

## Ultrasonic studies on enhancement of detergent action by the enzyme-lipase

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### ABSTRACT

Enzyme Technology is an area of considerable interest and development. Enzymes are biological catalysts and have been used for many years as isolated agents particularly in the food industries. In the present work, lipase is coupled with the three detergents, and the efficiency of the detergents in the removal of dye colour has been investigated by acoustical studies. In order to determine the concentration of the enzyme at which the interactions are maximum, ultrasonic velocities and densities are measured for the aqueous solution of the three detergents, in the presence of lipase at various concentrations. Acoustical parameters and surface properties are calculated.

**Key words:** Detergents, enzyme-lipase, acoustical parameters, surface properties.

### INTRODUCTION

Enzyme Technology is an area of considerable interest and development. Enzymes are biological catalysts and have been used for many years as isolated agents particularly in the food industries<sup>1</sup>. Biosurfactants are surface-active compounds produced by microorganisms. Biological surfactants possess a number of potential advantages over their synthetic counterparts, which include lower toxicity, biodegradability, and a wide variety of possible structures and ease of synthesis. Consequently, biosurfactants have wide applications in numerous areas, particularly to enhance oil recovery, and also in foods, beverages, cosmetics and pharmaceutical preparations. Enzymes are proteins that are capable of removing stains caused by organic substances because of stronger interactions between enzymes and molecules responsible for stains. These groups of detergents are called bio-detergents.

Several researchers have given importance on enzymes as detergents<sup>2-3</sup>. At present, proteases and amylases are used as bio-detergents<sup>4</sup>. Recently, lipases are also found to be suitable in detergent preparation. Bio-detergents are effective even in micro-amounts and they are biodegradable. They do not leave harmful residues. Bio-detergents have low negative impact on sewage-disposal processes and do not pose a threat to the aquatic life.

### EXPERIMENTAL

AnalaR grade (SDS) samples of Sodium lauryl Sulphate (SDS), Cetyl-N,N,N-Trimethyl ammonium bromide (CTAB), Triton X-100 (TX100), Lipase and the dyes crystal violet (CV), quinoline yellow (QY) and methylene blue (MB) were used as such, and triple distilled water was prepared by distillation technique. Accurately weighed amounts of the sample were dissolved in triple distilled water to obtain solution of various concentrations. The

ultrasonic velocities of the solutions were measured using an ultrasonic interferometer (F-81) with a single crystal at a frequency of 2MHz. The accuracy in the velocity measurement is  $\pm 0.5\%$ . The densities of solutions were measured at 303K using specific gravity bottles with an accuracy of  $\pm 0.001\text{kg/m}^3$ . The cell temperature was maintained constant at 303K by an ultra-thermostat (Julabo U-3) maintained at an accuracy of  $\pm 0.1\text{K}$ .

### Theoretical Formulations

The acoustical parameters such as adiabatic compressibility ( $k$ ), free length ( $L_f$ ), surface tension ( $\gamma$ ), molar surface energy ( $E$ ) and surface area ( $Y$ ) were calculated from the measured ultrasonic velocity ( $U$ ) and density ( $\rho$ ) of aqueous solutions of the surfactants, at 303K. The following equations<sup>4</sup> were used in these calculations.

Adiabatic Compressibility

$$k = (1/U^2\rho) \text{ kg}^{-1}\text{ms}^2 \quad \dots (1)$$

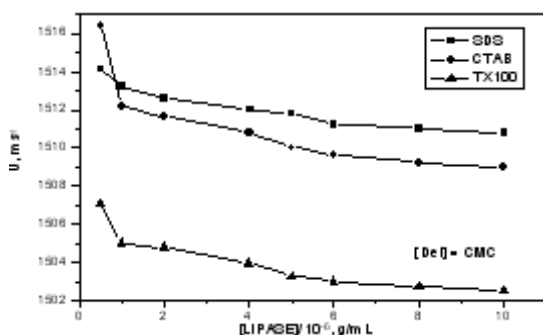


Fig. 1: Plots of ultrasonic velocity vs concentration of detergent with lipase at 303 K

Free length

$$L_f = k \times (k)^{1/2} \text{ A}^\circ \quad \dots(2)$$

Surface tension

$$\gamma = (U^{3/2}) \times (6.3 \times 10^{-4}) \times \rho \text{ N m}^{-1} \quad \dots(3)$$

