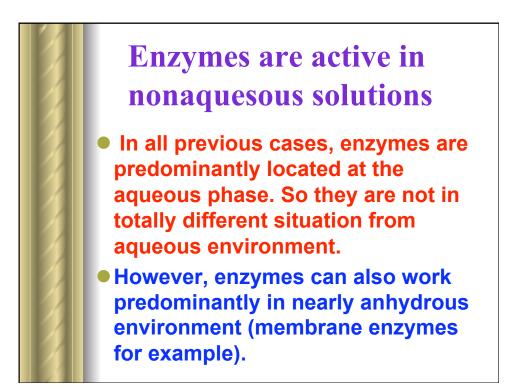
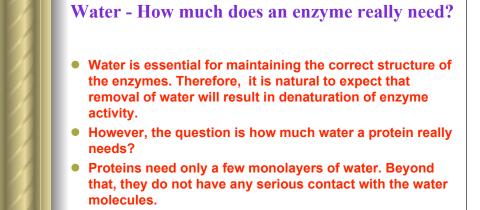


## Early approaches

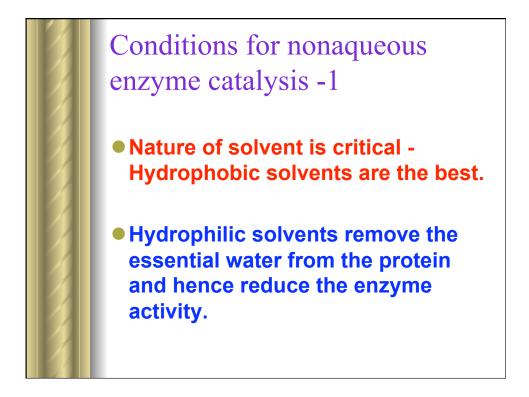
- Water- organic solvent (such as acetone or ethanol) mixture: Predominantly water. So many enzymes can work in this environment.
- Biphasic mixture: Use of water phase for enzymes and organic phase for substrates. Subtrate diffuses from organic phase to water, gets converted to product and goes back to organic phase. Reducing the size of aqueous droplets allows easy mass transfer. Reverse micelles, micro emulsions are all variation of this approach.
- Nearly Nonaqueous phase: They contain very few percentage of water. Ultimate step is to use enzymes in organic solvents.



Enzymes can act in organic solvents that contain less than 0.01% water (typically anhydrous).
Both chymotrypsin and subtilisin work in organic solvents although they are proteolytic enzymes needing water as a co-substrate.
So if they can work in anhydrous solvents other enzymes can also work.



 So if we provide the first one or two monolayers of water necessary fo rthe protein, then it should exhibit biological activity in anhydrous solvents.

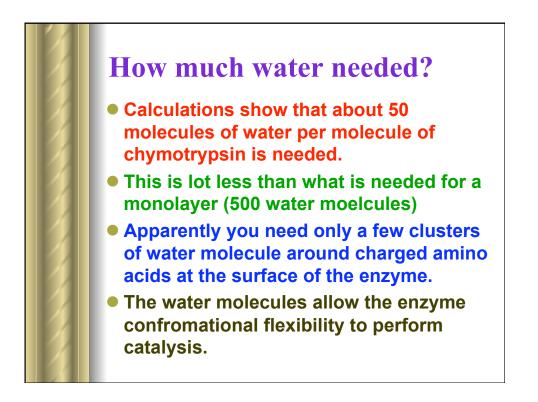


	•	4 1	•
Chvm	otrypsin	cata	VSIS

In anhydrous solvent such as octane, chymotrypsin works well. Water on the enzyme is also quite high in this hydrophobic solvent. Surprisingly in hydrophilic solvents, enzyme activity is lowered due to stripping of essential water molecules from the enzyme.

