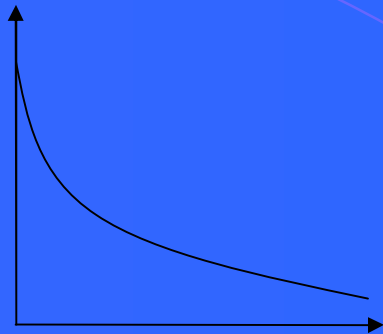


# 蛋白质的吸附、变性及污染防治

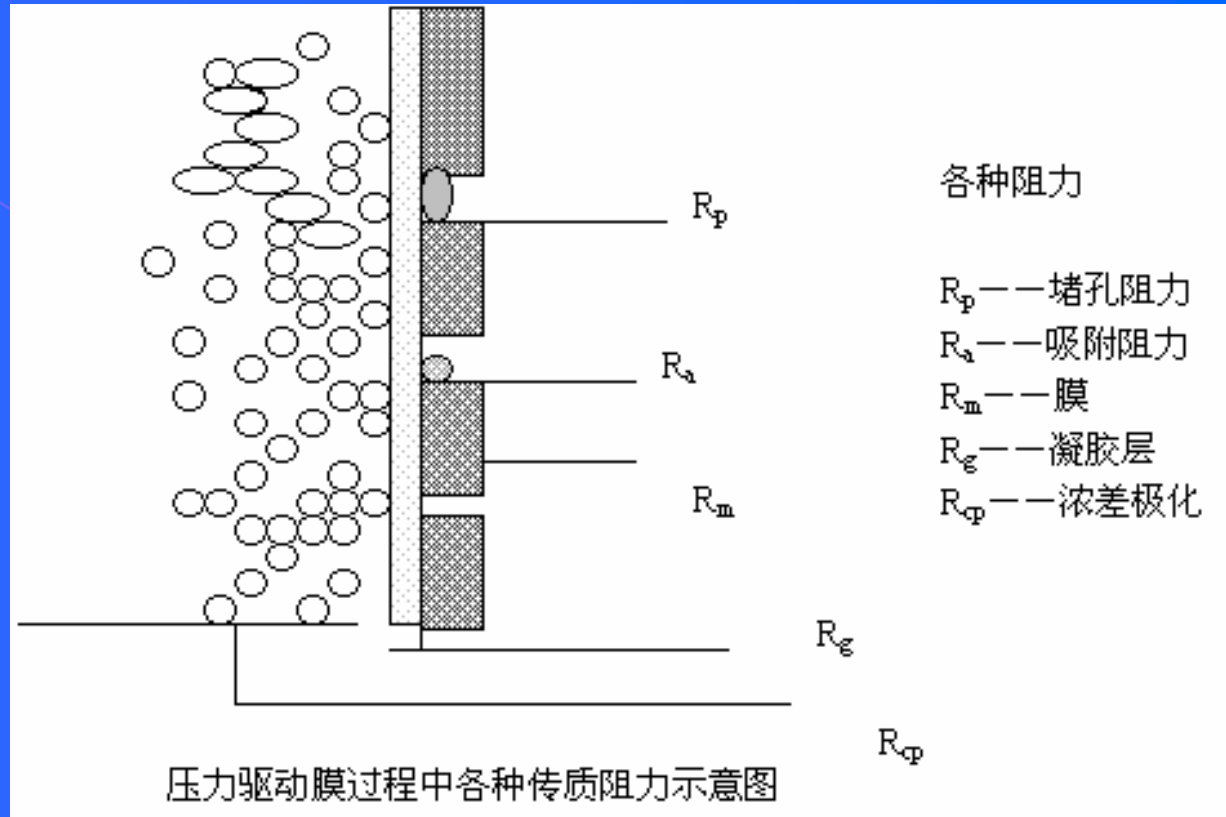
- | 引起通量下降的主要原因
- | 蛋白质的吸附过程
- | 膜过程的污染防治与清洗

# 引起通量下降的原因

时间

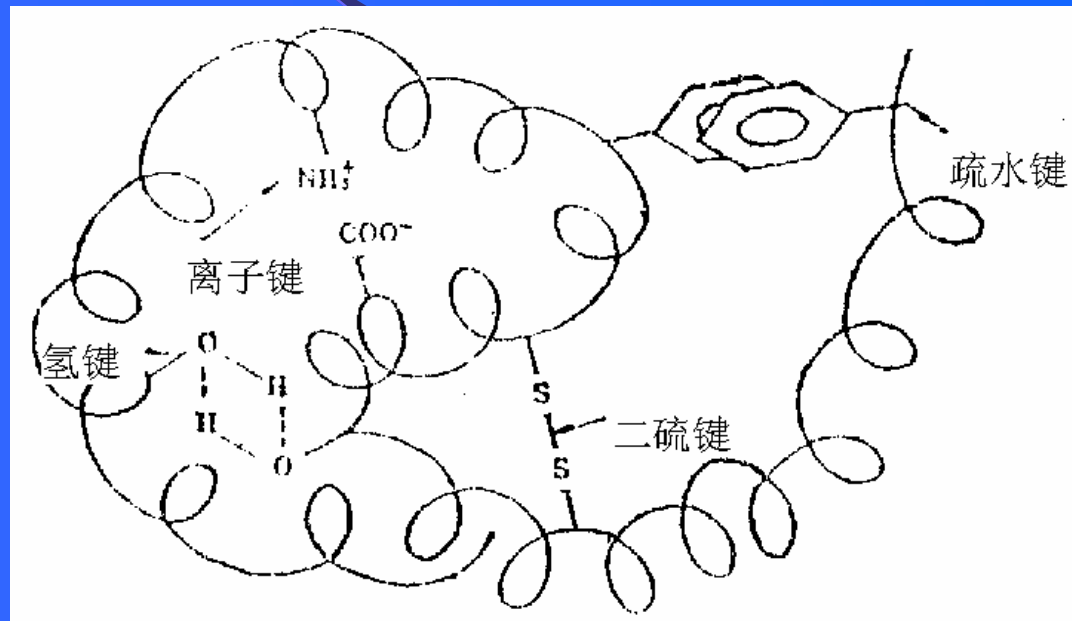


通量





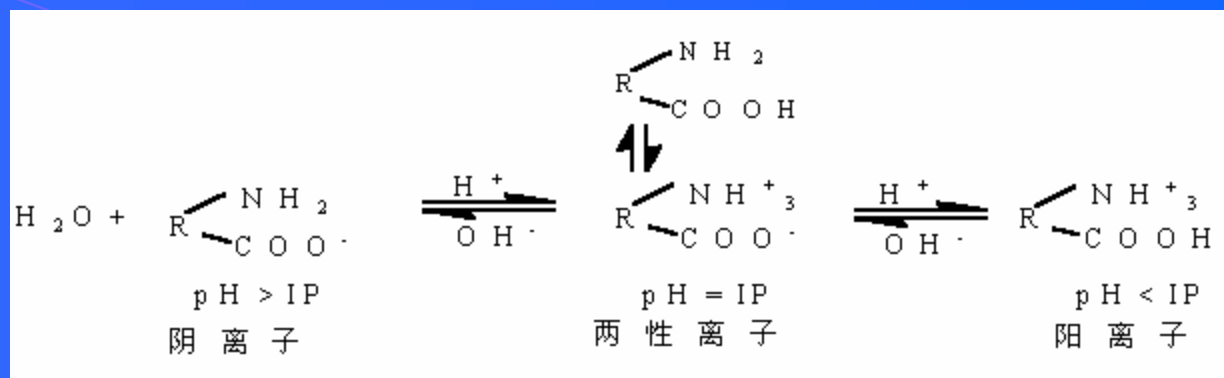
蛋白质除了上述的一级结构外，还有二级结构（ $\alpha$ -螺旋结构、 $\beta$ -折叠结构、转角结构、自由回转等）、三级结构（在二级结构基础上形成的很不规则的构象）和四级结构（由两条或两条以上的具有三级结构的多肽链聚合而成特定的构象），因此蛋白质分子除通常主要的化学键外还有许多其他重要的化学键如氢键、二硫键、离子键、酯键、疏水键和范德华力（图7-7）<sup>(5-6)</sup>，这些键中的一种或几种都影响蛋白质的吸附过程。尽管不是每种蛋白质都包含有上述所有键，但是对于任何一种蛋白质由于其固有的氨基酸结构，在 $1541\text{cm}^{-1}$ （Amide II）和 $1642\text{cm}^{-1}$ （Amide I）附近有较强的吸收峰。



# 主要蛋白质的分子量

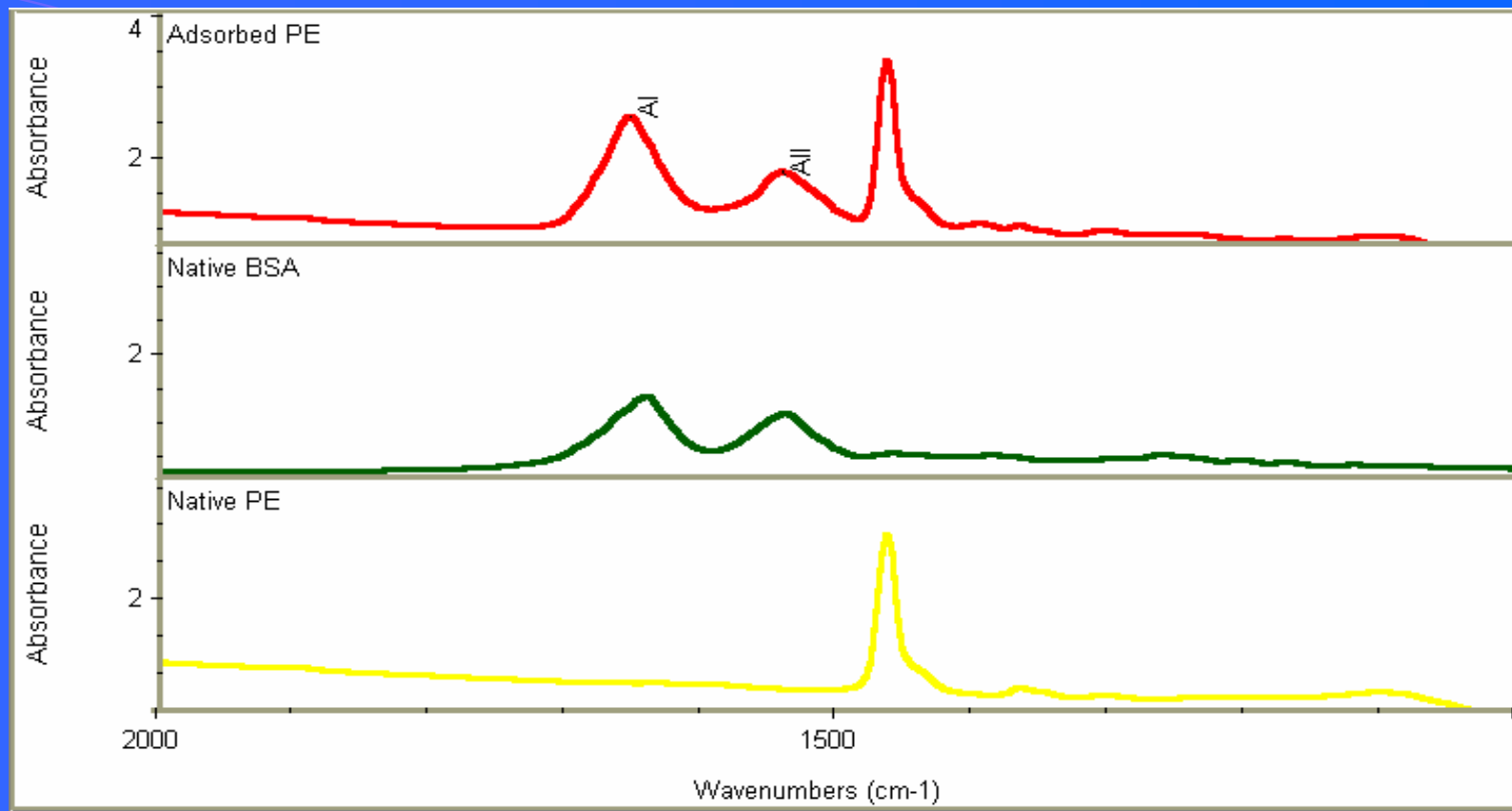
蛋白质	分子量	蛋白质	分子量
胰岛素	5734	天冬酰胺酶	255000
细胞色素C	12393	脲酶	483000
RNA酶	13683	醇脱氢酶	150000
溶菌酶	14300	RNA聚合酶	880000
$\alpha$ -淀粉酶	97600	烟草花叶病毒	40000000
血红蛋白（人）	64500	蛋白	

