近红外光谱仪之定性分析

——鱼粉掺假鉴别（三聚氰胺）

授课教师：贾　刚

授课对象：动科

授课时间：2008年

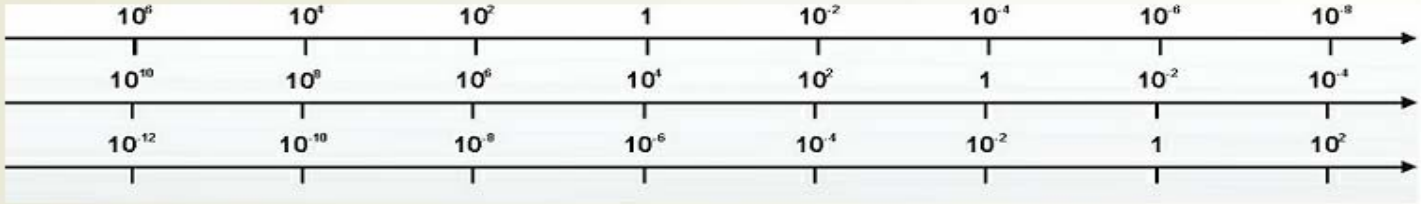
1. 实 验 目 的

- 掌握利用近红外光谱作定性分析的基本原理
  - 合格性测试
  - 聚类分析
  - 定性鉴定
- 掌握利用NIR进行定性鉴定的基本过程
  - 定性模型的建立、验证、维护、更新
  - 光谱扫描
  - 光谱分析
- 了解使用近红外光谱仪的主要操作步骤

