



# Organic chemistry experiment

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## The isolation of casein (from milk)

Experimental center of chemical education  
Fudan University

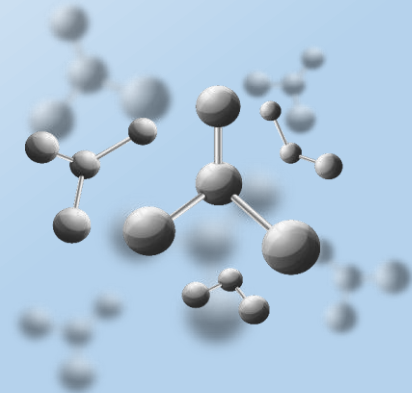




# Target

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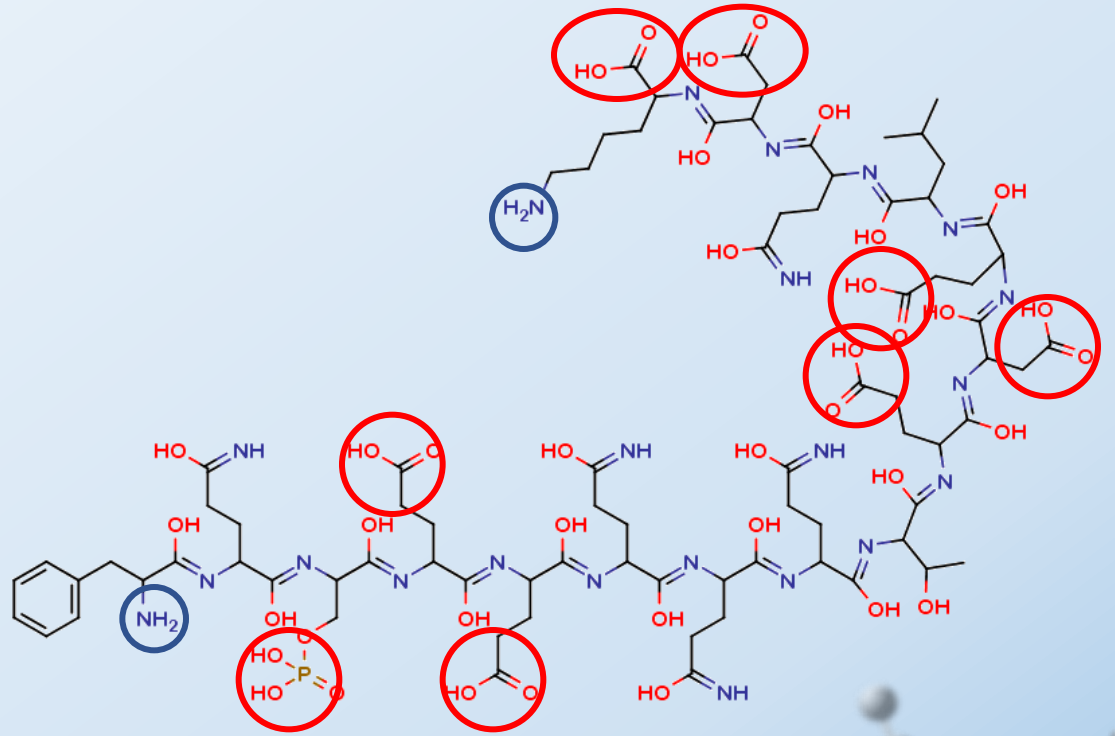
1. Comprehend the concept of pI
2. Isolate the protein by adjusting the pH
3. Identify the protein through biuret test



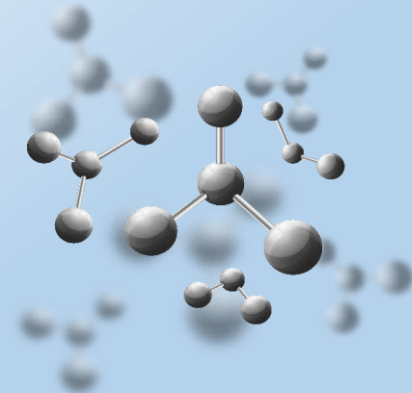


# Background

Casein



pI=4.8

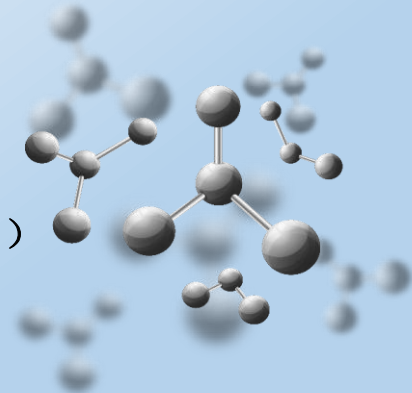
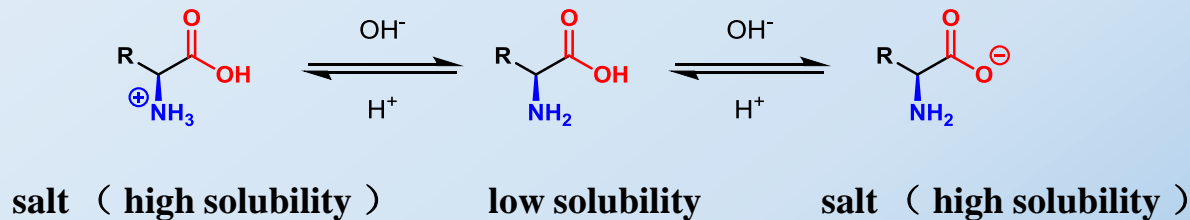