# 几种蛋白质的等电点



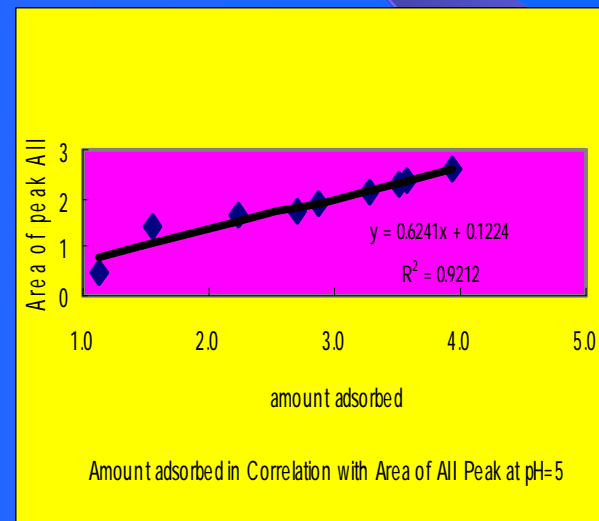
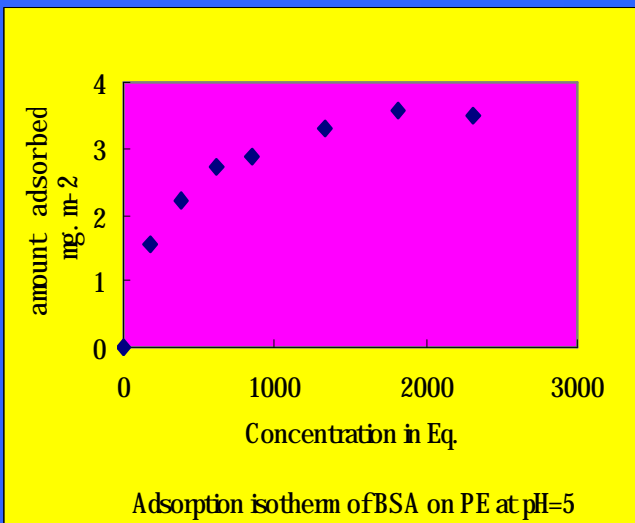
蛋白质名称	等电点	蛋白质名称	等电点
鱼精蛋白	12.0-12.40	血纤维蛋白质	5.5-5.8
胸腺组蛋白	10.8	胰岛素(牛)	5.30-5.35
溶菌酶	11.0-11.2	明胶	4.7-5.0
细胞色素C	9.8-10.3	血清清蛋白(人)	4.64
RNA酶	7.8	鸡蛋清蛋白	4.55-4.9
血红蛋白	7.07	胰蛋白酶(牛)	5.0-8.0
血清γ <sub>1</sub> -球蛋白(人)	5.8-6.6	丝蛋白(家蚕)	2.0-2.4

# Preliminary measurements on PE



# Adsorptional isotherm

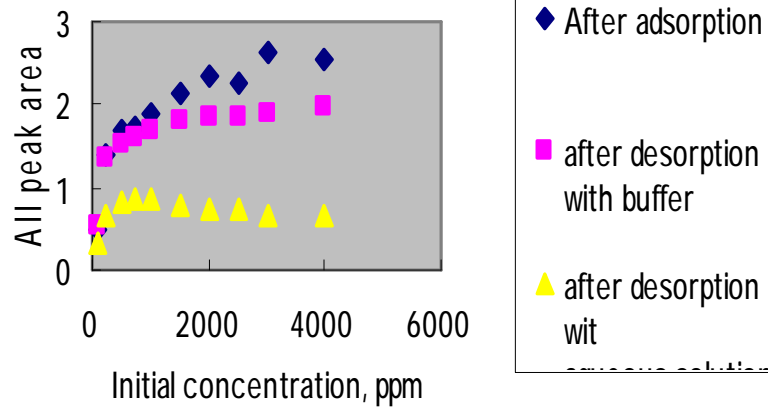
## —Dependence on Concentration



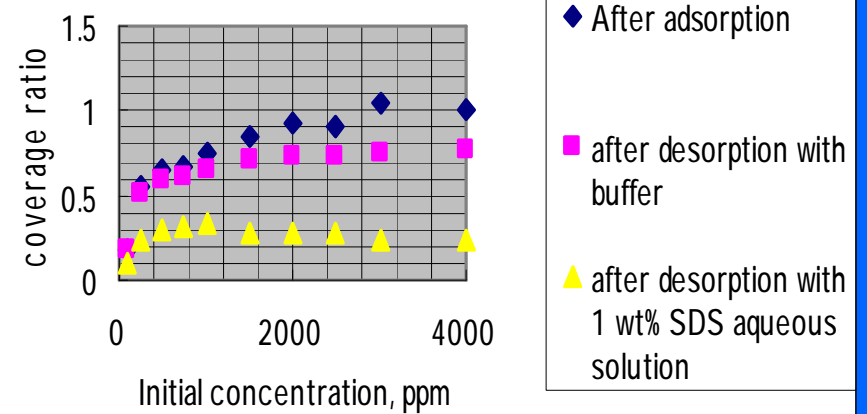


# Peak All area and coverage ratio changes with concentration

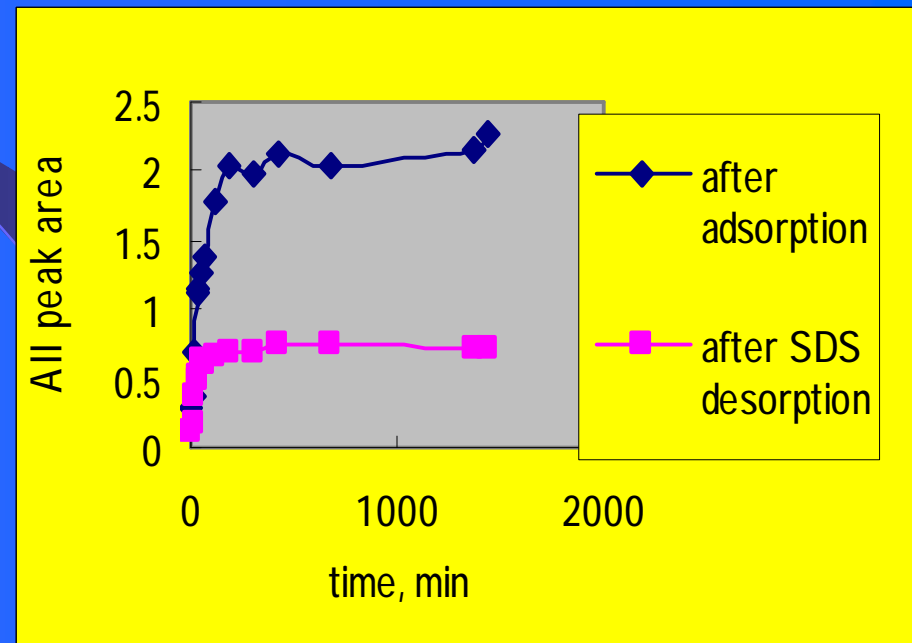
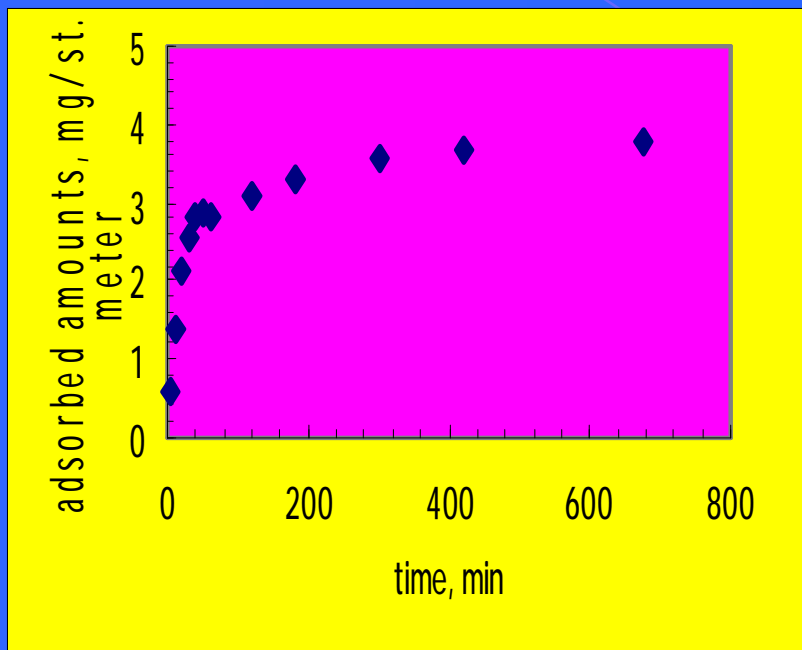
Change of All peak area due to adsorption and desorption at pH=5



Change of coverage ratio due to adsorption and desorption at pH=5



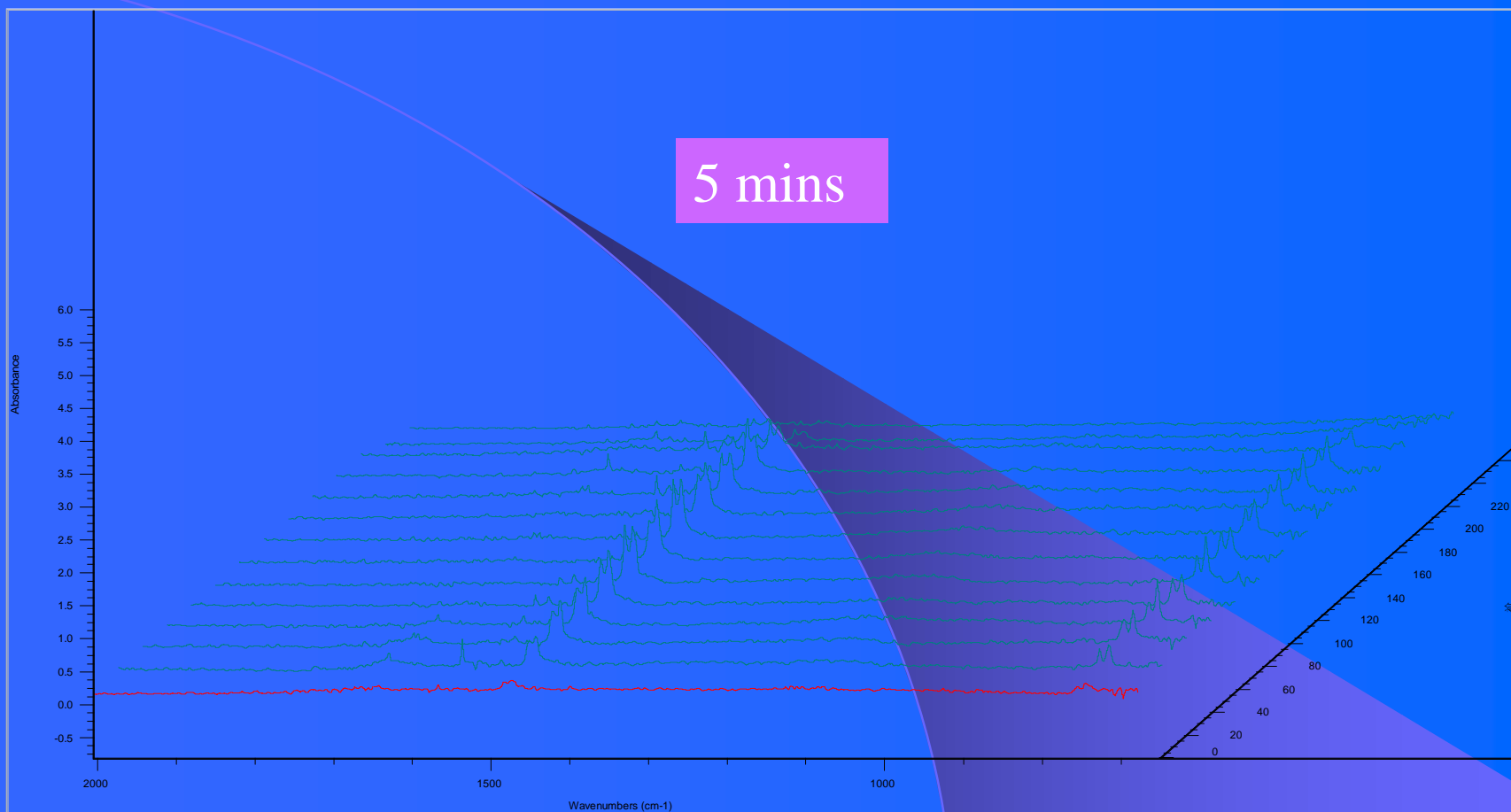
# Dependence on time

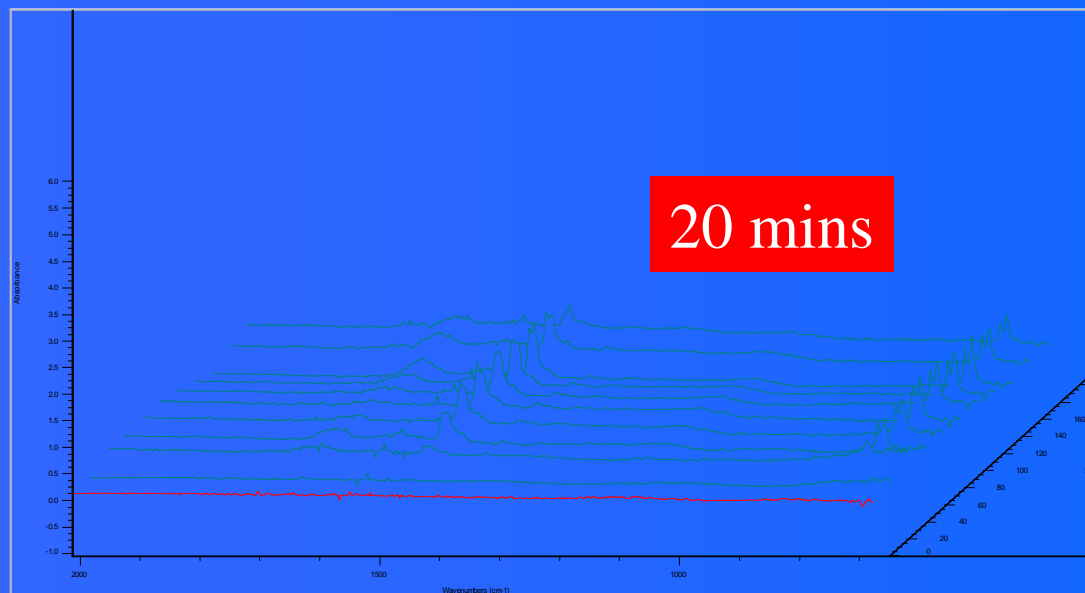
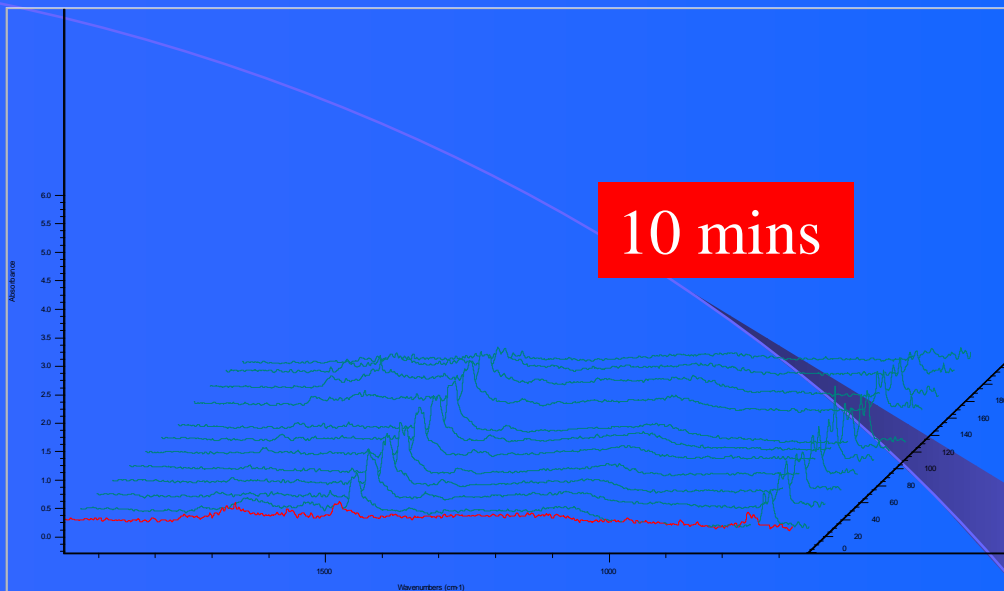