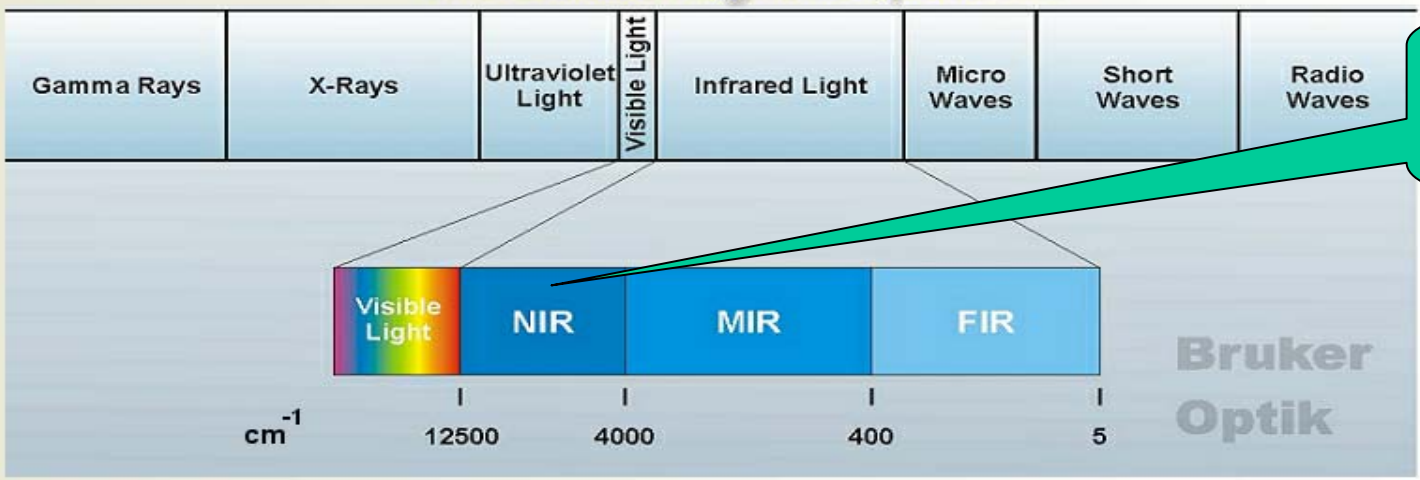
2. 基 本 原 理

BRUKER Optics

Introduction
Page 3



The electromagnetic spectrum



怎么产生？

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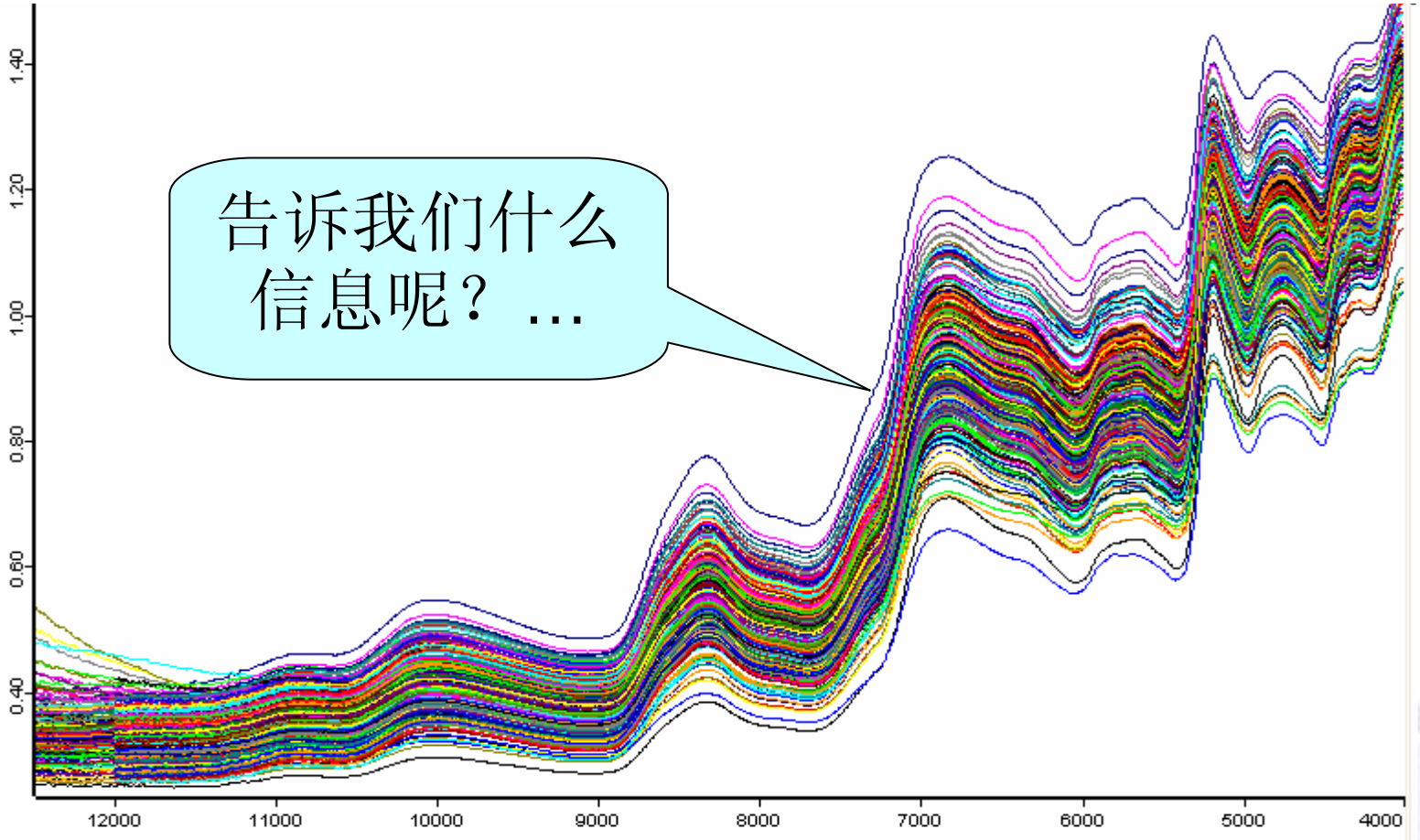
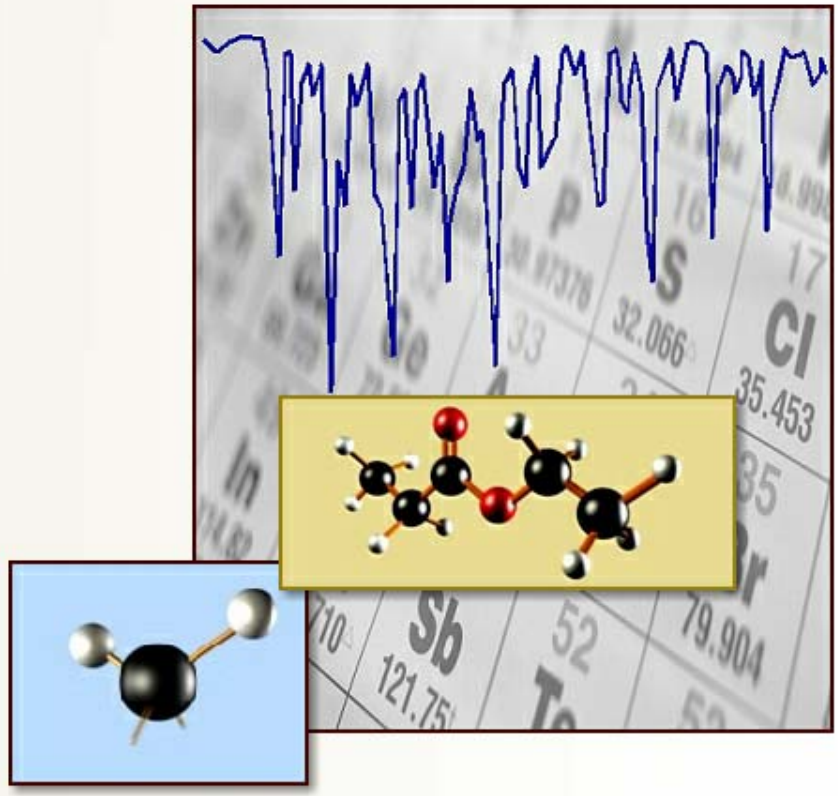
NIR吸收光谱


告诉我们什么
信息呢？…
1.40
1.20
1.00
0.80
0.60
0.40
12000
11000
10000
9000
8000
7000
6000
5000
4000

对光谱的分析——定性、定量

Evaluation of spectra

Infrared spectroscopy is an extremely efficient analytical method due to modest operating expenditure. The analytical results are provided within a short period of time without the need of extensive sample preparation. In particular, infrared spectroscopy provides data which can be evaluated by quantity as well as by quality. The following will describe the qualitative and quantitative evaluation of acquired spectra.



Qualitative evaluation of spectra

1. Identify an unknown substance
2. Check the identification of a known substance

Quantitative evaluation of spectra

Tutorial "Evaluation of Spectra"

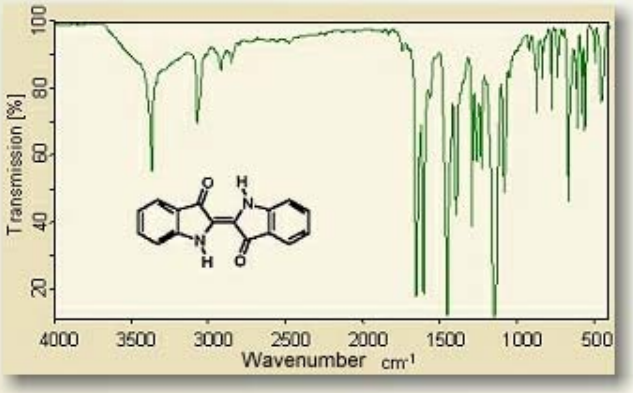
对光谱的分析——定性

Identify an unknown substance

a) Structural determination by interpreting spectra

A functional group within a molecule is considered as a harmonic oscillator (see vibration theory) which in a first approximation vibrates without being affected by the rest of the molecule. This results in the fact that a particular functional group shows IR absorption bands within characteristic spectral ranges: this is called group vibrations.

This fact serves as the basis for spectral interpretation, whereby the position, (relative) intensity and half-width of a band decide whether a band can be assigned to a specific structural group.



Many functional groups of organic molecules show characteristic vibrations corresponding to absorption bands within defined ranges of the IR spectrum. These molecular vibrations are mainly restricted to the functional group and do not affect the remaining molecule, i.e. such functional groups can be identified by their absorption band.

This circumstance, apart from a straightforward acquisition technique, makes IR spectroscopy to be one of the simplest, fastest and most reliable methods when assigning a substance to its specific class of compounds. The position and intensity of the absorption bands are extremely specific in the case of a pure substance. This enables the IR spectrum, similar to the human fingerprint, to be used as a highly characteristic feature for identification.

