

# 实验室装柱



GE imagination at work

# 实验室装柱

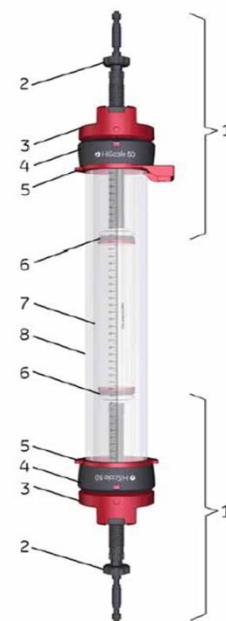
- 确定装柱方案
- 装柱流程
- 柱效测定

# 确定装柱方案

- 装柱规模：根据试验所需
- 层析柱类型：压力范围，尺寸
- 凝胶填料种类：粒径大小，耐压范围，流速....
- 装柱缓冲液：水，盐溶液，有机溶剂
- 装柱泵



# 实验室常用层析柱



XK column



Tricorn



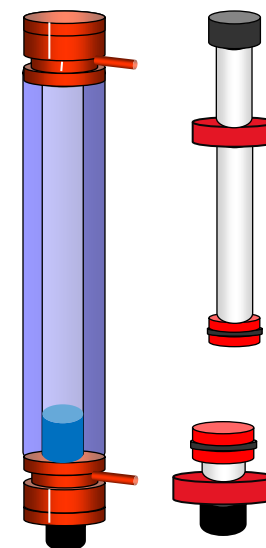
Hiscale

# 实验室层析空柱

	XK	Tricorn	Hiscala
长度	20-100 cm	2-60 cm	20-40 cm
直径范围	1.6-5 cm	0.5-1 cm	1.6-5 cm
耐压	0.5 Mpa XK 16,26 0.3 Mpa XK 50 0.2 Mpa (jacket)	10 Mpa, Tricorn 5 5 Mpa, Tricorn 10	2 Mpa

# 确认装柱方案

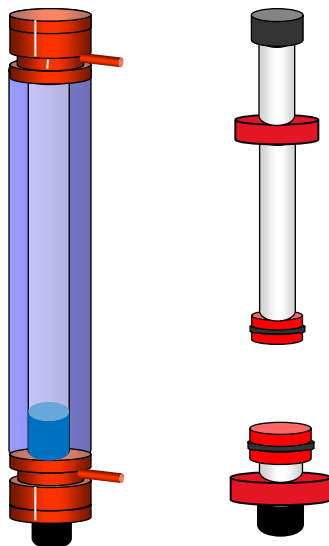
填料: Q Sepharose Fast Flow  
平均粒径: 90  $\mu\text{m}$   
装柱体积: 53.1ml  
层析柱: XK 26/20  
层析柱筛网: 23  $\mu\text{m}$  < 1/3 90 $\mu\text{m}$   
内径: 2.6 cm  
横截面积: 5.31  $\text{cm}^2$   
装柱高度: 10 cm  
压缩因子: 1.25  
所需介质体积: 53.1ml  $\times$  1.25 = 66.375 ml  
装柱缓冲液: 去离子水