# General adsorptional steps

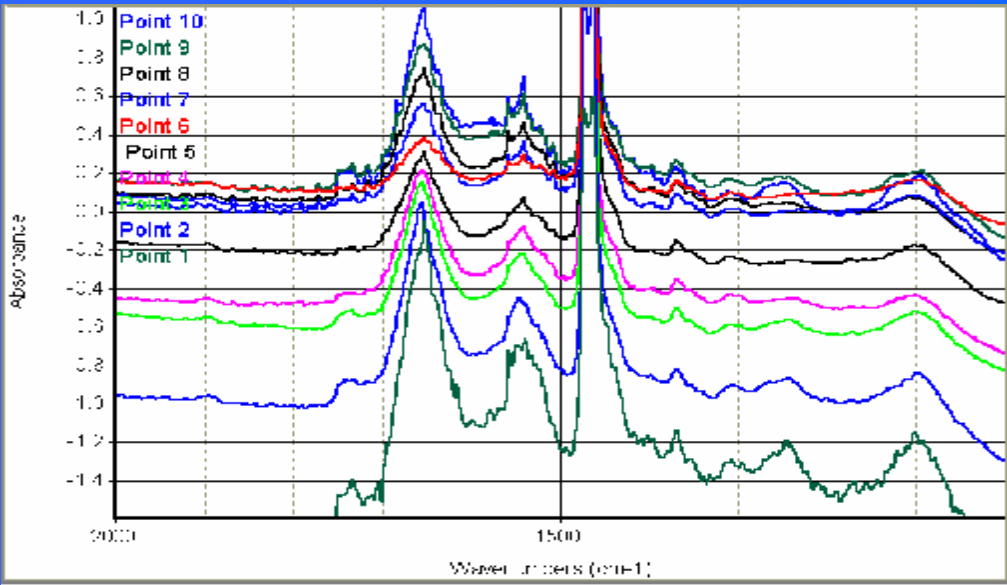
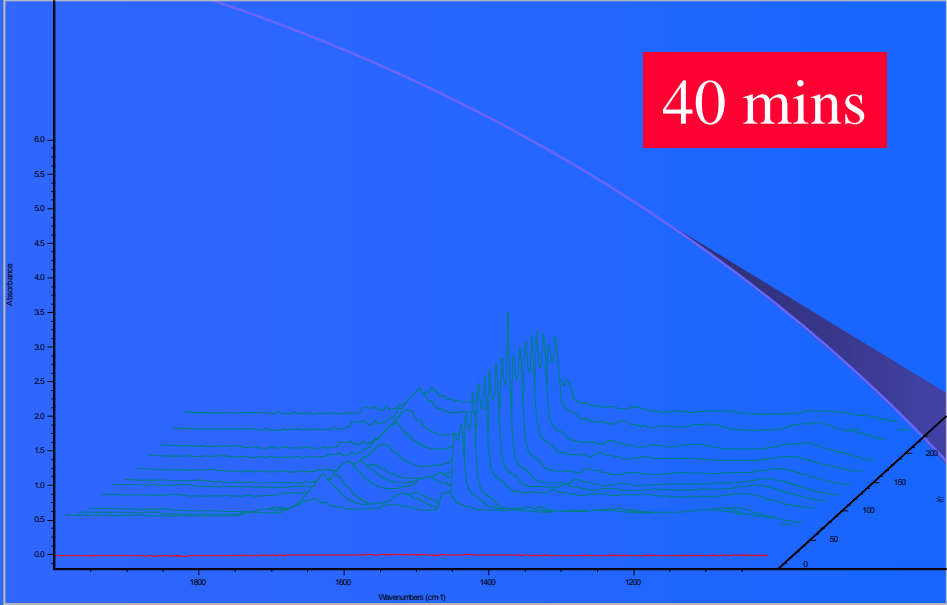
- n Diffusion of protein molecules from bulk to the interface
- n Attachment of protein molecules to active sites on the surface
- n Reformation of the structure of the protein molecule after adsorption.

# Time-FTIR mapping

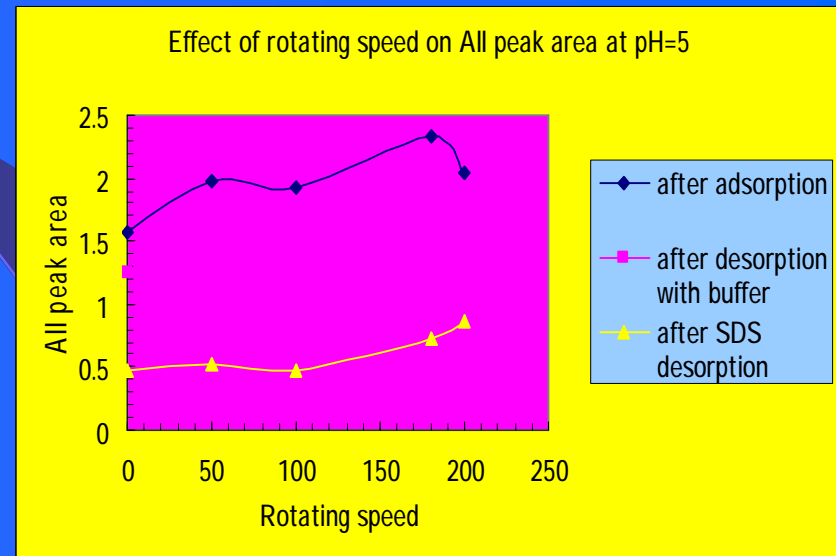
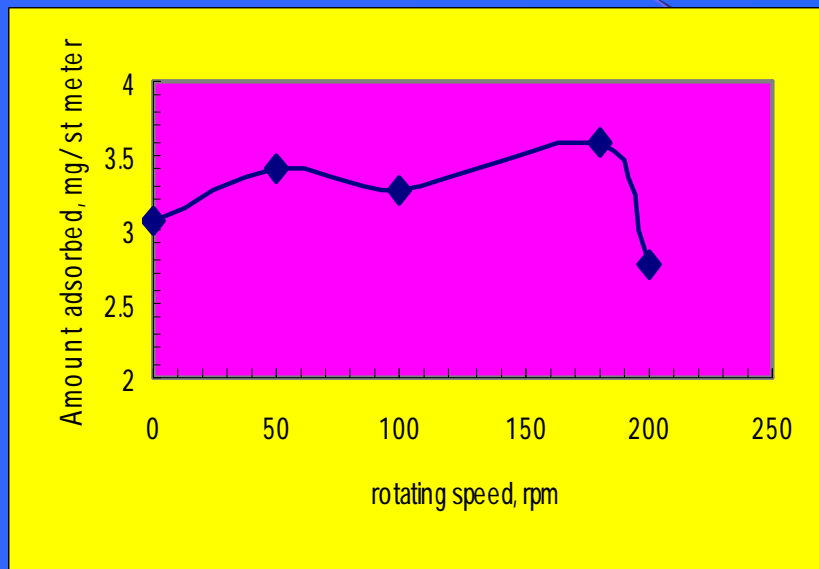




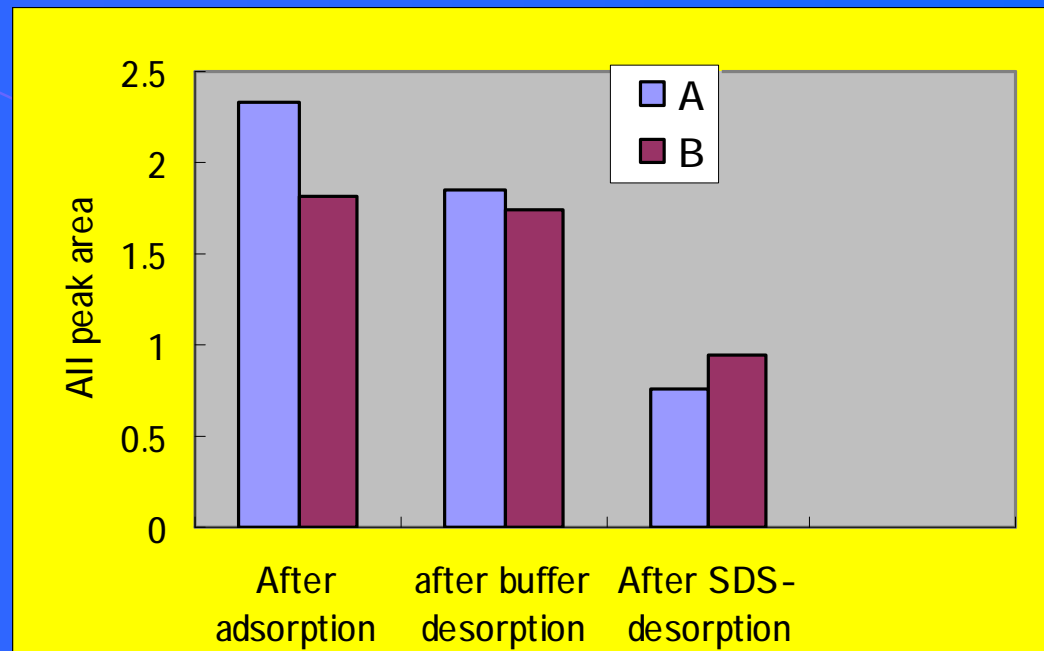
40 mins



# Diffusional speed



# Additional test

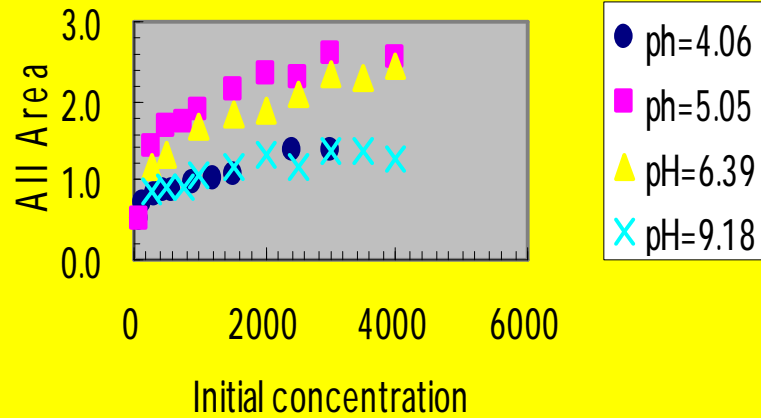


Effect of the way that BSA is added on the adsorption. The condition of the both cases are completely same, but A: the BSA is added one time to make a 2000 ppm solution for adsorption; B- The equal amount of BSA is added 6 times respectively into the equal medium volume at every 4hrs adsorption interval.