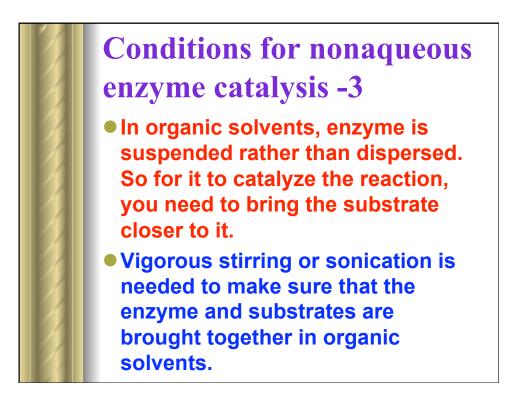
Solvent Octane	k <sub>cat</sub> /K <sub>m</sub> (M <sup>-1</sup> min <sup>-1</sup> ) <u>63</u>	% water in enzyme 2.5
Toluene	4.4	2.3
Tetrahydrofuran	0.27	1.6
Acetone	0.022	1.2
Pyridine	<0.004	1.0

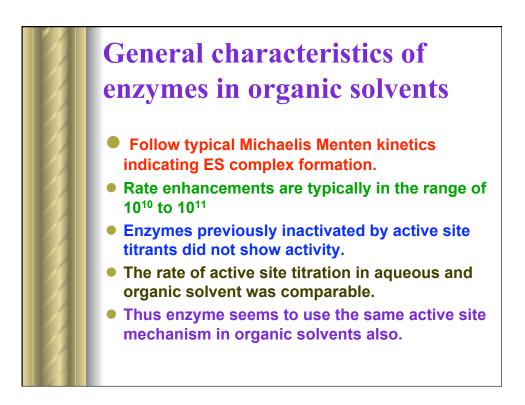
Addition of water where restores the enzyme activ By adding water to the en- case, one could regain as increase in activity.	vity dramati nzyme in the	cally. e following
Chymotrypsin in:	K <sub>cat</sub> /K <sub>m</sub> (M <sup>-1</sup> min <sup>-1</sup> )	% water in enzyme
Octane	63	2.5
Acetone (without added water)	0.022	1.2
Acetone [with added water (1.5%)]	22	2.4



# **Conditions for nonaqueous enzyme catalysis -2**

- Enzymes used in organic solvents should be either precipitated (or lyophilized) from aqueous solutions with appropriate pH in which it shows optimal activity. So that the enzyme can have pH memory.
- At pH optimum, the group at and around active site are appropriately ionized to perform the catalysis. By precipitating or lyophilizing the enzyme under this conditions, we allow the enzyme to remember this structure when placed in organic solvent.





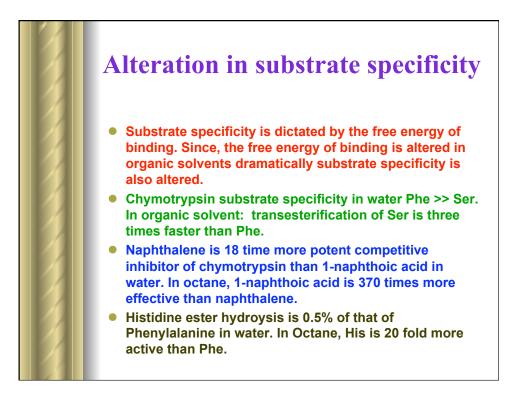
Increase in	therma	al stability
need free water. Si	bility. To inact nce both these ic solvents, the	tivate a protein you also
<ul> <li>Accordingly, chym in water.</li> </ul>	otrypsin is mo	ore stable in octane than
Chymotrypsin in	Temperature °C	Half life
water	60	Few minutes
Octane	100	Few hours

## Storage

- In addition to thermal stability, enzymes in organic solvents also show remarkable capacity for storage.
- Chymotrypsin retains full activity even after 6 months in octane at 20 °C, where as its half life is only a few days in water.

#### Liagnd induced enzyme memory

• Lyophilization of subtilisin from aqueous solutions containing competitive inhibitor followed by their removal results in a rate enhancement of 100 fold in organic solvent.



### **Alteration of stereospecificity**

 Stereoselectivity of L- isomers over D isomer for hydrolysis of N-acetyl alanyl chloroethyl ester for elastase, chymotrypsin, subtilisin, trypsin and αlytic protease is of the order of 10<sup>3</sup> to 10<sup>4</sup> in water. In nonaqueous solvents it does not exceed even 10. (more hydrophobic the solvent is less stereospecific the reaction is)