# 实验室装柱

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# 装柱流程1：层析柱的准备



## 提示：

- 筛网定期用0.5M NaOH 超声清洗，用20% 酒精排除气泡
- 各个部件组装完毕后，检查密闭性
- 垂直固定

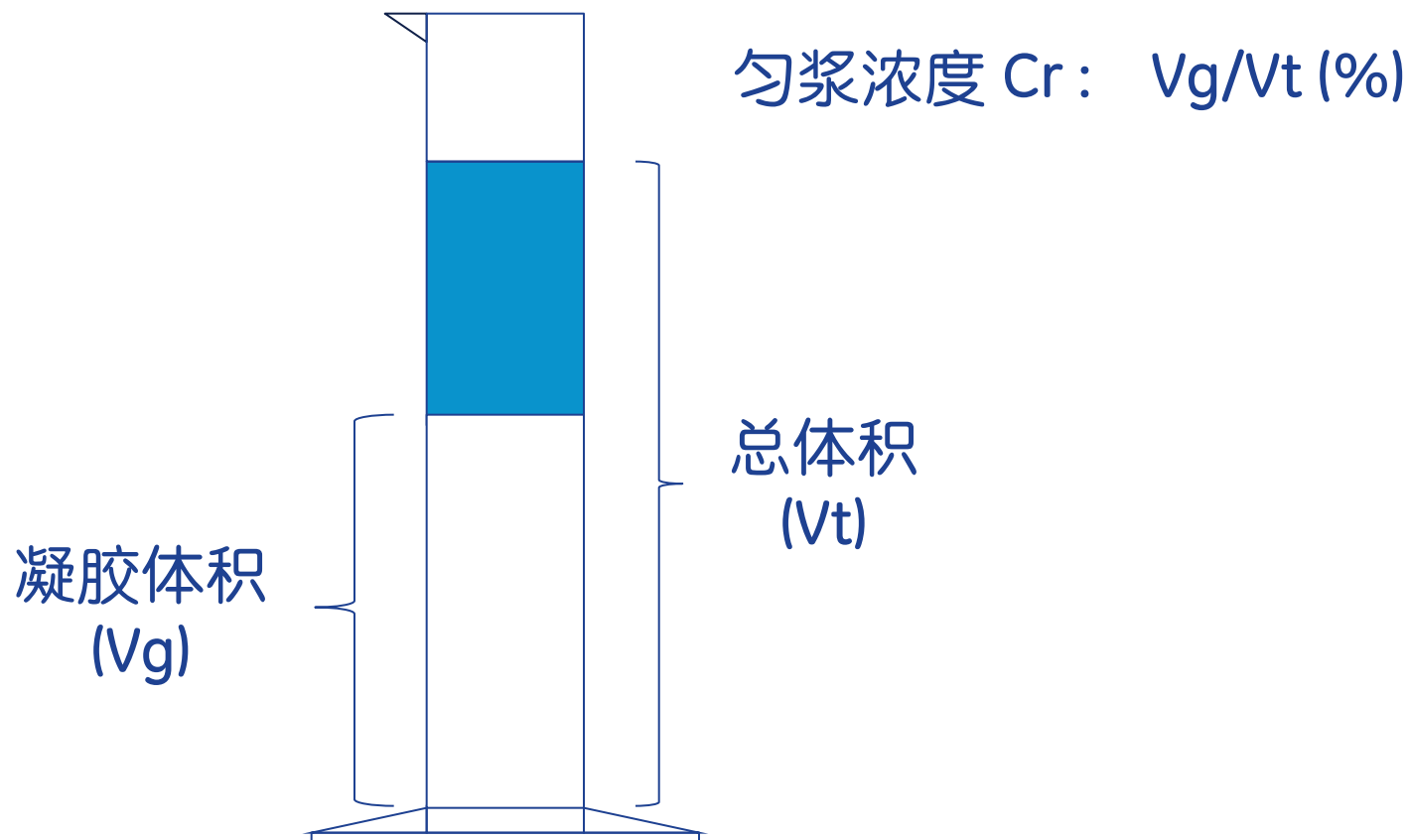


## 装柱流程2： 填料匀浆的准备

- 置换填料缓冲液至装柱缓冲液中
- 匀浆浓度建议在50%-75%之间

$$\text{所需凝胶悬液的体积 (ml)} = \frac{\text{装柱体积 (ml)} \times \text{填料的压缩比}}{\text{凝胶悬液的浓度}}$$

# 计算填料匀浆浓度



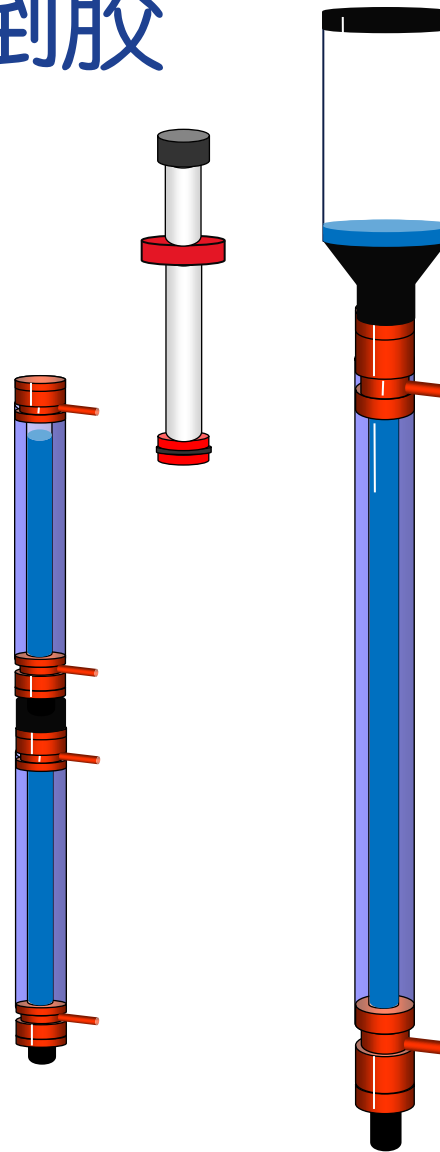
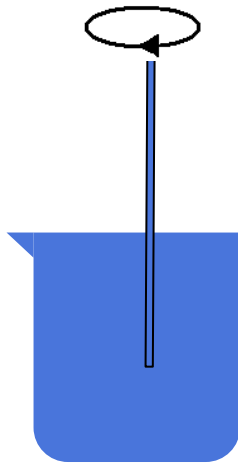
# 常用介质的压缩因子