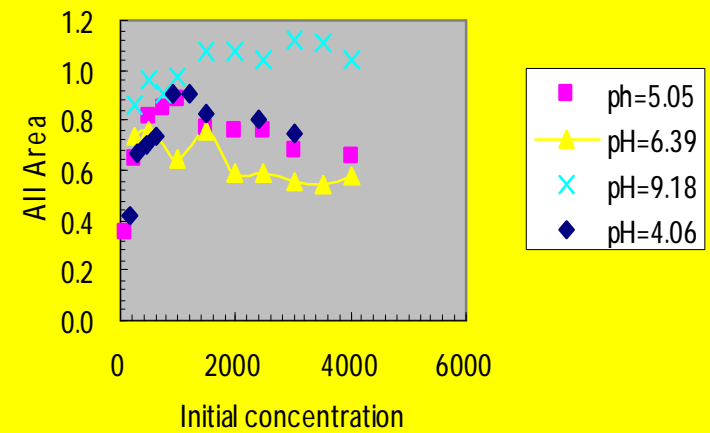


# pH effect

Change of Amide II area with pH after adsorption at different concentration

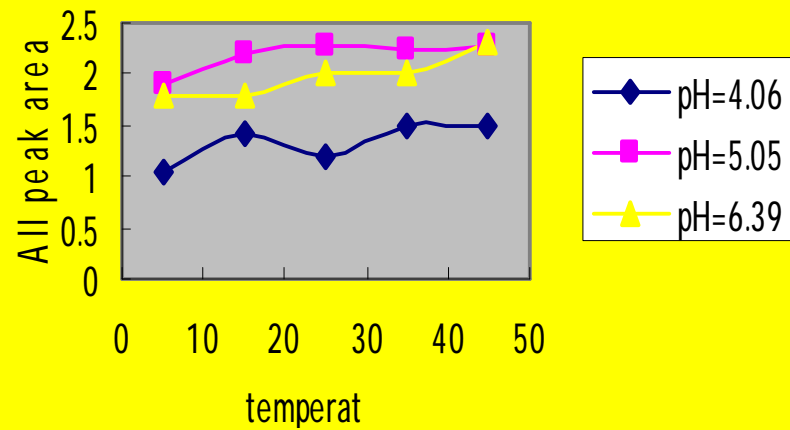


Change of Amide II area after the adsorbed samples in Fig.11 are desorbed with SDS

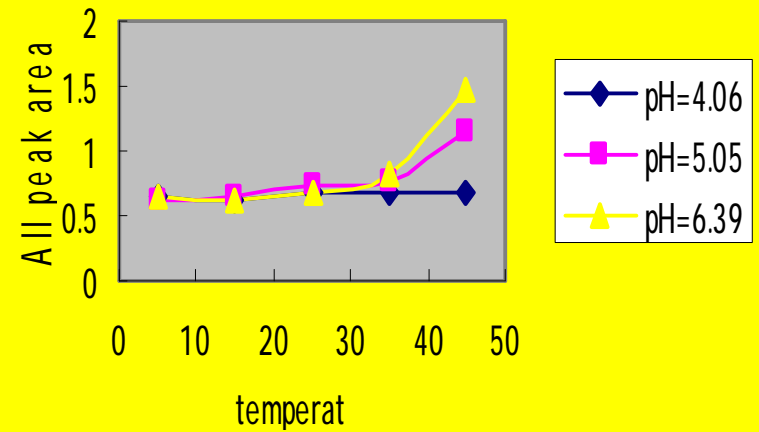


# Temperature effect

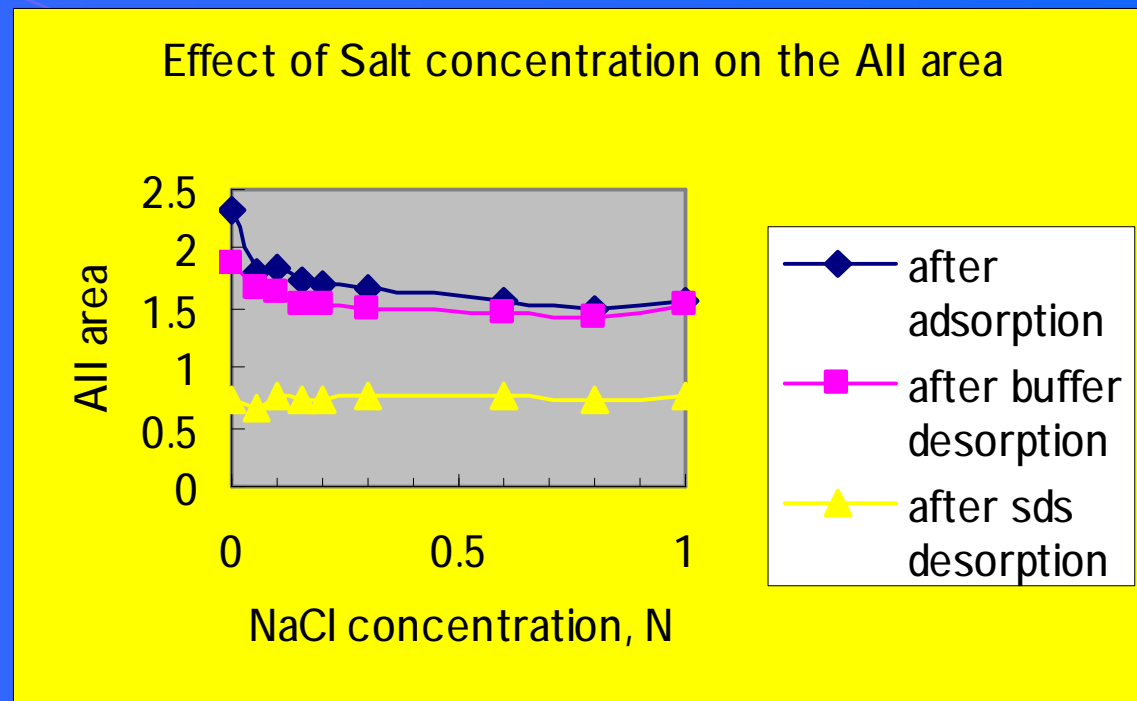
Effect of temperature on the All peak area after adsorption of BSA on PE



Effect of temperature on the All peak area after sds-desorption of BSA on PE

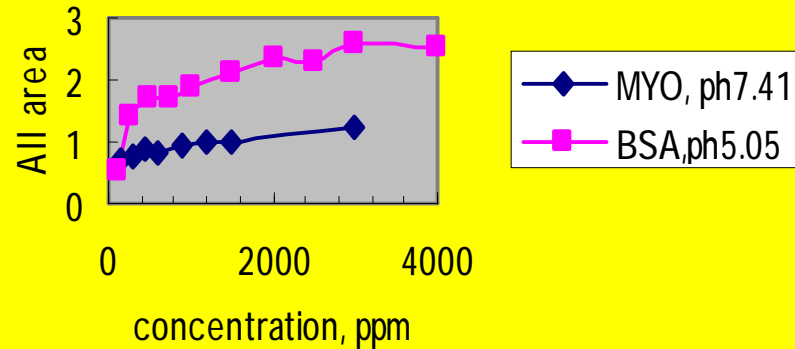


# Salt effect

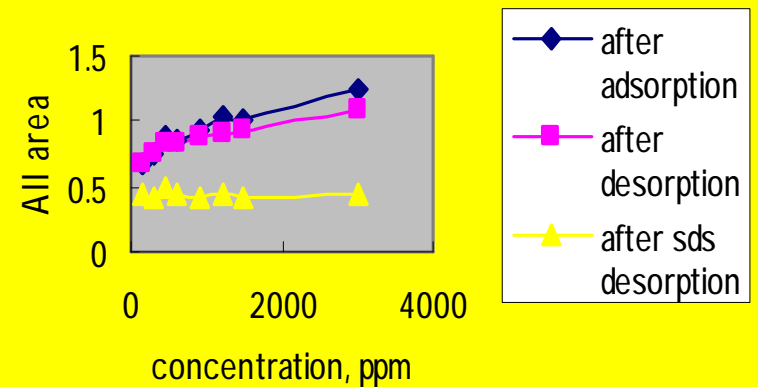


# Adsorption of Myoglobin

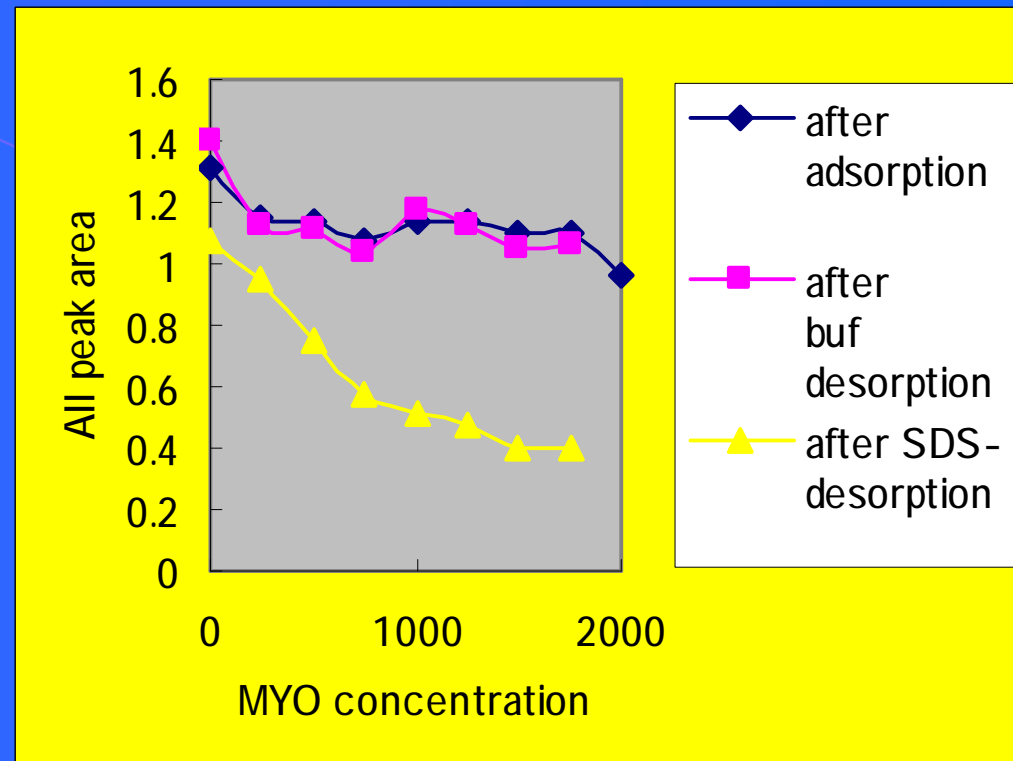
All area changes with concentration of BSA and MYo after adsorption



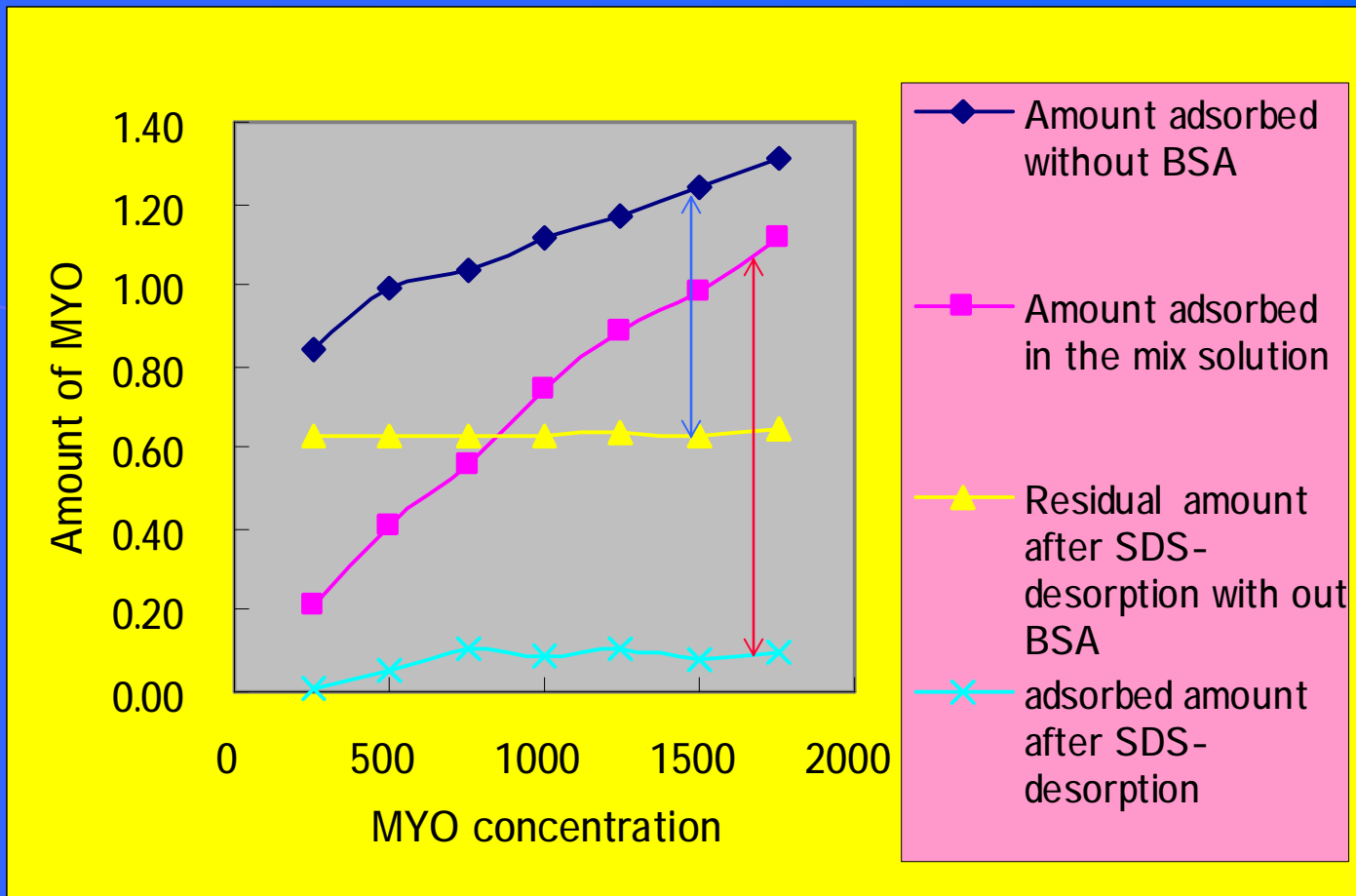
Change of All peak area due to MYO adsorption and desorption at pH=7.41



# Competitive adsorption



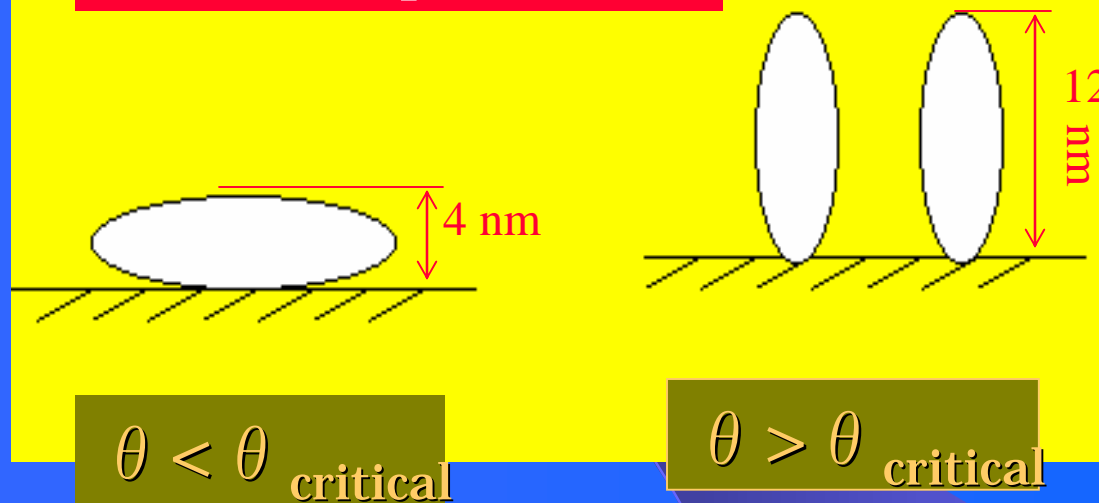
All peak area changes with MYO concentration with total concentration remaining at 2000 ppm



