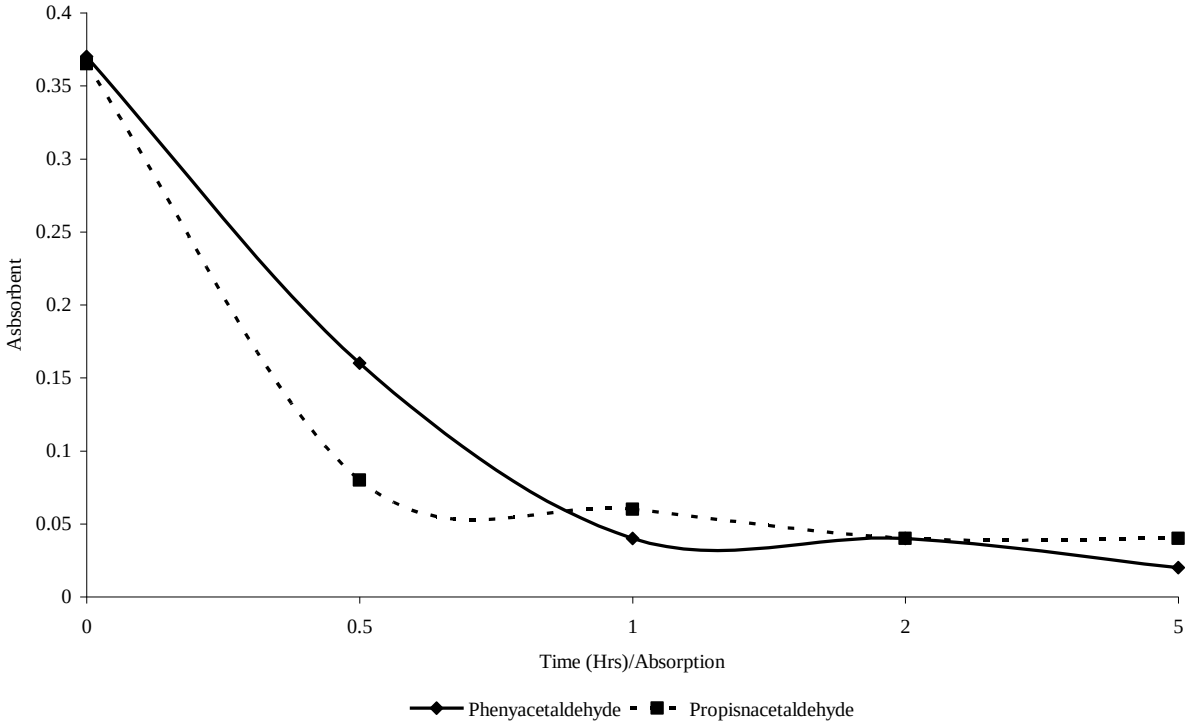

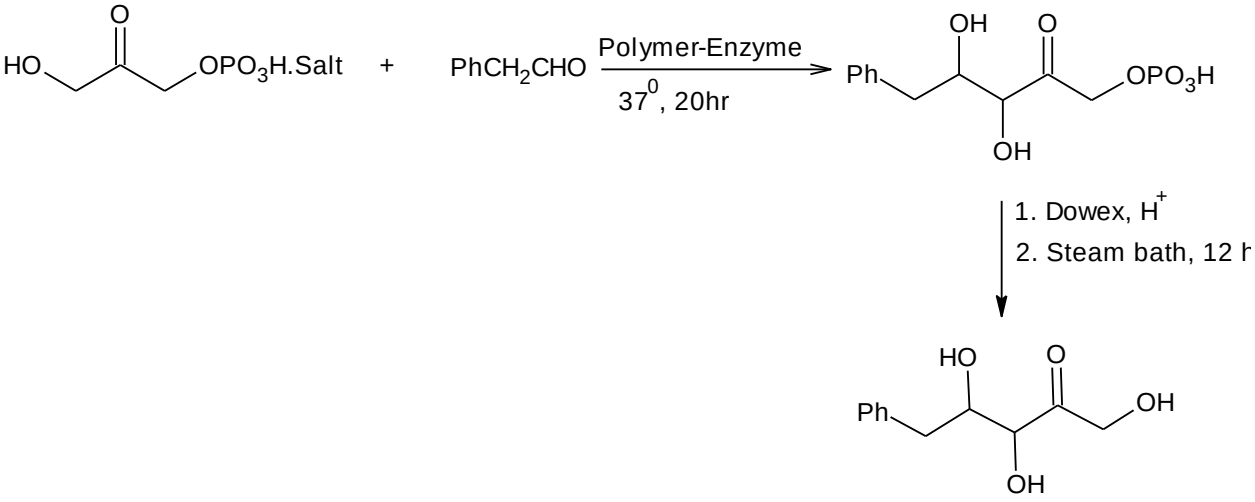

Scheme 1

A kinetic run of the reaction between DHAP salt and phenylacetaldehyde mediated by polymer bound aldolase was run in 1:3 DMSO-H_2O at 37^o. These latter two aldehydes are non natural substrates for aldolase. (Fig. 8). It was found the reaction went faster for propionaldehyde than phenylacetaldehyde. This was then followed by a reaction of DHAP salt and phenylacetaldehyde on a 0.5 millimolar scale (scheme 1). In 1:3 DMSO-H_2O at 37^o, the phosphorylated intermediate obtained was subjected to hydrolysis (Dowex H+ 12 hrs) HPLC in 1:4 H_2O MeCN of this hydrolysed product gave a peak with retention time of 5.5

min., a peak that was wholly absent in the unhydrolysed intermediate.

DISCUSSION

Kinetic runs in organic solvents found that reactions were in general faster with increase in the proportion of water in the solvents system for all the organic solvents employed in the runs. Noteworthy however was for the entire system of organic, aqueous-organic solvents employed, that of the 1:3 DMSO-H_2O system was found to be the fastest (figure 6), faster than in water, the medium in which the enzyme normally catalyse such reactions in nature.

Spontaneous decomposition of reactions during the kinetic runs remains a possibility. But a kinetic analysis of spontaneous decomposition of DHAP salts in 1:3 DMSO-H_2O revealed this not to be a factor in the microgel-enzyme catalysed reaction (figure 7). No decomposition of DHAP slat was observed two hours after the kinetic run was started, whereas in the kinetic run of DHAP salt and D-glyceraldehydes catalysed by polymer bound aldolase in 1:3 DMSO-H_20, reaction was complete in less than 1 hour. Hence the reaction that took place in this system was a genuine chemical reaction.

Having established that the reaction of DHAP salt and D-glyceraldehyde goes faster in 1:3 DMSO-H_2O than in water, kinetic runs were made between DHAP and phenylacetaldehyde, DHAP and propionaldehyde in this solvent system, the reaction going faster with propinaldehyde than with phenylacetaldehyde. A reaction of DHAP salt and phenylacetaldehyde on a 0.5 millimolar scale yielded the intermediate monophosphate which on acidic hydrolysis yielded dephosphorylated product whose HPLC characteristics were wholly different from that of the intermediate product.

Conclusion. Polymer bound aldolase has been found to catalyse reactions between DHAP and non natural acceptors. The reaction has been found to go faster in aqueous-organic solvent than in aqueous solvent which is the medium in which the enzyme normally catalyse reactions. Conjugation of microgel to the nature enzyme has enhanced its ability to catalyse such reactions.

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