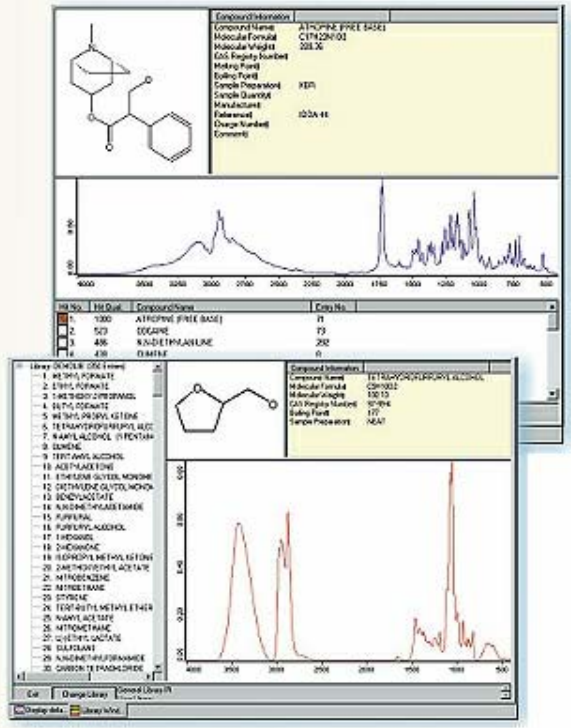
Tutorial "Evaluation of Spectra"

对光谱的分析——定性

Identify an unknown substance

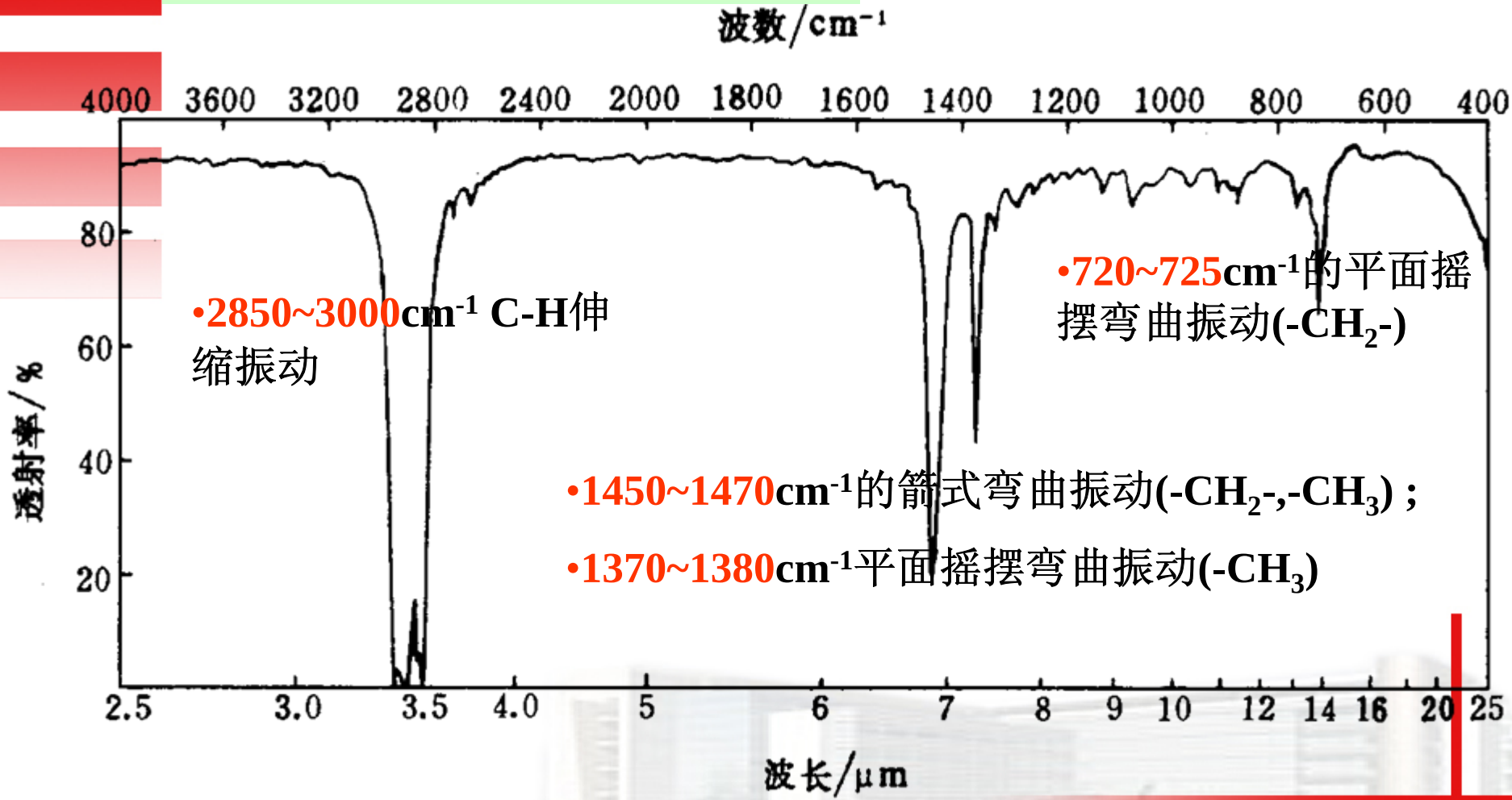
b.) Comparing with spectral libraries

Besides basic spectral interpretation, various comprehensive digital spectral libraries have been compiled according to different chemical classes and groups of substance. These are provided, for example, by companies like Bruker and Sadtler. Apart from working with existing spectral libraries, it is possible to create your own libraries using modern spectroscopic software,see OPUS/SEARCH. Different spectra regarding the number of bands and half-width, may require different search algorithms. Therefore, OPUS/SEARCH has the flexibility in providing various search options.