Variation of the amount adsorbed of MYO after adsorption and SDS-desorption —comparison of pure and mix solutions

# The assumed models

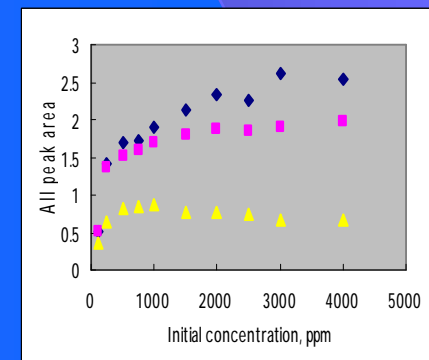
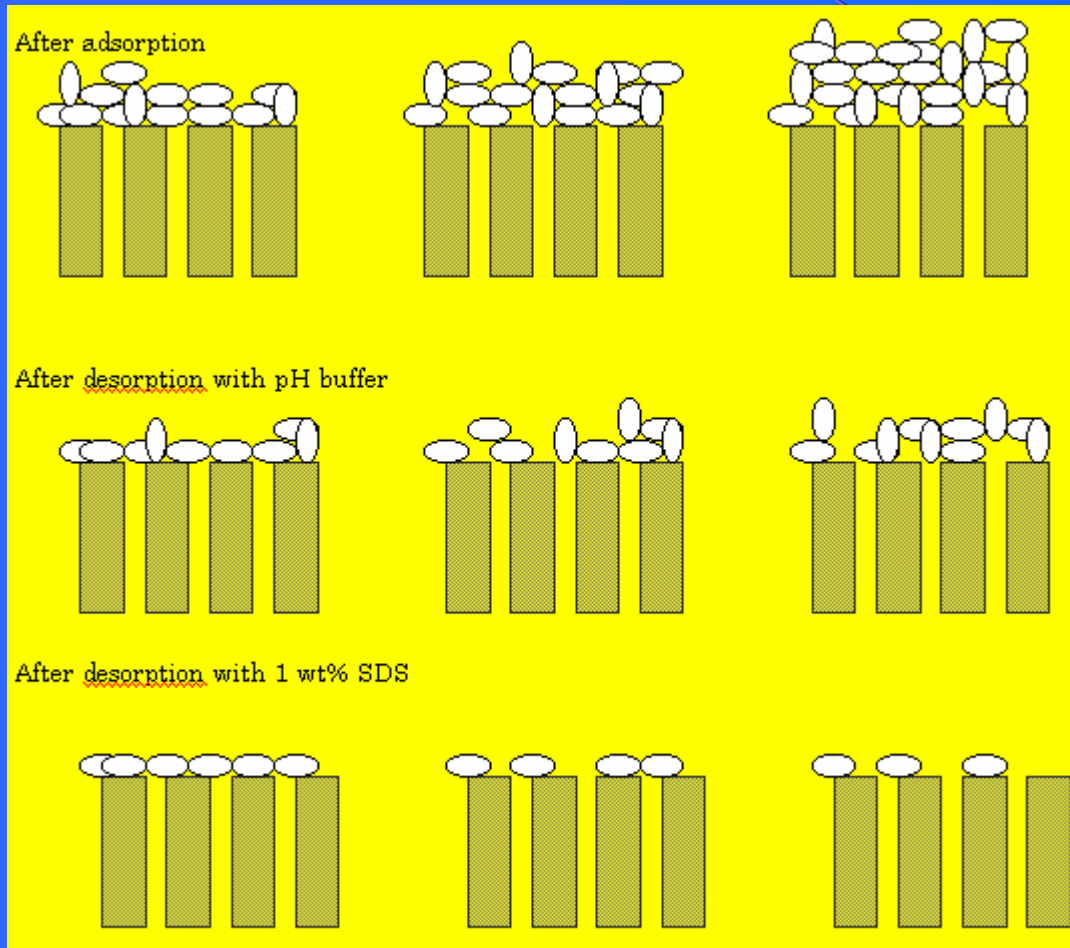
A well-accepted model



Staunton S and Quiquampoix. 1. J Colloid interface Sci., 1994, 166:89-94  
Lu D and Park K. J Colloid interface Sci., 1991, 144:271-281  
Urano H and Fukuzaki S. J. Bioscience and Bioengineering, 2000, 90:105-111

# The assumed models

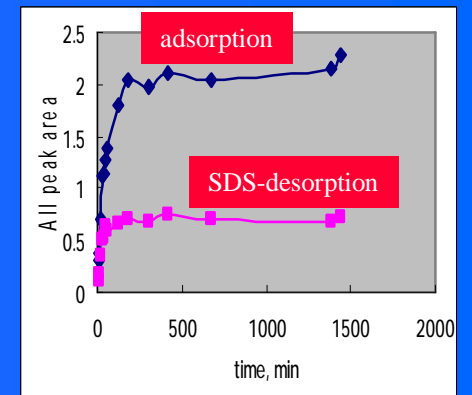
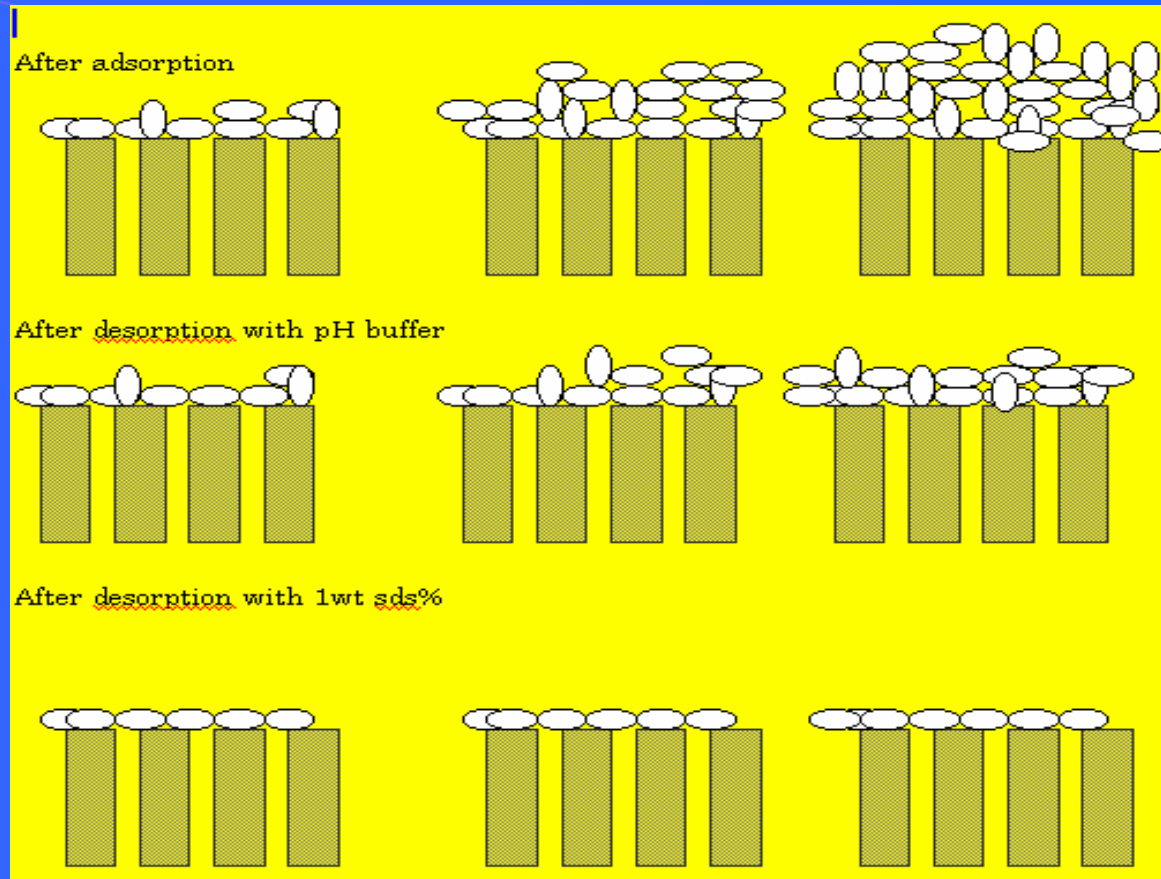
$C_{critical} < C_1 < C_2 < C_3$  ---loose or aggregation adsorption





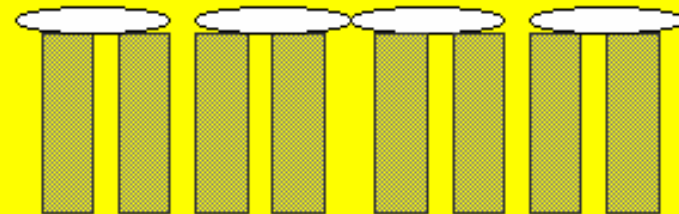
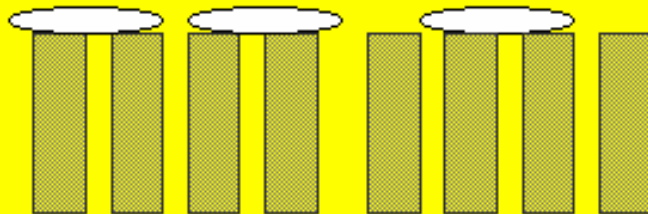
$$t_1 < t_2 < t_3 \quad (\theta > \theta_{\text{critical}})$$

-- above the critical concentration

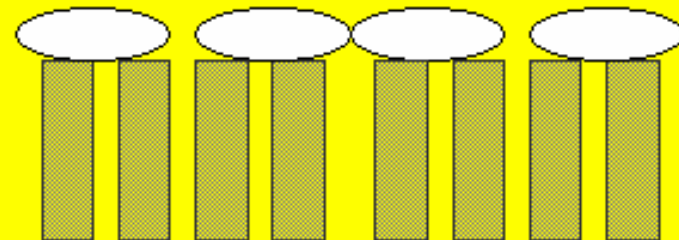
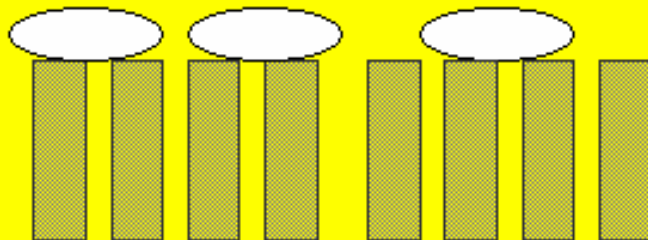


$t_1 < t_2$  ( $\theta < \theta_{\text{critical}}$ )  
----tightly or ordered adsorption

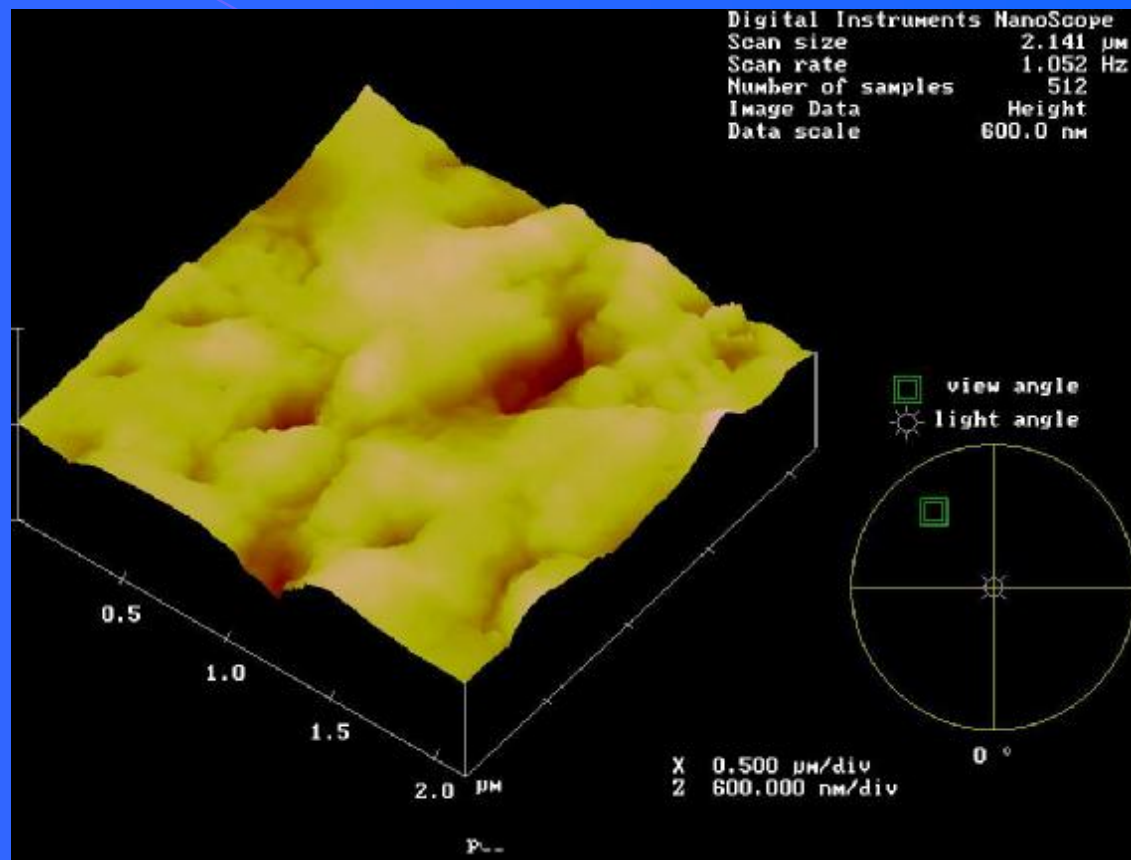
After adsorption -- low concentration (irreversible)