Molar surface energy

$$E = \gamma \times V_m^{2/3} \text{ J mol}^{-1} \quad \dots(4)$$

Surface area

$$Y = (36 \pi N V_a)^{1/3} \text{ m}^2 \text{ mol}^{-1} \quad \dots(5)$$

where  $U$  = Ultrasonic velocity (m/s),  $\rho$  = density ( $\text{kg/m}^3$ ),  $V_m$  = Molar volume ( $\text{m}^3$ ),  $V_a$  = Available volume ( $\text{m}^3$ ),  $k$  = Jacobson's constant.<sup>4</sup>

### RESULTS AND DISCUSSION

In the present work, lipase is coupled with the following three detergents,

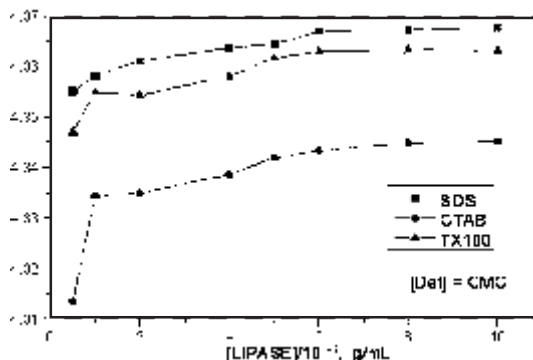


Fig. 2: Plots of Adiabatic compressibility vs concentration of detergent with Lipase at 303K

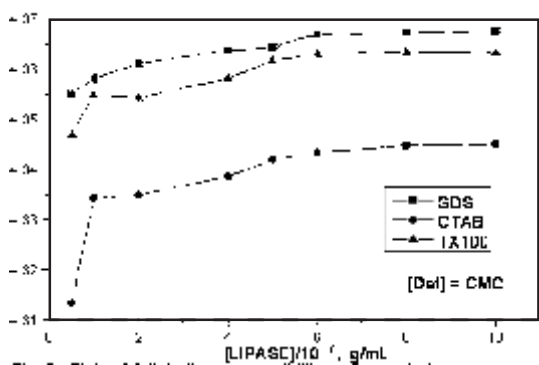


Fig. 3: Plots of surface tension vs concentration of detergent with Lipase at 303K

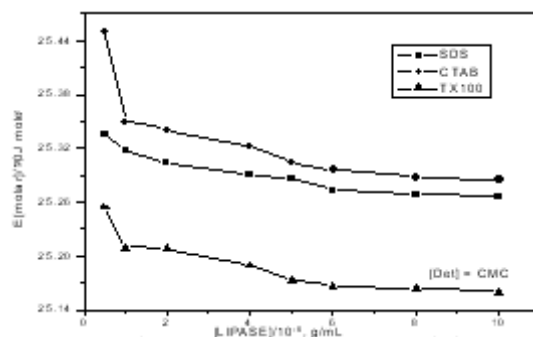


Fig. 4: Plots of molar surface energy vs concentration of detergent with Lipase at 303K

**Sodium Lauryl Sulphate [SDS],**

(Anionic surfactant;  $\text{CH}_3(\text{CH}_2)_{10}\text{CH}_2\text{OSO}_3\text{Na}$ ) Cetyl-  
 NNN-Trimethyl Ammonium Bromide [CTAB],  
 (Cationic surfactant;  $[\text{CH}_3(\text{CH}_2)_{15}\text{N}(\text{CH}_3)_3]^+\text{Br}$ ) Triton  
 X-100, [TX100]

(Non-ionic surfactant; p-tertiary octyl  
 phenol poly (oxyethylene)<sub>9,2</sub>), and the efficiency of

the detergents in the removal of dye colour has been investigated by acoustical studies. In order to determine the concentration of the enzyme at which the interactions are maximum, ultrasonic velocities and densities are measured for the aqueous solution of the three detergents, in the presence of lipase at various concentrations. Acoustical parameters and surface properties are calculated.

**Table 1 : Ultrasonic velocities (u), densities (ρ), adiabatic compressibilities (k), free lengths (L<sub>f</sub>), molar surface energies (e) and surface areas (y) of dye+[det]+lipase solution**

[DYE]=50mM,[SDS]=8.1mM,[CTAB]=0.33mM,[TX100]=0.2mM,[LIPASE]=1 X 10<sup>-5</sup>g/mL,TEMP=303K