Tutorial "Evaluation of Spectra"

例：正辛烷的红外光谱



- 2850~3000cm^{-1} C-H伸缩振动
- 720~725cm^{-1}的平面摇摆弯曲振动(-CH_2-)
- 1450~1470cm^{-1}的箭式弯曲振动(-CH_2-,-CH_3)；
- 1370~1380cm^{-1}平面摇摆弯曲振动(-CH_3)

对光谱的分析——定性

Check the identity of a known substance

Infrared spectroscopy is a perfect analytical tool for quality control. It gives the answer to the following question: "D oes the quality of the raw material delivered to the receiving department comply with the specifications?" The underlying concept is very easy:

identical material = identical IR spectrum

The identification is done by comparing measured spectra with reference spectra already saved. The method is based upon the following considerations:

chemically different materials result in different spectra

real spectral differences exceed the reproducibility of repeated measurements

reference samples represent the expected sample variations caused by supplier, batch, season, purity, grain size etc.

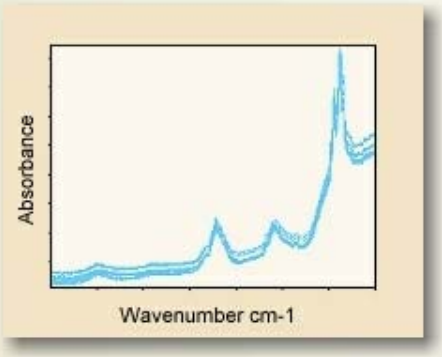
The same type of oil? Test it moving the mouse-pointer above the two samples...

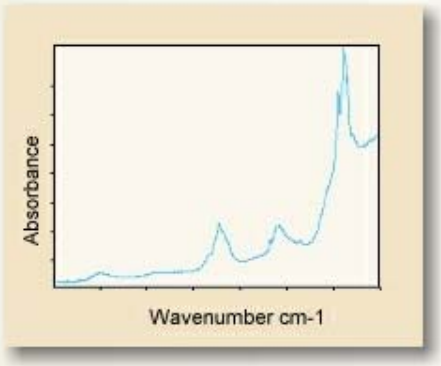
对光谱的分析——定性

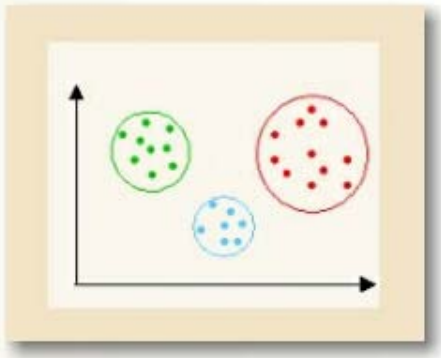
Reference library structure

It is important to note that the reference samples can vary to a certain degree, a circumstance that is experienced within quality control every day. The spectrum of the material to be identified is compared with the reference sample by means of a valid tolerance previously defined. How to create a reference library and to compare spectra will be described in the following.

1.) Measure reference sample → 2.) Calculate average spectrum & threshold values → 3.) Library structure & validation







对光谱的分析——定性

Identifying new samples

It is important to note that the reference samples can vary to a certain degree, a circumstance that is experienced within quality control every day. The spectrum of the material to be identified is compared with the reference sample by means of a valid tolerance previously defined. How to create a reference library and to compare spectra will be described in the following.

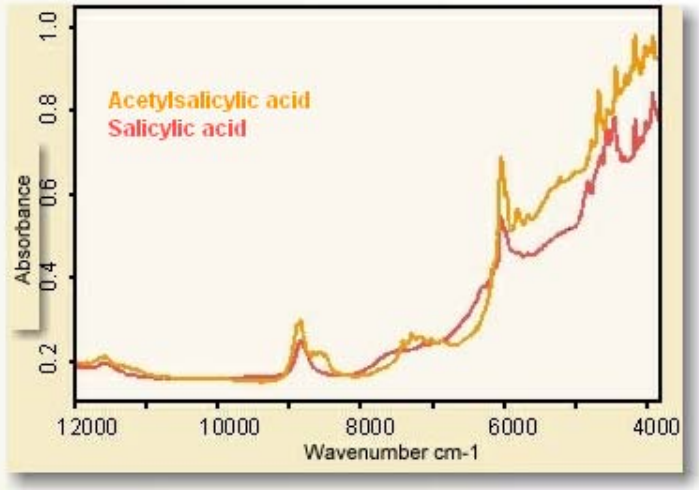
1.) Measure new samples → 2.) Compare with library → 3.) Identify material

对光谱的分析——定性

Comparing spectra

Basically, to compare one spectrum with a reference library, the spectral distance between two spectra is calculated, which also defines the similarity between these two spectra. There are several algorithms available to quantify the spectral distance. Depending on the substance (or spectrum) and considering your type of problem you have to select the most suitable comparison method. The following describes the calculation of the Euclidean distance and the comparison by factor analysis.

The Euclidean distance is the sum of all single differences when comparing two spectra point by point, i.e. the difference between two spectra is reduced to one single numerical value. The Euclidean distance can be used very well as the measured value when comparing spectra. However, the size of this numerical value is not standardized and has always to be considered as a relative value. Generally, this method can be used for all kinds of spectra



1.) Euclidean distance

Comparing spectra " point by point" :

$$D = \sqrt{\sum_i \left(A_{\text{Sample}}(\lambda_i) - A_{\text{Reference}}(\lambda_i)\right)^2}$$

A(λi): Absorbance value at wavelength λi
1,2....i: Data points within the selected spectral range

对光谱的分析——定性

Comparing spectra

2.) Factor analysis

Factor analysis is a variance analysis which is widely used as a general statistical method to analyze data. It is based on the search of differences (variances) within the reference data record. Factor analysis is also called principal component analysis, PCA. The main features comprise:

orthogonal data transformation

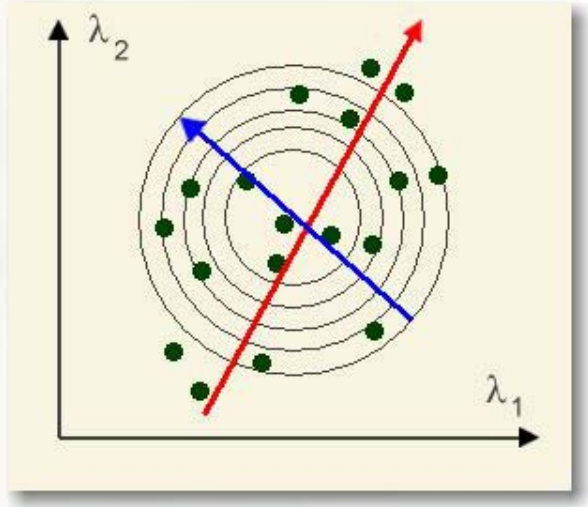
substantial data compression: represents the data

record by only a few latent variables

Advantages of factor analysis:

data compression

reduces considerably noise components



Factor analysis
1. The factor loading collects the largest part of the variance in the data record
2.) The factor loading collects the largest part of the remaining variance

and so on ...