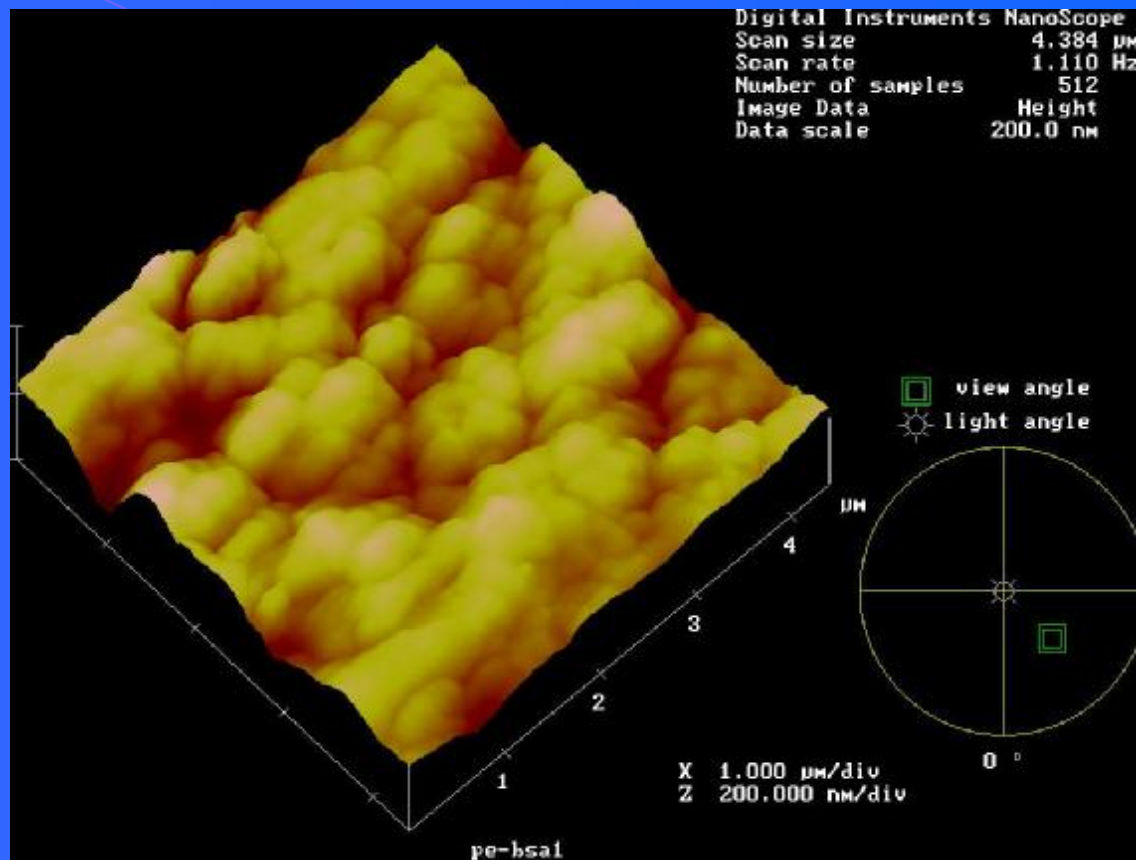
After adsorption -- high concentration (irreversible)



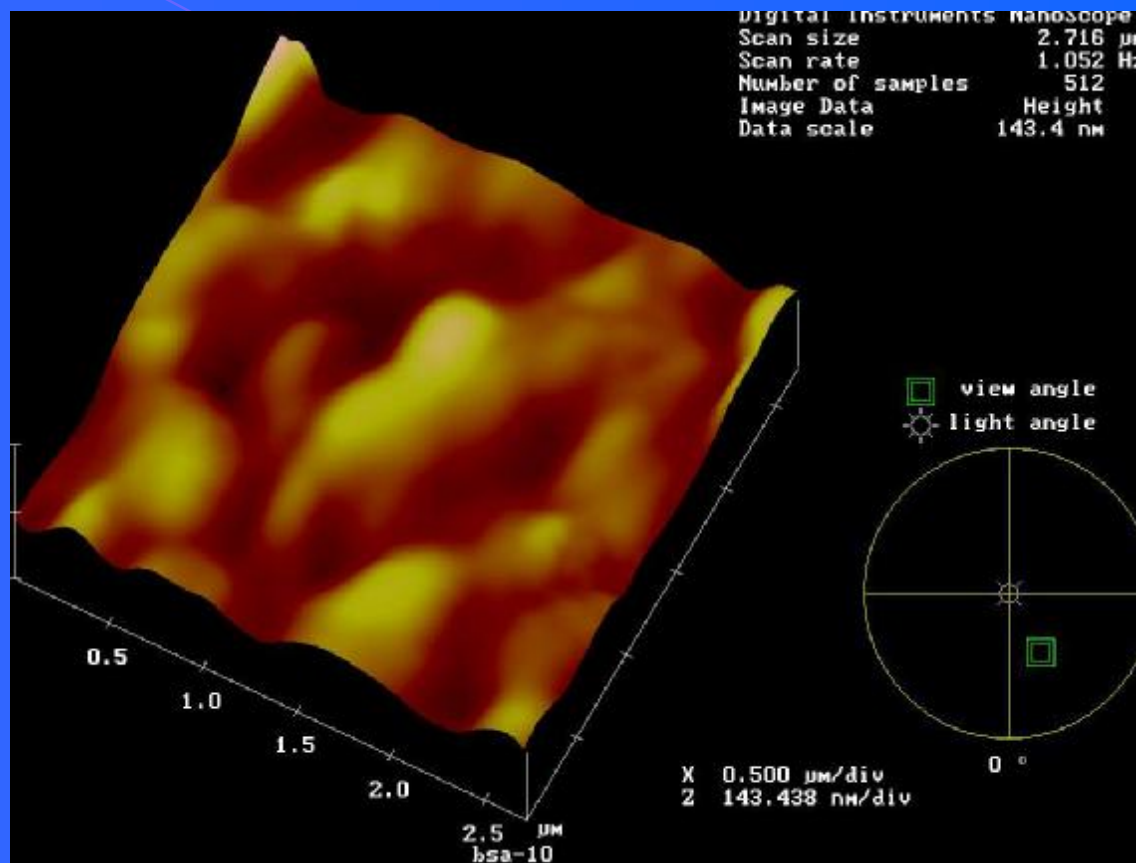
# AFM for PE



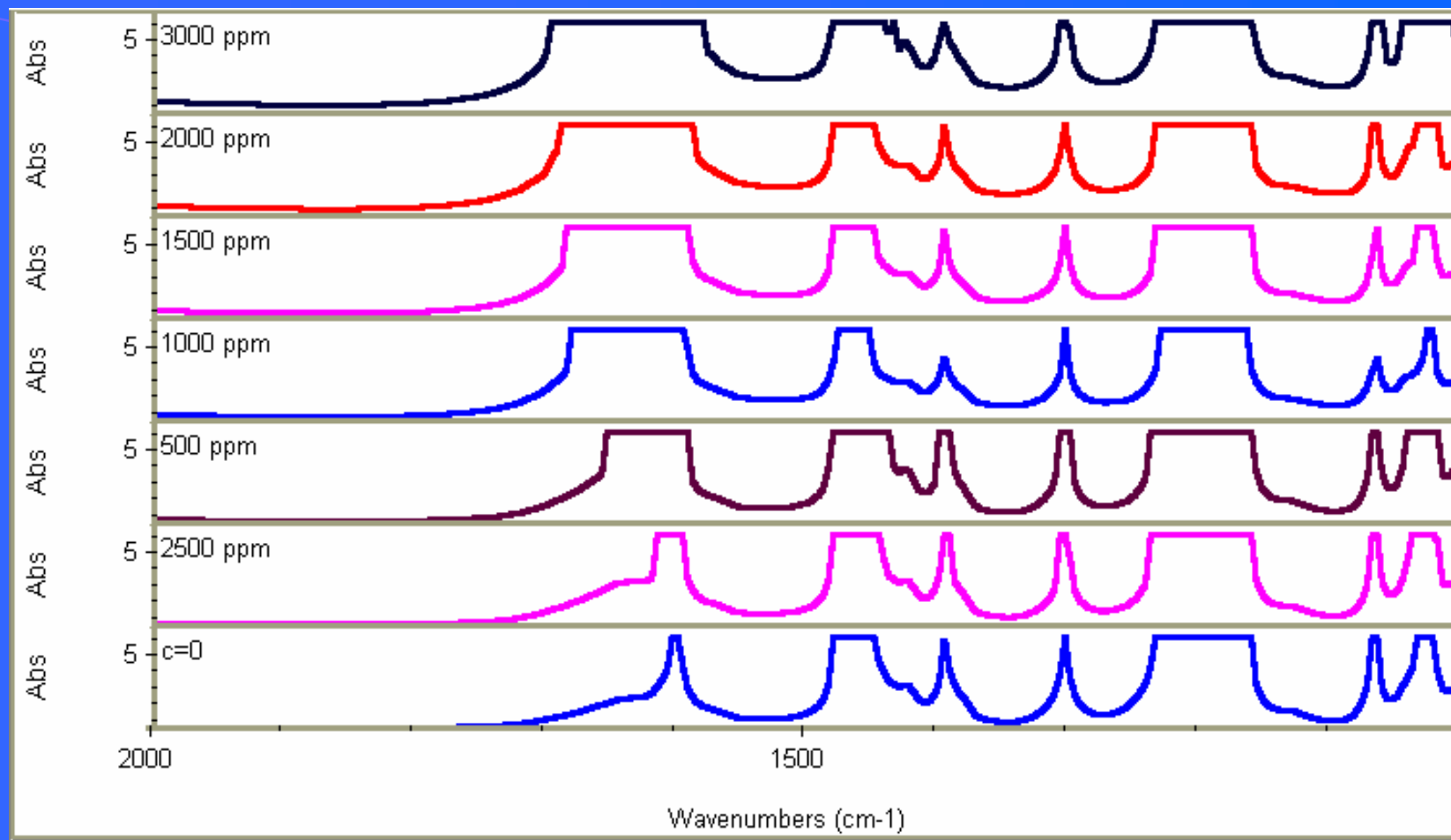
# AFM for adsorbed PE (100mg/l)



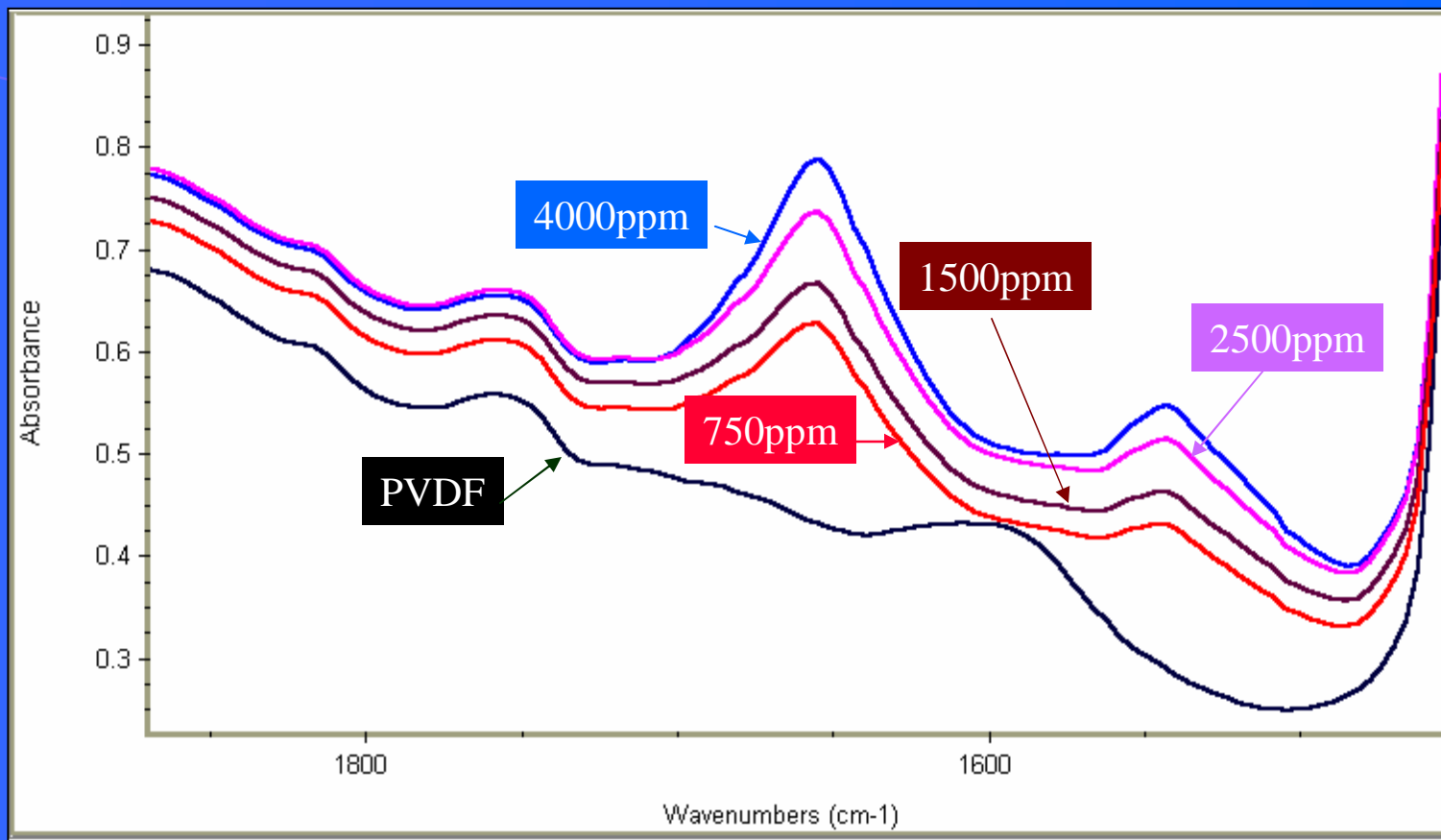
# AFM for adsorbed PE (1000mg/l)