Solution	U, ms <sup>-1</sup>	ρ, kgm <sup>-3</sup>	k/10 <sup>-10</sup> kg <sup>-1</sup> ms <sup>2</sup>	L <sub>f</sub> Nm	E[molar] /10 <sup>2</sup> J mole <sup>-1</sup>	Y /10 <sup>9</sup> m <sup>2</sup> /mole
CV	1300.1	1018.2	5.810	4.78	22.58	48.64
CV+SDS	1310.2	1020.5	5.708	4.74	22.86	48.89
CV+SDS+LIP	1320.4	1022.6	5.609	4.70	23.14	49.15
CV+CTAB	1315.2	1020.8	5.663	4.72	22.99	49.02
CV+CTAB+LIP	1325.6	1022.8	5.564	4.68	23.28	49.27
CV+TX100	1320.3	1021.2	5.618	4.70	23.13	49.14
CV+TX100+LIP	1330.2	1023.2	5.523	4.66	23.40	49.39
QY	1513.6	1020.0	4.279	4.10	28.38	53.83
QY+SDS	1526.0	1022.4	4.200	4.07	28.75	54.12
QY+SDS+LIP	1551.9	1024.8	4.052	3.99	29.51	54.73
QY+CTAB	1530.1	1022.7	4.176	4.05	28.87	54.22
QY+CTAB+LIP	1557.0	1025.2	4.024	3.98	29.65	54.85
QY+TX100	1535.0	1023.2	4.148	4.04	29.01	54.34
QY+TX100+LIP	1565.0	1027.8	3.972	3.95	29.91	55.04
MB	1720.1	1021.8	3.308	3.61	34.40	58.62
MB+SDS	1732.6	1023.2	3.256	3.58	34.79	58.90
MB+SDS+LIP	1760.2	1026.0	3.146	3.52	35.65	59.53
MB+CTAB	1735.5	1023.9	3.243	3.57	34.88	58.97
MB+CTAB+LIP	1768.3	1028.1	3.111	3.50	35.93	59.71
MB+TX100	1740.2	1025.1	3.221	3.56	35.04	59.08
MB+TX100+LIP	1775.0	1030.8	3.079	3.48	36.16	59.86

Plots of ultrasonic velocity vs lipase concentration for the three systems are given in Fig.1. It is found that the interactions are maximum, even at a very low concentration of lipase for all the three detergents. Plots of adiabatic compressibility (k) vs concentration (Fig. 2) are also in support of these observations. The plots of surface tension (γ) vs concentration is in Fig.3, while Fig.4 contains

plots of molar surface energy vs concentration of lipase. Thus, the detergent action of all the three detergents used in the investigation is enhanced by the presence of lipase.

In order to establish the enhancement of detergent action by lipase, acoustical properties are calculated for the three different dyes, namely,

crystal violet (CV), quinoline yellow (QY) and methylene blue (MB). In these studies the dye concentration is kept constant [50 mM]. The detergent concentration is fixed at CMC and the lipase concentration was taken as  $1 \times 10^{-5}$ g/mL. It was found that the addition of SDS with dye removed the colour slowly, but when lipase was added the colour removal was instantaneous. The acoustical parameters for crystal violet system are presented in Table 1. When the dye colour is removed, the ultrasonic velocity increases which is found from the data in Table 1. When lipase is added to CV-SDS system, the ultrasonic velocity increases and there is corresponding decrease in  $k$  and  $L_r$  values. Thus, lipase enhances the detergent action of SDS. This is also supported by the change in  $g$ ,  $E$  and  $Y$  values. Similar observations are made in the case of the other two detergents CTAB and TX100. Thus, the detergent action is enhanced by the addition of lipase in the colour removal of dye.

Acoustical studies for QY system, in presence of SDS and lipase are given in Table 1. These data also indicate that the detergent action of three detergents in the colour removal of QY is enhanced by lipase. It may be noted that the increase in ultrasonic velocity in the presence of detergent and lipase is much more than the increase in ultrasonic velocity value in the CV system.

Therefore, the detergent-lipase combination used in the present investigation is more effective in the colour removal of QY. The surface properties, such as surface tension ( $\gamma$ ), molar surface energy ( $E$ ) and surface area ( $Y$ ) are much greater for QY system than that of CV system. Similar observations are also made in the case of methylene blue (Table 1) and the efficiency of the three detergents can be increased to a greater extent by the addition of lipase. This is supported by the highest values of  $\gamma$ ,  $E$  and  $Y$  for the systems containing methylene blue.

### CONCLUSION

Lipase functions as a bio-detergent. It enhances the detergent action of the common surfactants in the removal of dye colours. This is established by the measurement of the acoustical properties and surface properties for solutions containing dye, dye-detergent and dye-detergent-lipase mixtures.

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