对光谱的分析——定量

Quantitative evaluation of spectra

The basic principle for quantitative evaluation in optical spectroscopy as well as in IR spectroscopy is the Bouguer-Lambert-Beer Law which had already been defined in 1852. Quantitative determinations by means of IR spectroscopy are preferably performed in solution. Transmission T of a sample is defined as:

$$T = I / I_0$$

I_0 is the intensity of the incident light beam, I is the intensity of the light beam leaving the sample.

The percentage transmission (%T) is 100 • T.

When traversing the measurement cell, the light intensity decreases exponentially:

$$I = I_0 \bullet \exp(-\varepsilon \bullet c \bullet b)$$

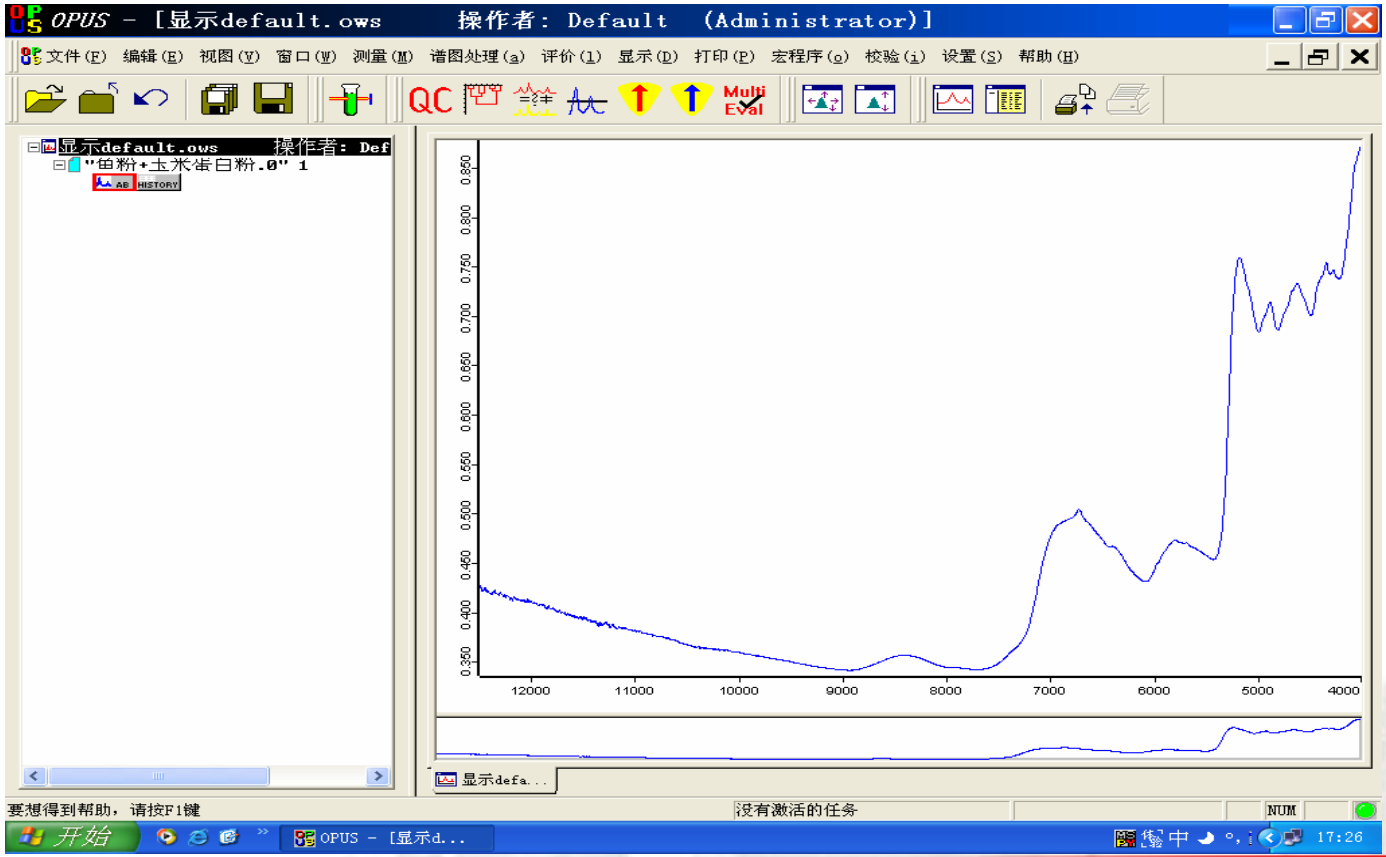
Where ε is the molar absorption coefficient (in L mol-1 cm-1), c is the sample concentration (in mol L-1) and b the thickness of the measurement cell (in cm). The absorption coefficient ε is a value which depends on either the wavelength or the wavenumber, which is typical for the compound analyzed. From the equation above, it follows that:

$$\log (I / I_0) = -\varepsilon \bullet c \bullet b \qquad A = \log (I_0 / I) = \varepsilon \bullet c \bullet b$$

where A is the absorbance. Because of the Bouguer-Lambert-Beer Law, the relationship between absorbance and concentration of the absorbing substance is a linear function.