# FTIR Spectra of SPPO by direct determination

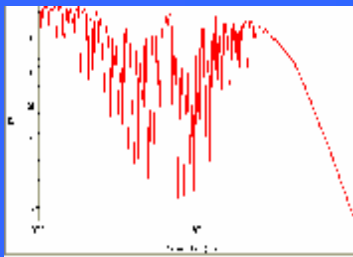


# Typical spectrum of BSA-adsorbed PVDF

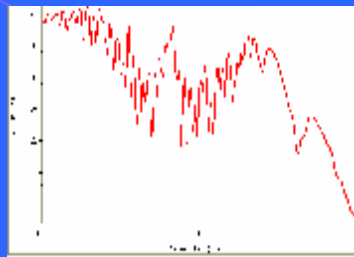


# FTIR steps for SPPO

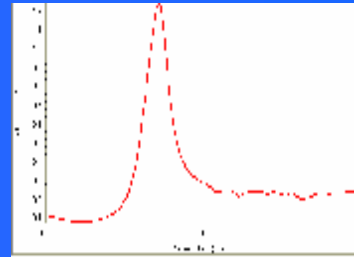
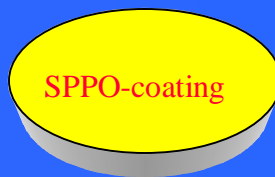
Spectrum BSA = BSA in liquid-Buffer reference( Back ground 1)



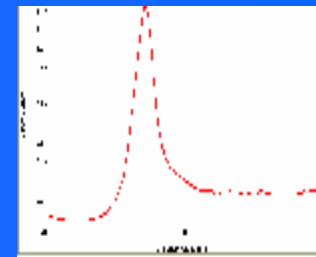
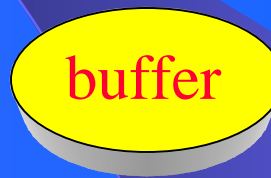
Background 1



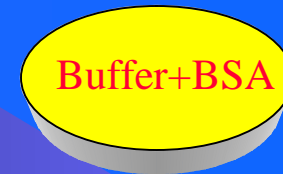
Background 2



Reference



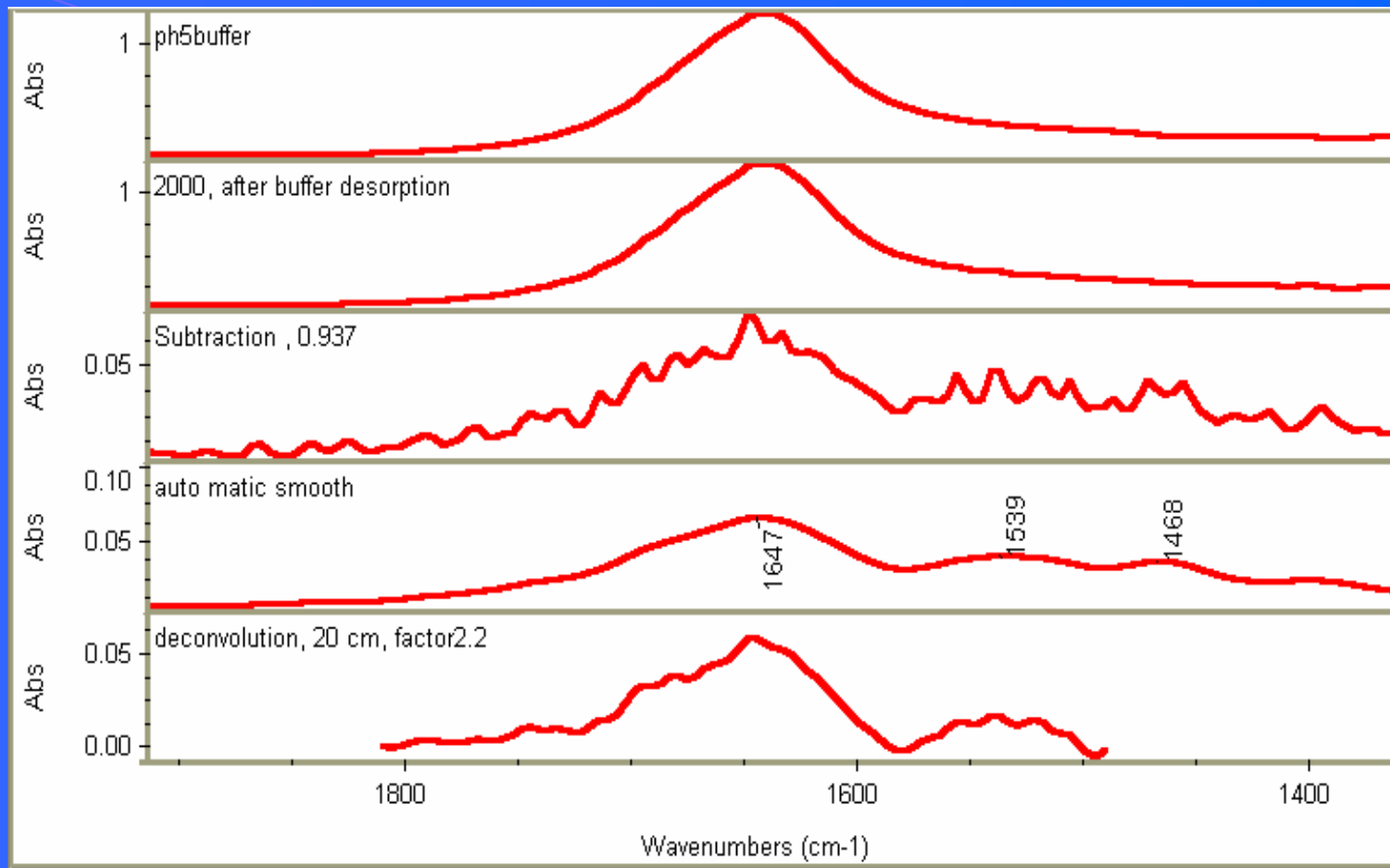
Sample



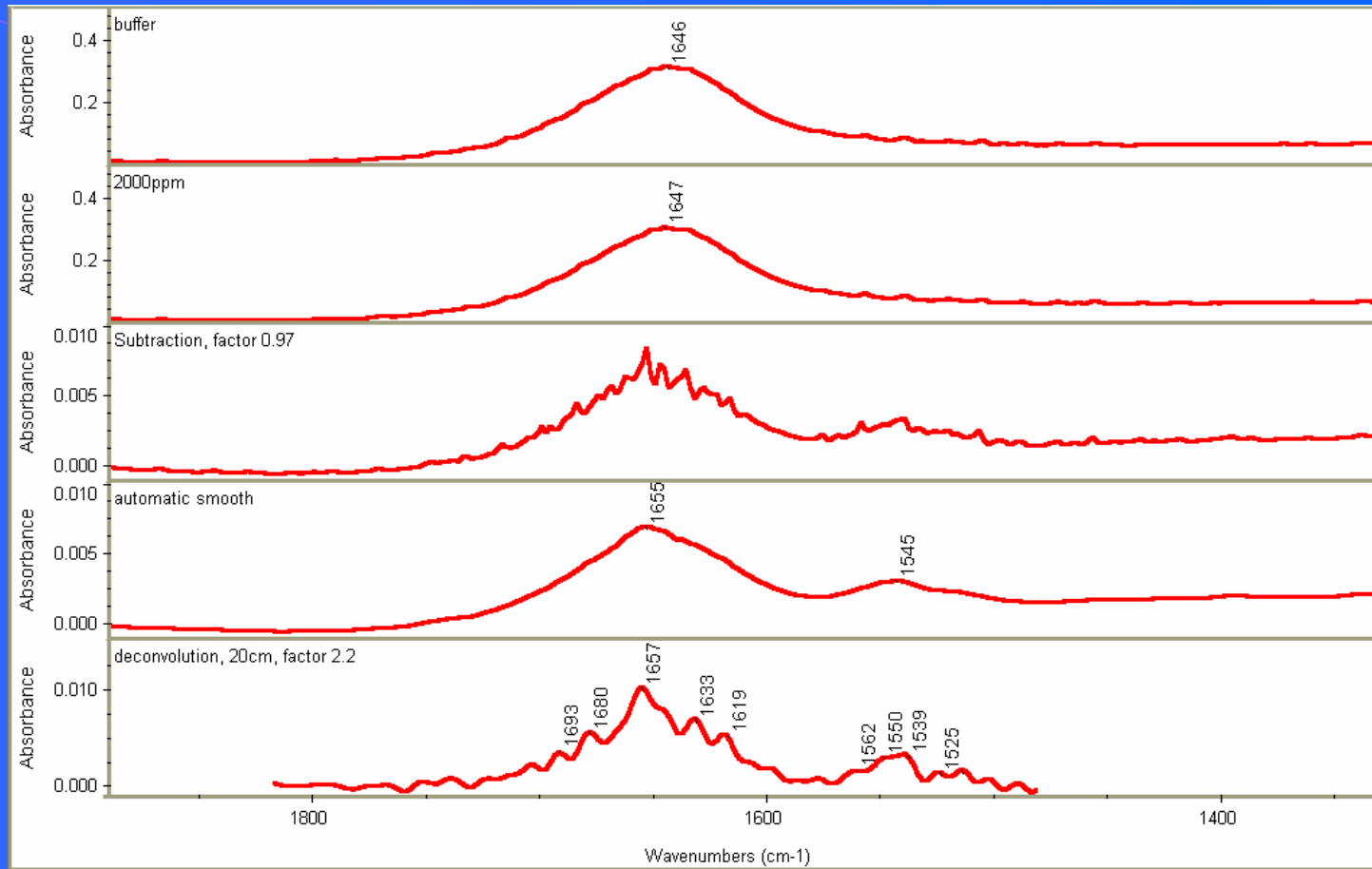
Spectrum for adsorbed BSA = (BSA+SPPO+buffer) - Buffer reference( Back ground 2)



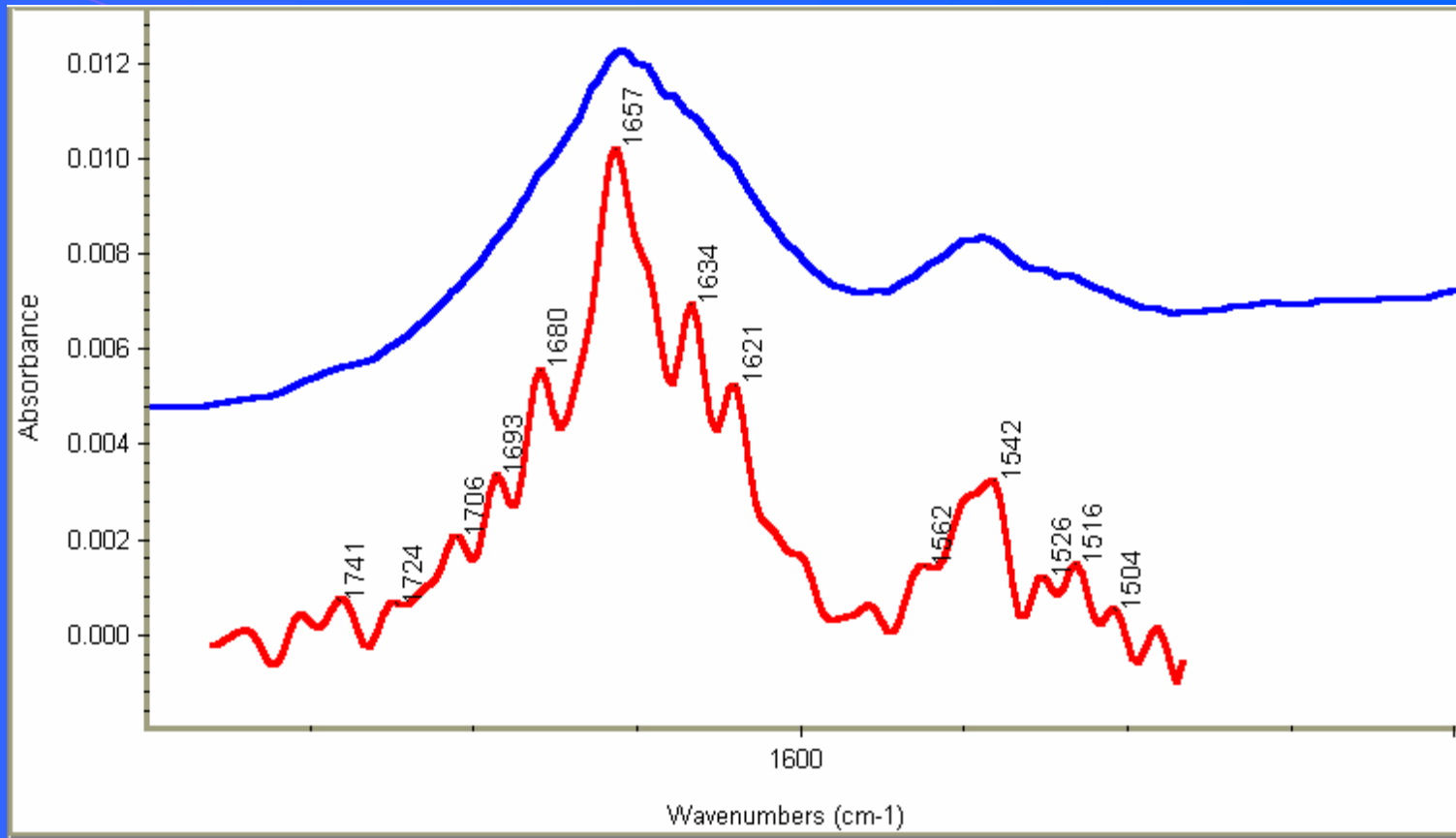
# Deconvolved spectra of adsorbed BSA on SPPO at pH 5.05