# Experimental principle

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1. Casein is a kind of complex phosphoprotein, and due to its nature of being amphoteric compound, the acid-base property of the solution will obviously affect the charge on protein. When the pH of milk adjusted close to the pI of casein (pI=4.8), the protein becomes neutral. Since its lowest solubility, sediment will form, which could be separated through centrifugation.

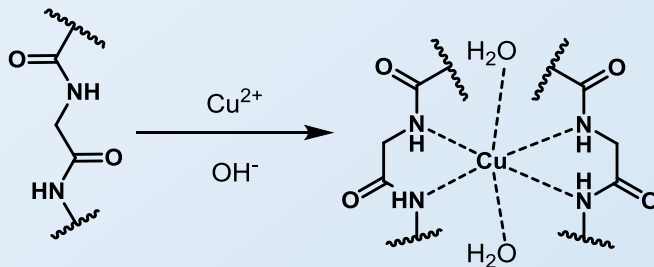




# Experimental principle

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2. **Biuret test** : The compound with polypeptide skeleton can react with  $\text{Cu}^{2+}$  under basic condition, which will form purple complex.



Protein or polypeptide

Purple complex





# Reagents

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Reduced-fat milk

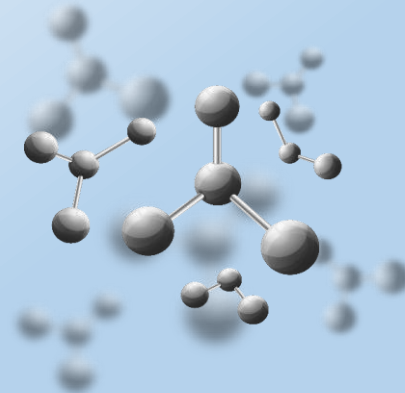
Dilute acetic acid (1:9)

Ethyl alcohol

Diethyl ether

1%  $\text{CuSO}_{4(\text{aq})}$

0.4 M NaOH in saline

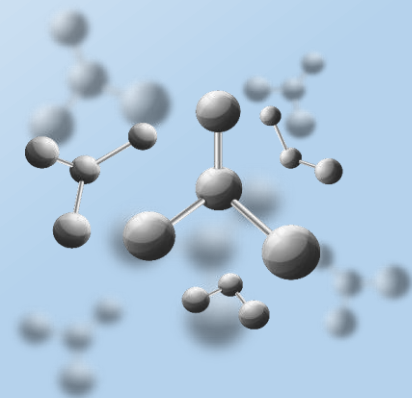




# Reagents

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	M.w.	m.p.	b.p.	d.
Diethyl ether	74.12	-116	35	0.713
EtOH	46.07	-114	78	0.789

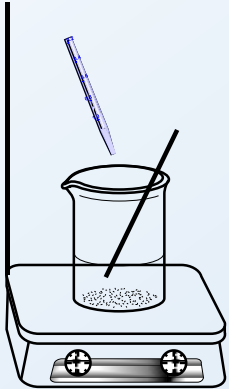




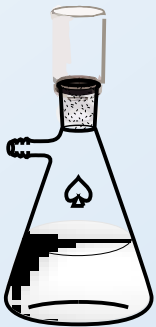


# The isolation of casein

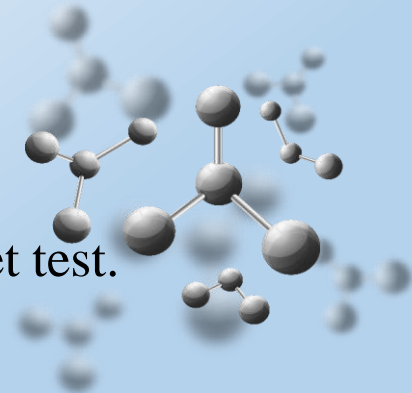
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1. 50 mL Reduced-fat milk, 100 mL beaker 40 °C  
Dilute acetic acid ( 1:9 ) 2 mL
2. Cool down for 10 min, 3000 r/min 15 min centrifugation



3. Separate the sediment, mixed with 95% EtOH 20 mL ,  
suction filtration
4. 10 mL EtOH: Et<sub>2</sub>O = 1:1 wash twice, 5 mL Et<sub>2</sub>O wash  
suction filtration
5. Dry and weigh
6. Solute 0.5 g product in 5 mL 0.4 M NaOH, biuret test.







# Attention

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1. The milk could not be long-placed, or the lactose will transform to lactic acid, which may affect the isolation.
2. Excess acetic acid is not allowed, since the acetic acid will accelerate the hydrolysis of lactose.