3. 实验结果及光谱分析

——鱼粉样品的NIR吸收光谱



3.定性分析示例

- 3.1 合格性测试：
  - 待测样品的光谱必须每个波长都在参比样品的*置信范围*(CI)内。
  - $CI = (A_{reference,i} - A_{sample,i}) / \delta_{reference,I}$
  - 合格性测试主要用于特定产品的质量控制

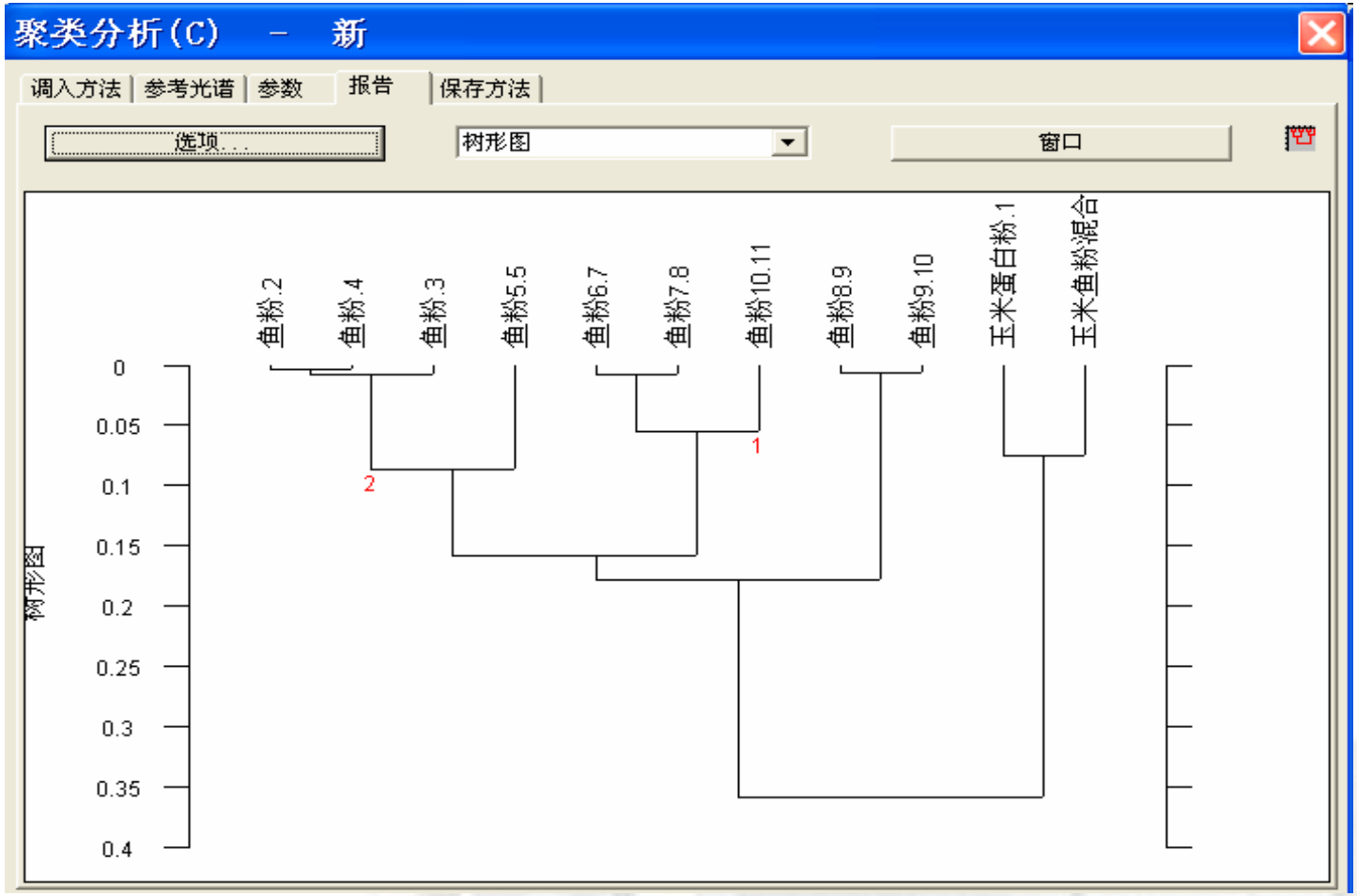
3.1 合格性测试结果示意

合格性测试报告	值
方法文件	1.cft - 2007/06/29 10:03:28 (GMT+8)
合格性测试模式	CI 范围
合格性索引范围	3.0
总和范围	0.50
使用签名的 CI 值	No

合格性测试	代码	最大 CI 值	在频率	标准偏差	和 1	和 2	数据点 > CI 范围
失败	0	17.55	4802.08	1.57E-003	0.559	2.25	341

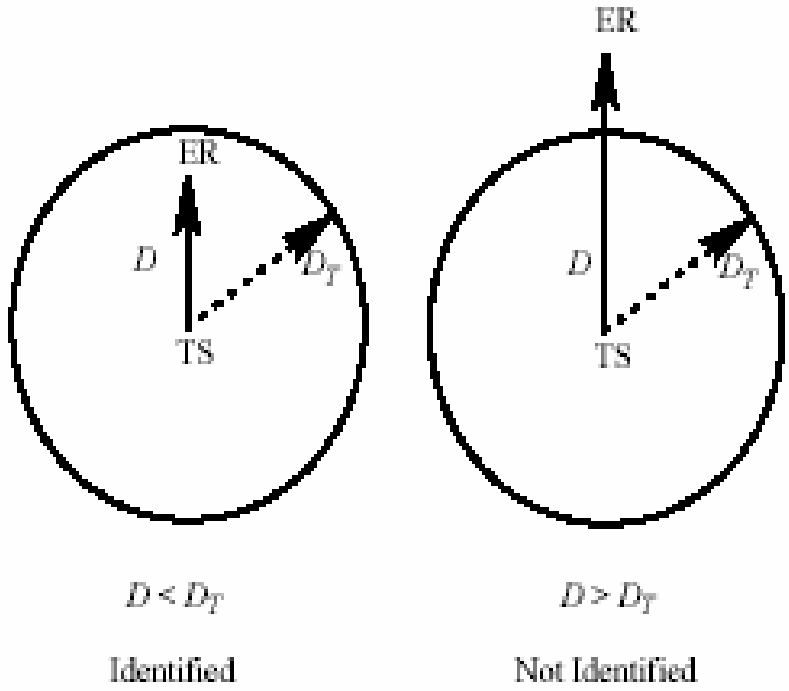
- 3.2 聚类分析
- 利用标准算法、因子法、第一范围标定法、重现水平归一化法等计算光谱距离
- 标准算法：$$D = \sqrt{\sum_{k} (a(k) - b(k))^2}$$
- 光谱的距离表明了谱图的相似度，两张谱图的光谱距离为零则表明它们是一样的；