介质	压缩因子
Q Sepharose Fast Flow	1.25
DEAE Sepahrose Fast Flow	1.25
SP Sepharose Fast Flow	1.25
CM Sepharose Fast Flow	1.25
Phenyl Sepharose Fast Flow	1.25
Sepharose 4 and 6 Fast Flow	1.2
Sphacryl High Resolution	1.2
Superdex 75 and 200 Prep grade	1.2
Q and SP High Performance	1.2
Phenyl High Performance	1.2
Sephadex G-25	250g/L

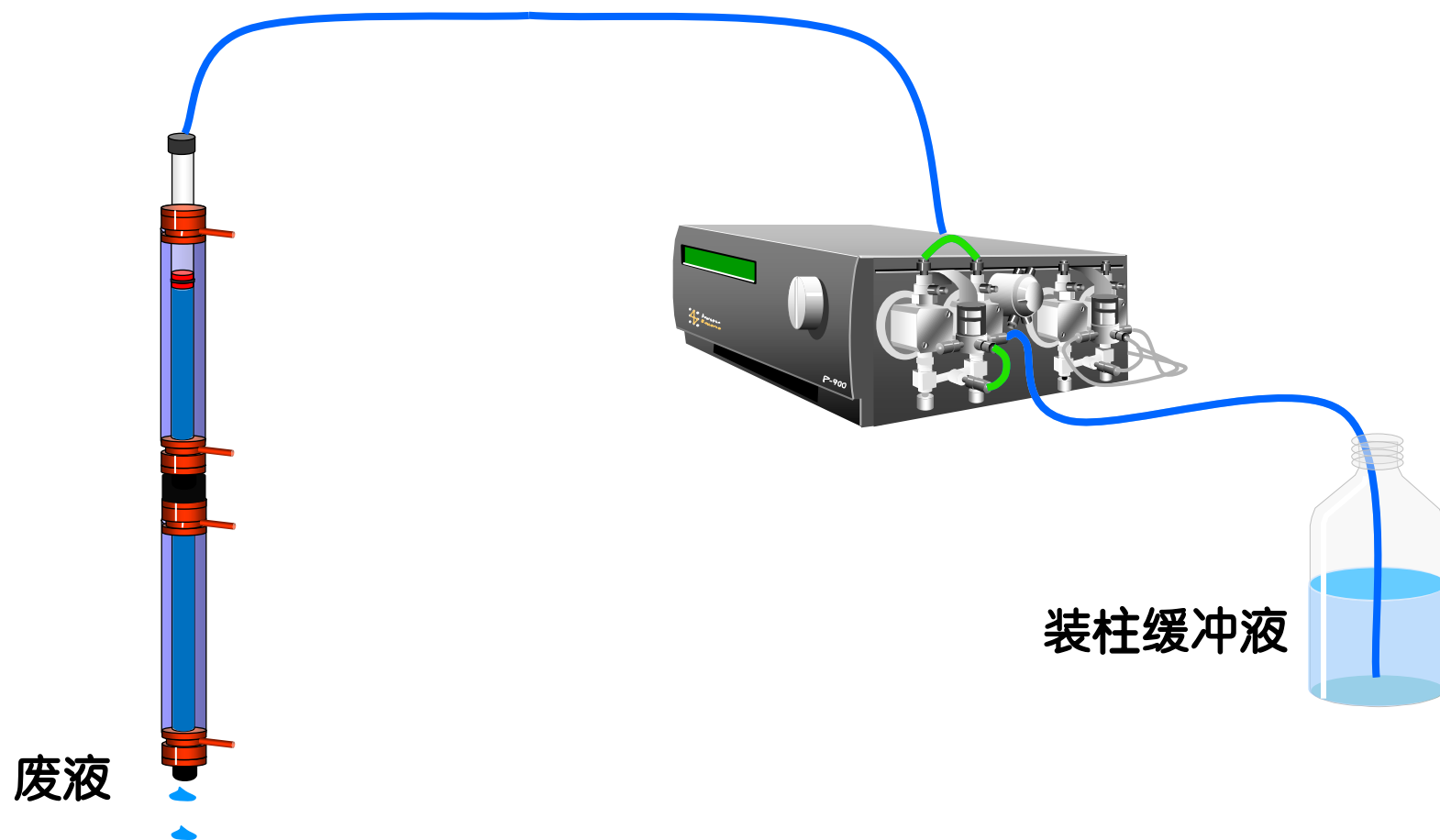


# 装柱流程3：匀浆，倒胶

- 保持层析柱垂直
- 快速，温和操作，避免气泡进入层析柱



# 装柱流程4：压胶



## 装柱流路图

# 压胶：恒流和恒压

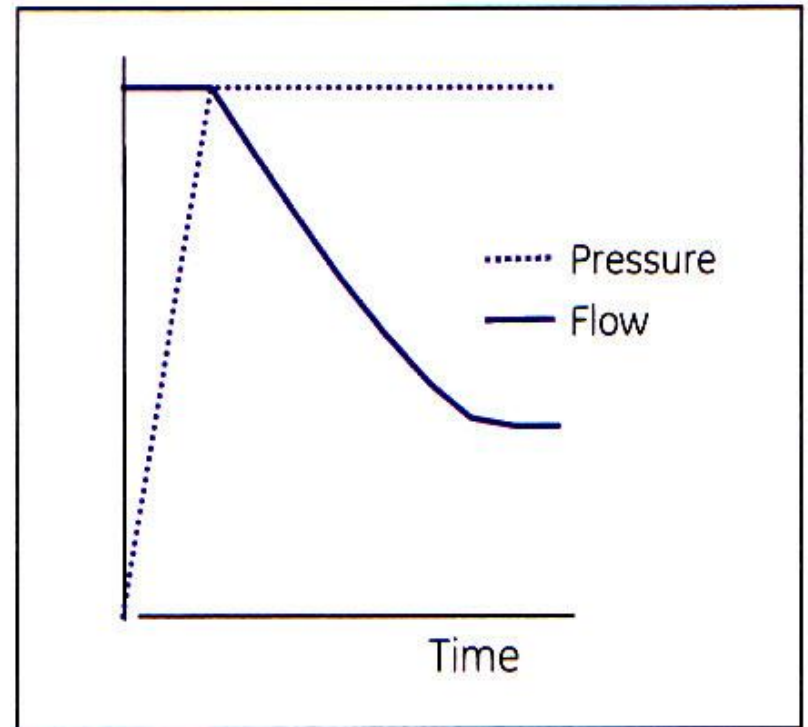
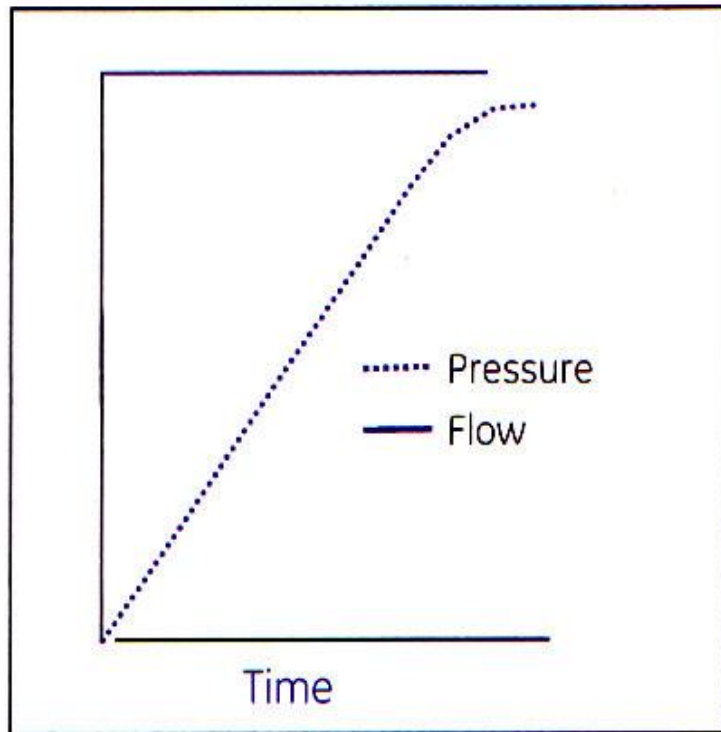
恒流:

The screenshot shows the 'system2 Pump Instructions' interface. On the left, under 'Instructions', the 'Pump' radio button is selected. A list of instruction types is shown, with 'Flow' selected. On the right, under 'Parameters', the 'FlowRate' is set to 2 ml/min, with a range of [0.00 - 100.00].

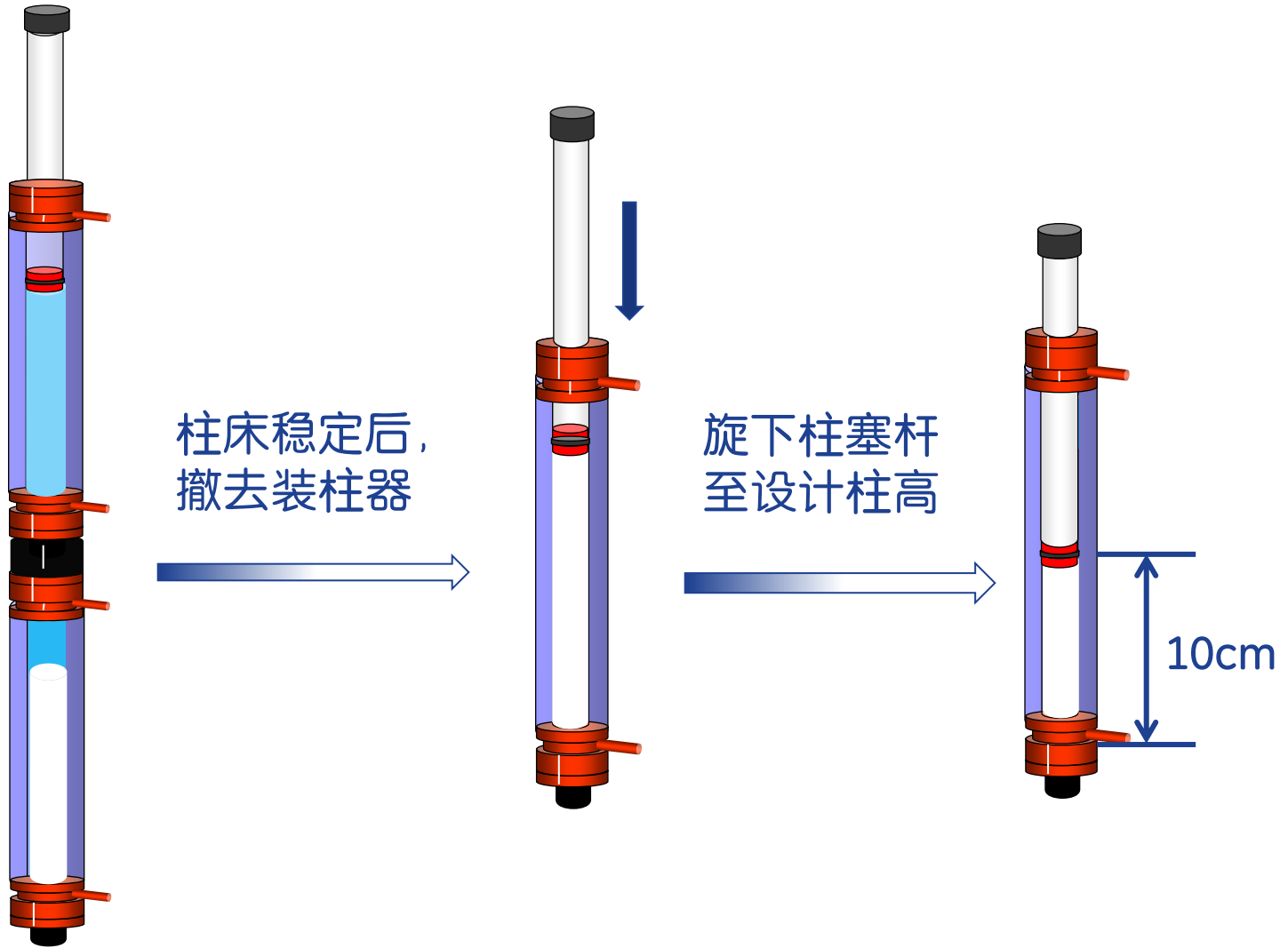
恒压:

The screenshot shows the 'system2 Pump Instructions' interface. On the left, under 'Instructions', the 'Pump' radio button is selected. A list of instruction types is shown, with 'SystemPumpControlMode' selected. On the right, under 'Parameters', the 'Mode' is set to 'PressFlowControl' (radio button selected). The 'PressLevel' is set to 0.3 MPa, with a range of [0.00 - 10.00]. The 'MinFlow' is set to 0.10 ml/min, with a range of [0.00 - 100.00].

# 恒压 VS 恒流



# 装柱流程5：完成





# 实验室装柱

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- 柱效测定

# 柱效测定

- 柱效高低是纯化成败的关键之一

- 柱效包括：

每米理论塔板数/理论塔板高度 (HETP)

对称因子 (As)

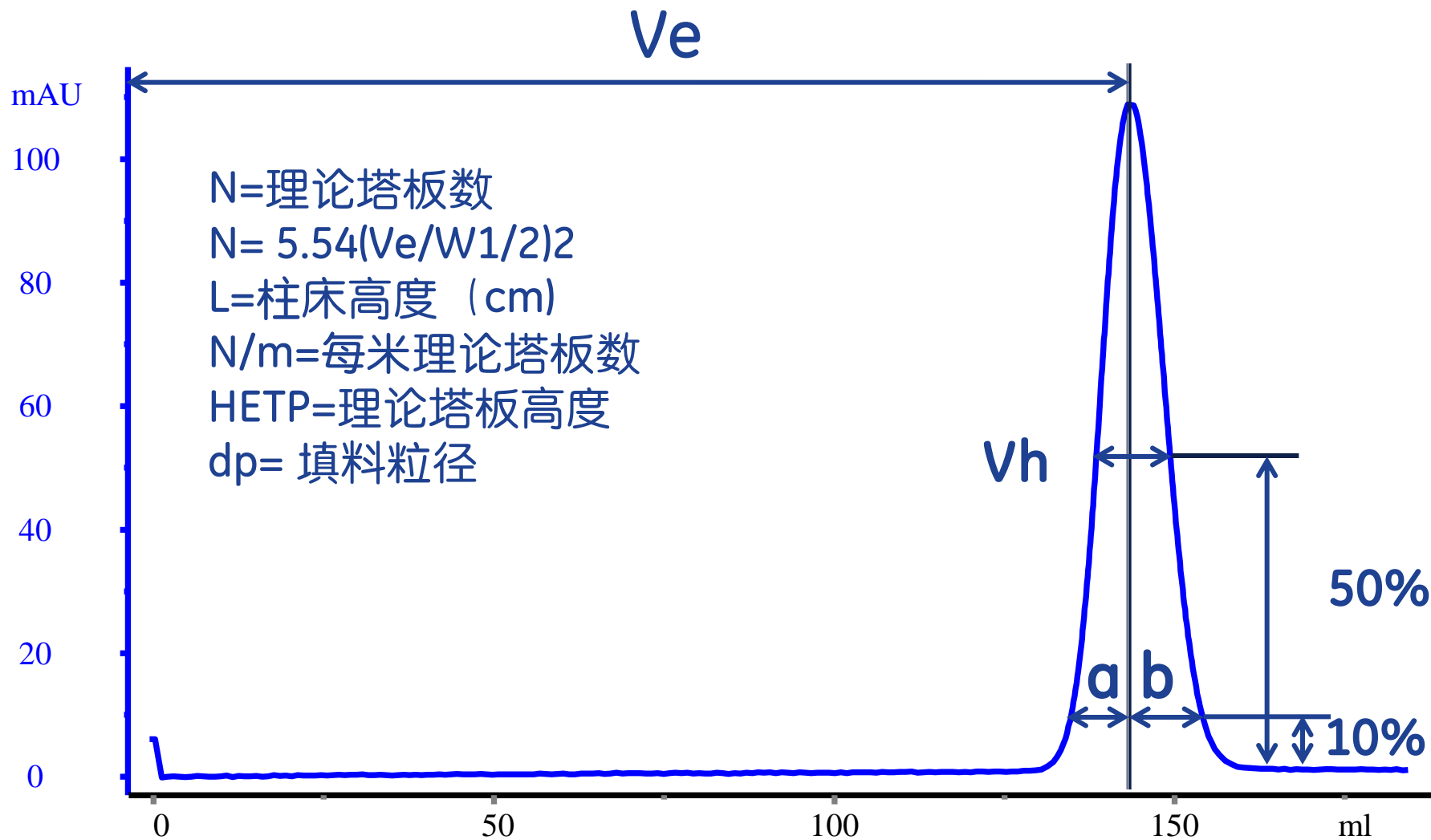
- 测柱效的方法：

流速 15-30 cm/h

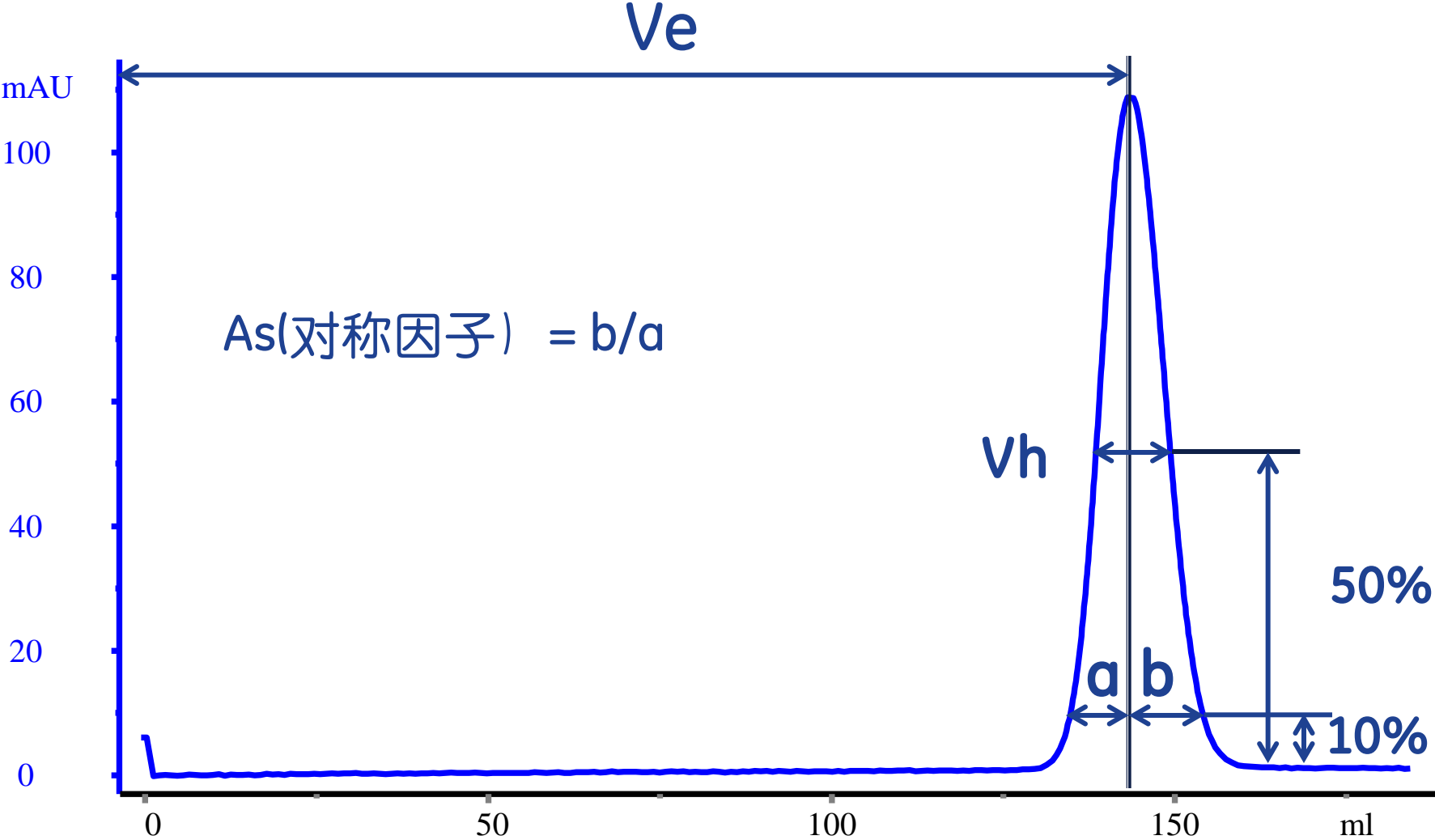
1%丙酮 (280nm 检测) 或 2M NaCl (电导检测)