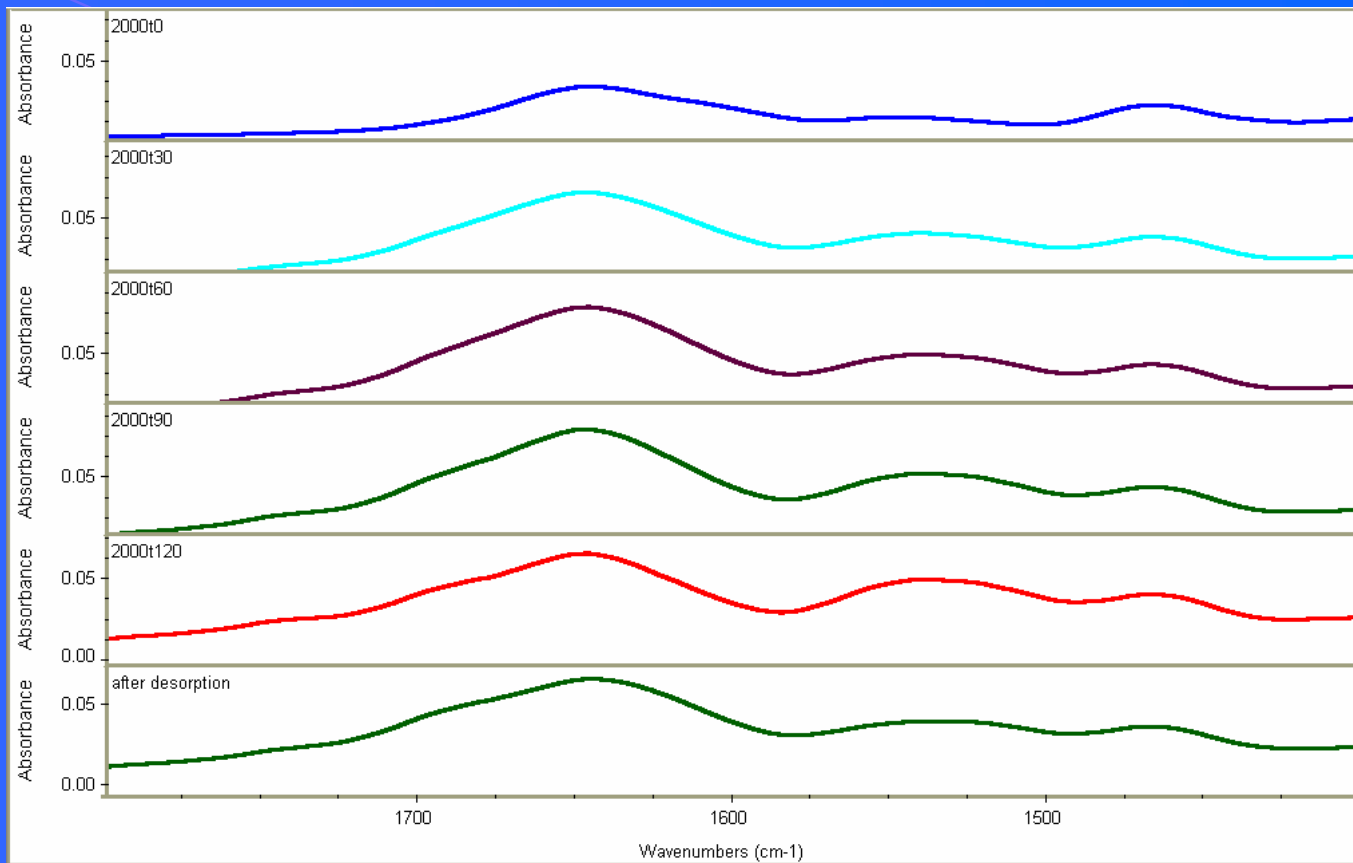
# Deconvolved spectra of BSA in solution



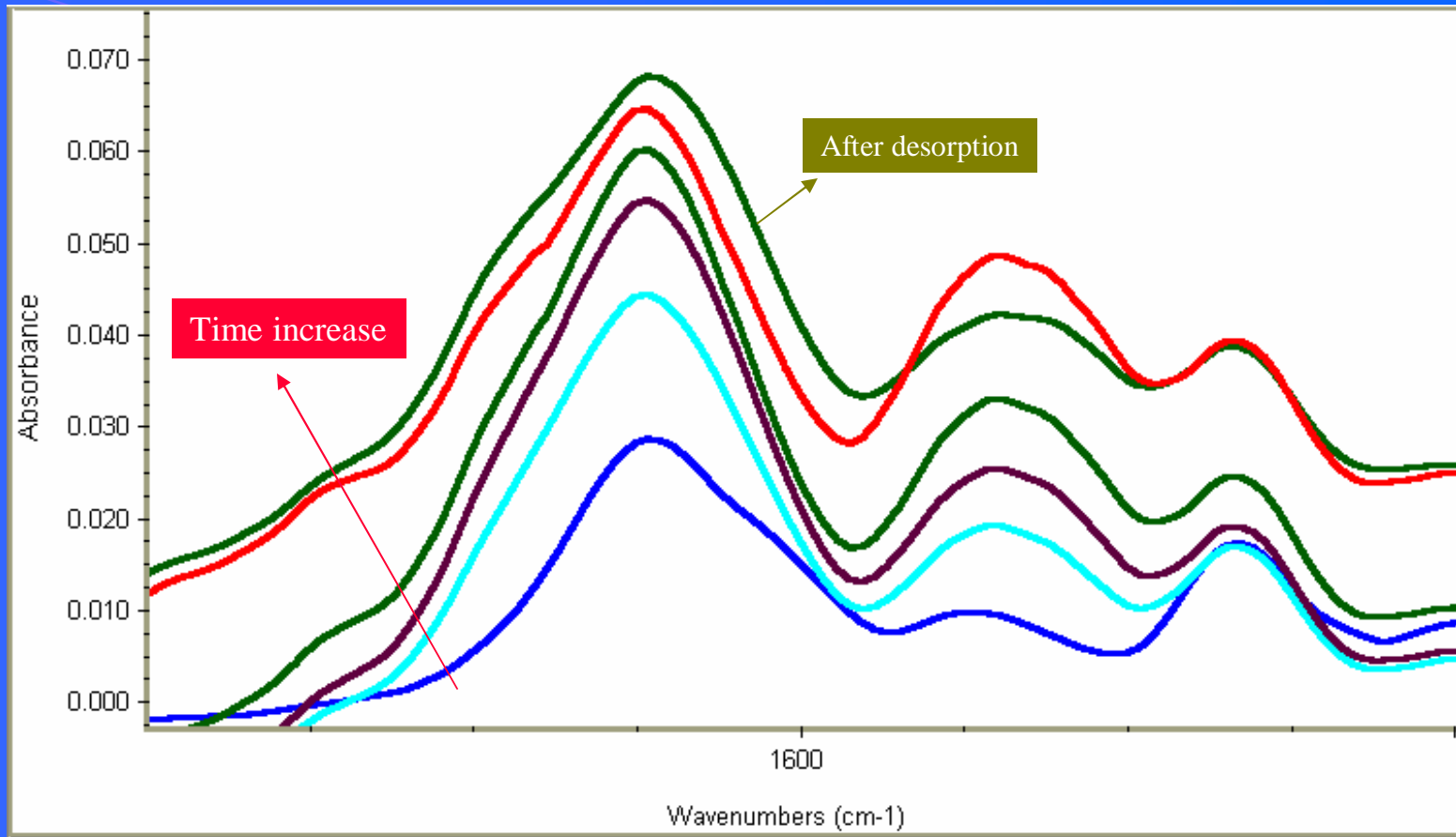
# Deconvolution of BSA in solution at pH 5



# Spectra changes with time for BSA adsorbed in SPPO



# Spectra changes with time for BSA adsorbed in SPPO



# 膜污染防治

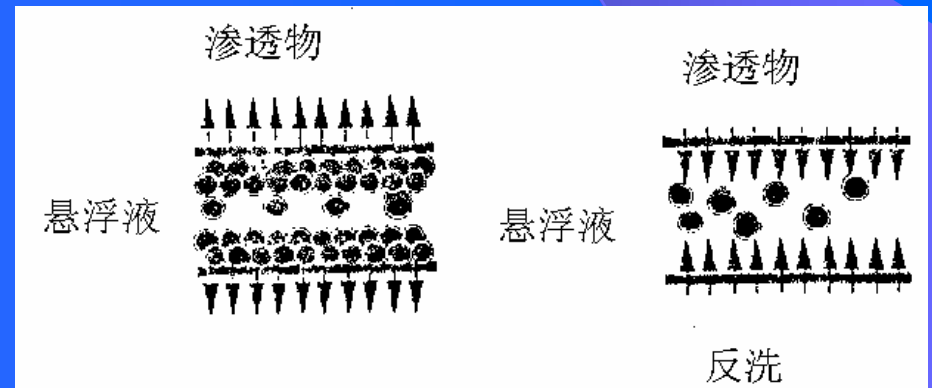
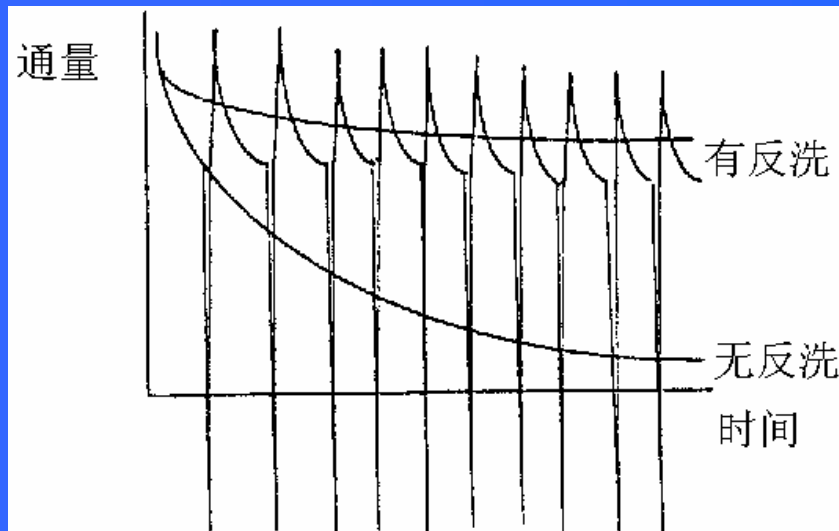
- 丨 膜材料的选择
- 丨 膜孔径或截留分子量的选择
- 丨 组件结构选择和特殊结构设计
- 丨 原料液预处理
- 丨 操作条件优化
- 丨 进行定时和不定时冲洗
  - 丨 提高料液水温
  - 丨 使用消毒试剂
- 丨 正确维护和保养

# 膜的清洗与再生

- 丨 i) 水力学清洗;
- 丨 ii) 机械清洗;
- 丨 iii) 化学清洗
- 丨 iv) 电清洗

# 水力学清洗

水力学清洗方法主要是反洗（只适用于微滤膜和疏松的超滤膜），即以一定频率交替加压、减压和改变流向。





# 机械清洗

- 机械清洗原只适用于微滤/超滤管式系统（用海绵球擦洗）。但近年来开发出一种外压式的帘式膜，可以很方便地进行机械冲洗。

# 化学清洗

- 酸（较强的如 $\text{H}_3\text{PO}_4$ ，或较弱的如乳酸）；
- 碱（ $\text{NaOH}$ ）；
- 洗涤剂（碱性、非离子型）；
- 酶（蛋白酶、淀粉酶、葡聚糖酶）；
- 络合剂（EDTA、聚丙烯酸酯、六偏磷酸钠）；
- 清毒剂（ $\text{H}_2\text{O}_2$ 和 $\text{NaOCl}$ ）；
- 蒸汽和气体（环氧乙烷）消毒。

# 电清洗

电清洗是一种十分特殊的清洗方法。在膜上施加电场，则带电粒子或分子将沿电场方向迁移。通过在一定时间间隔内施加电场且在无需中断操作的情况下从界面上除去粒子或分子。这种方法的缺点是需使用导电膜及安装有电极的特殊膜器，对膜和装置的要求较高。

# 本章小节

- 膜通量下降的原因
- 蛋白质的性质
- 不同条件下蛋白质吸附效果
- 防治膜污染的主要措施
- 膜的清洗方法

# 本章习题

1. 为了考察混合蛋白质在PE微滤膜上的吸附特性，现用牛血清蛋白质（BSA）和肌红蛋白（MYO）配制成2000ppm的混合蛋白质溶液对PE膜进行吸附和SDS脱附实验，结果发现在BSA存在时，相同MYO浓度吸附和SDS脱附的差值更大。试说明：  
a. 哪种蛋白质对PE膜的污染更严重？  
b. 本题的结果在实际中有什么应用？
2. 若蛋白质在某种膜上的最大吸附量为 $4\text{mg}/\text{cm}^2$ ，请问吸附量为 $0.5\text{mg}/\text{cm}^2$ 的蛋白质可能以什么方式？

3. 用一个微滤过程将发酵液1100L浓缩到100L。所用膜器长为0.5 m，其中装有320根直径为2 mm的纤维，厚度为0.5mm，原料通入膜外侧（外压式），通量可表示为： $J_t = J_0 t^{-0.5}$

如 $J_0=100 \text{ L}/(\text{m}^2\cdot\text{h})$ ，完成上述过程需要多长时间？

4. 若3题采用“反冲”法，每10s有1/10孔体积被反冲，通量保持 $100\text{L}/(\text{m}^2\cdot\text{h})$ 不变，如膜总空隙率为50%，则体积减少会持续多长时间（反冲时间可忽略）。