3.2 聚类分析结果示意


聚类分析(C) - 新
调入方法
参考光谱
参数
报告
保存方法
选项...
树形图
窗口
鱼粉.2
鱼粉.4
鱼粉.3
鱼粉5.5
鱼粉6.7
鱼粉7.8
鱼粉10.11
鱼粉8.9
鱼粉9.10
玉米蛋白粉.1
玉米鱼粉混合
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
树形图
1
2

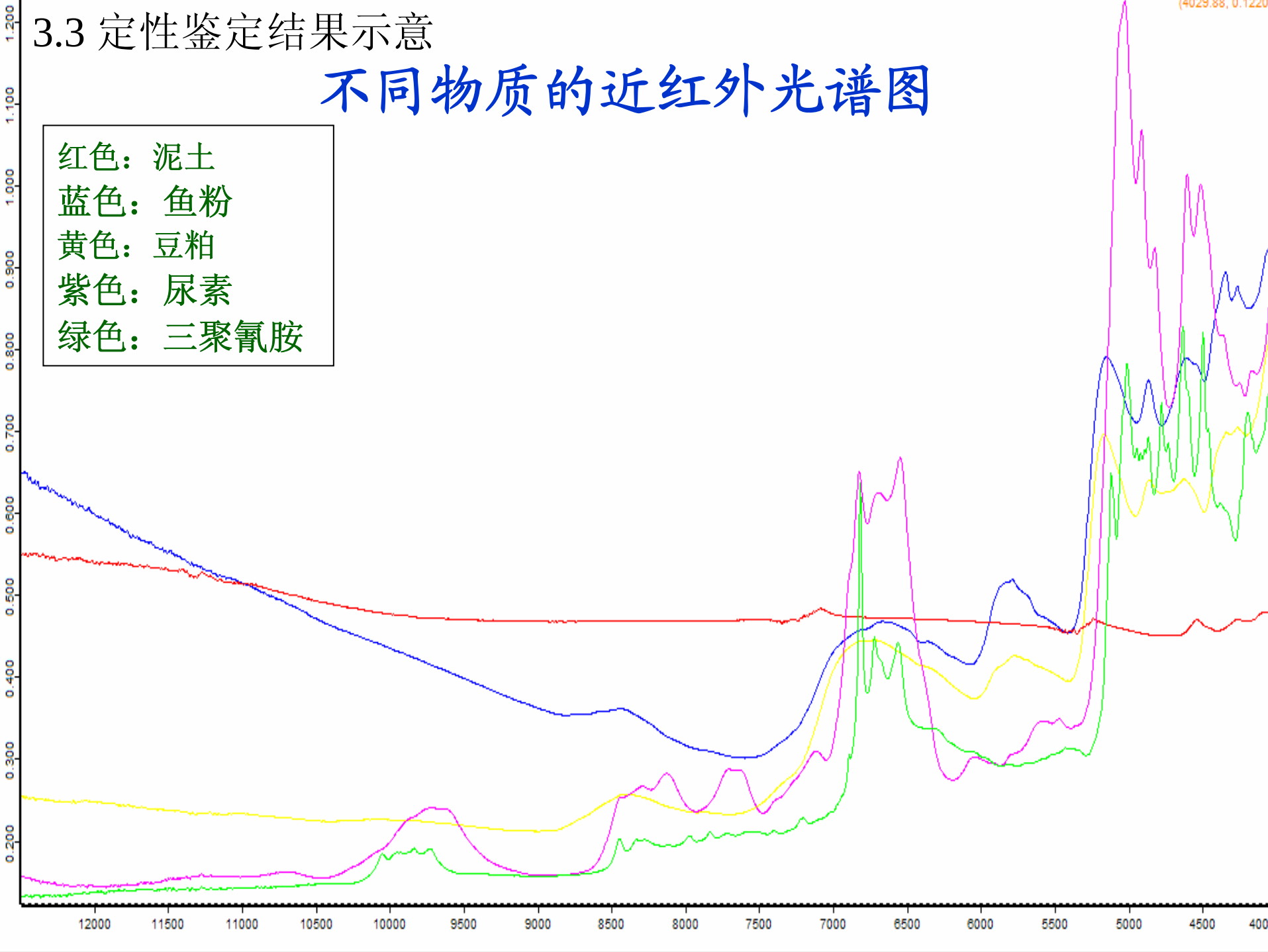
- 3.3 定性鉴定：
- 待测样品的光谱与参比标准样品的光谱的阈值的关系
- 阈值：对库中每一条平均参考光谱所定义的一个欧氏距离限度。例如：