上样体积1%-2%

# HETP 检测



# As检测



# Unicorn 软件计算HETP和As

Baseline: Calculate baseline   Accept negative peaks

Peak skim 10 ratio

Column height: 10 cm Column V 53 ml

**Chromatogram Layout: 10**

Curve Style and Color Edit Texts Layout Library

Header Curve Names Y-Axis X-Axis Curve Peak Table

Select table to display

- NONE
- Peaktable-A:
- Peaktable-B:
- Peaktable-C:
- Peaktable-D:
- Peaktable-E:
- Peaktable-F:
- Peaktable-G:
- Peaktable-H:

Select peak table columns

- Fraction tube id
- Baseline height
- Sigma
- Resolution
- Capacity factor
- K<sub>av</sub>
- Plate height (HETP)
- Asymmetry
- Conductivity height

# 结果评价

HETP (理论塔板高度)

理想情况

1-2 dp

非常好

2-4 dp

好

3-5 dp

差

5-10 dp

$A_s$  (对称因子)

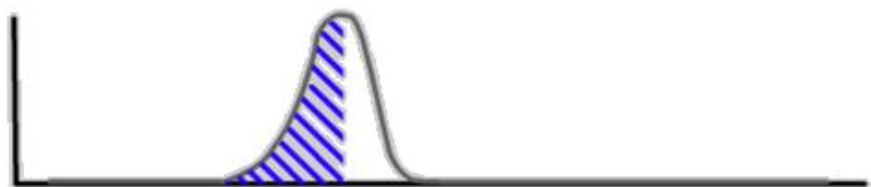
理想

1.0

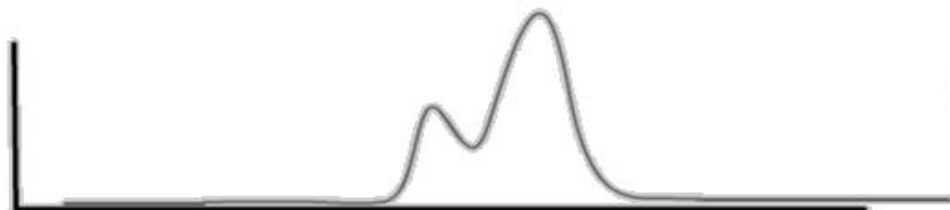
可接受范围

0.8-1.5

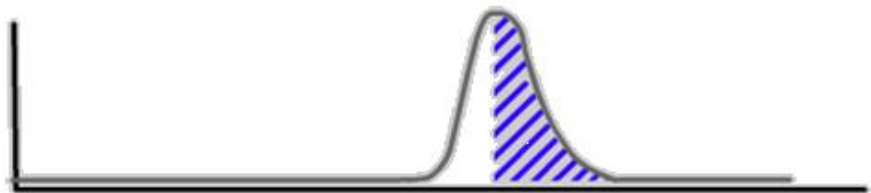
# 常见现象



Leading 前延, 太紧



Split 多峰, 装柱不均匀



Tailing 拖尾, 太松

# 装柱实验

填料	Capto Q
填料体积	20 ml
层析柱	XK 16/20
装柱缓冲液	脱气去离子水
柱效测定缓冲液	0.4 M NaCl
柱效测定溶液	1% 柱体积
柱效测定程序	HETP test

1. 清洗层析柱
2. 检查密封性
3. 筛网排气
4. 层析柱底部连接并垂直固定
5. 加入1 cm高的水
6. 连接柱头，排除筛网气泡
7. 混匀填料，玻璃棒引流导入
8. 16 ml/min，0.3 Mpa 报警压压柱
9. 待胶面不动时
10. 继续压胶 6 min，标记胶面
11. 暂停机器
12. 柱底部封口，松开入口
13. 下压柱头至胶面下约 2-3 mm
14. 重新连接柱子到 AKTA
15. 柱效测定



# Thank you very much for your attention