$$D_t = D_{max} + \frac{S_0}{4}$$

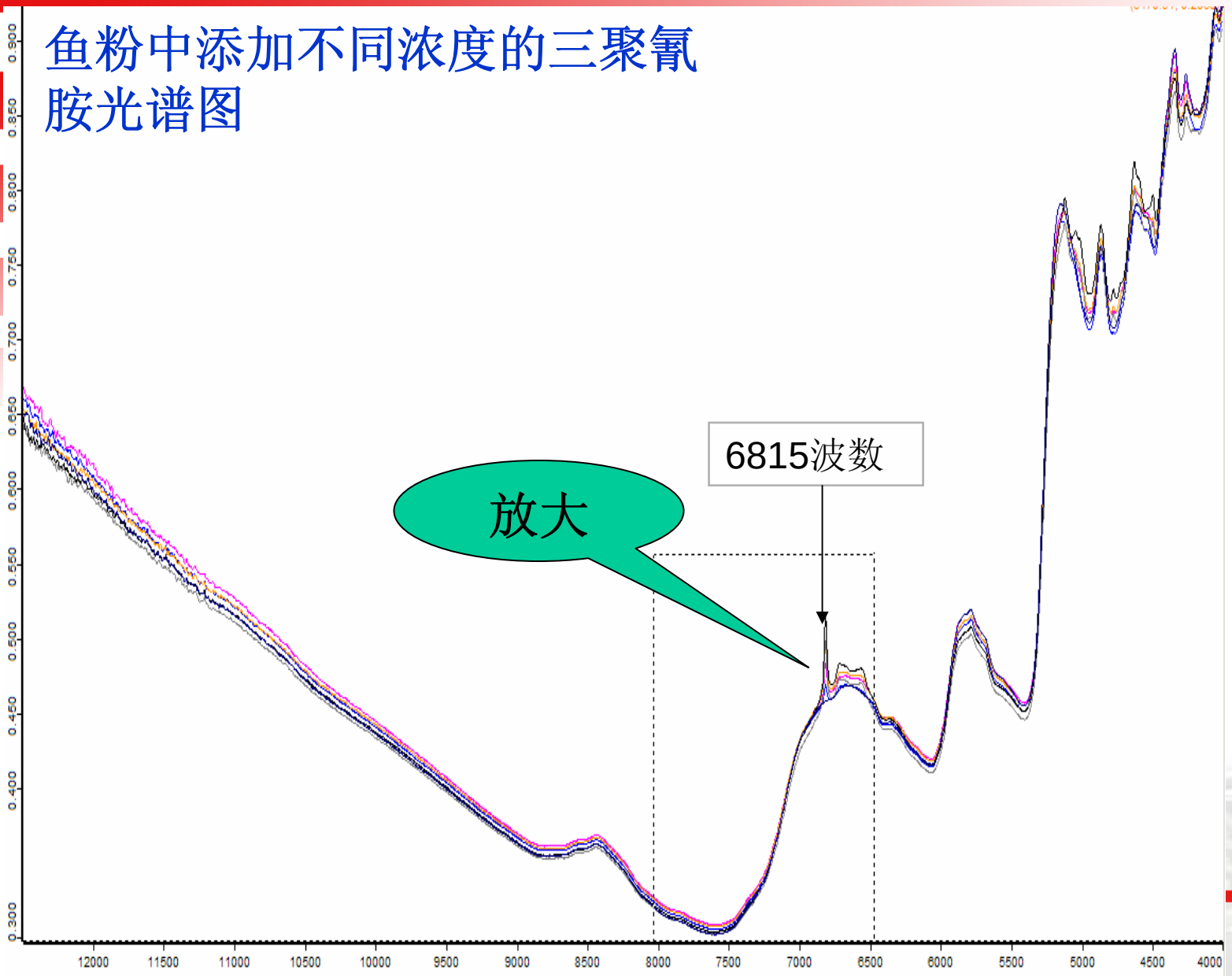


3.3 定性鉴定结果示意

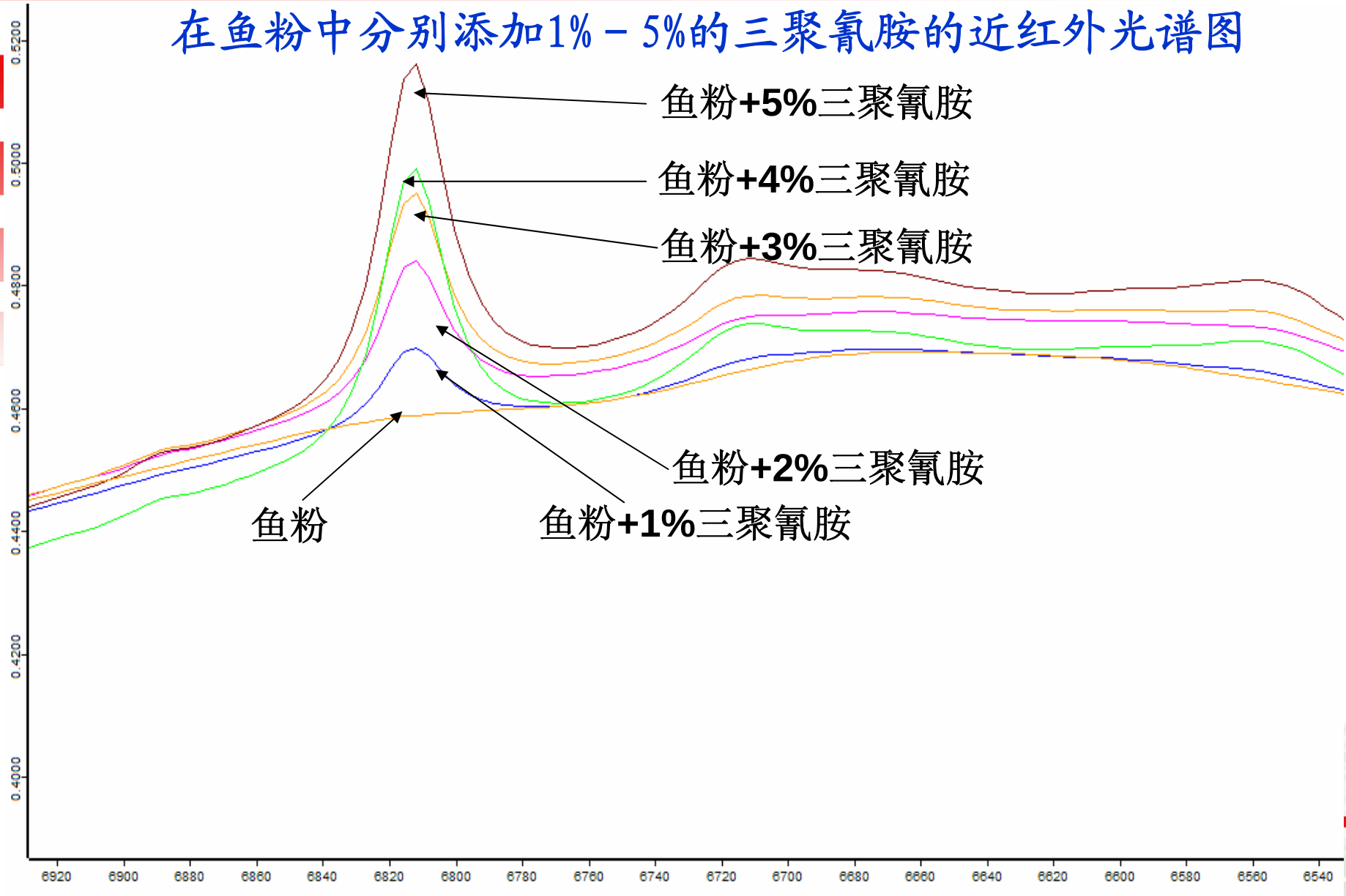
不同物质的近红外光谱图

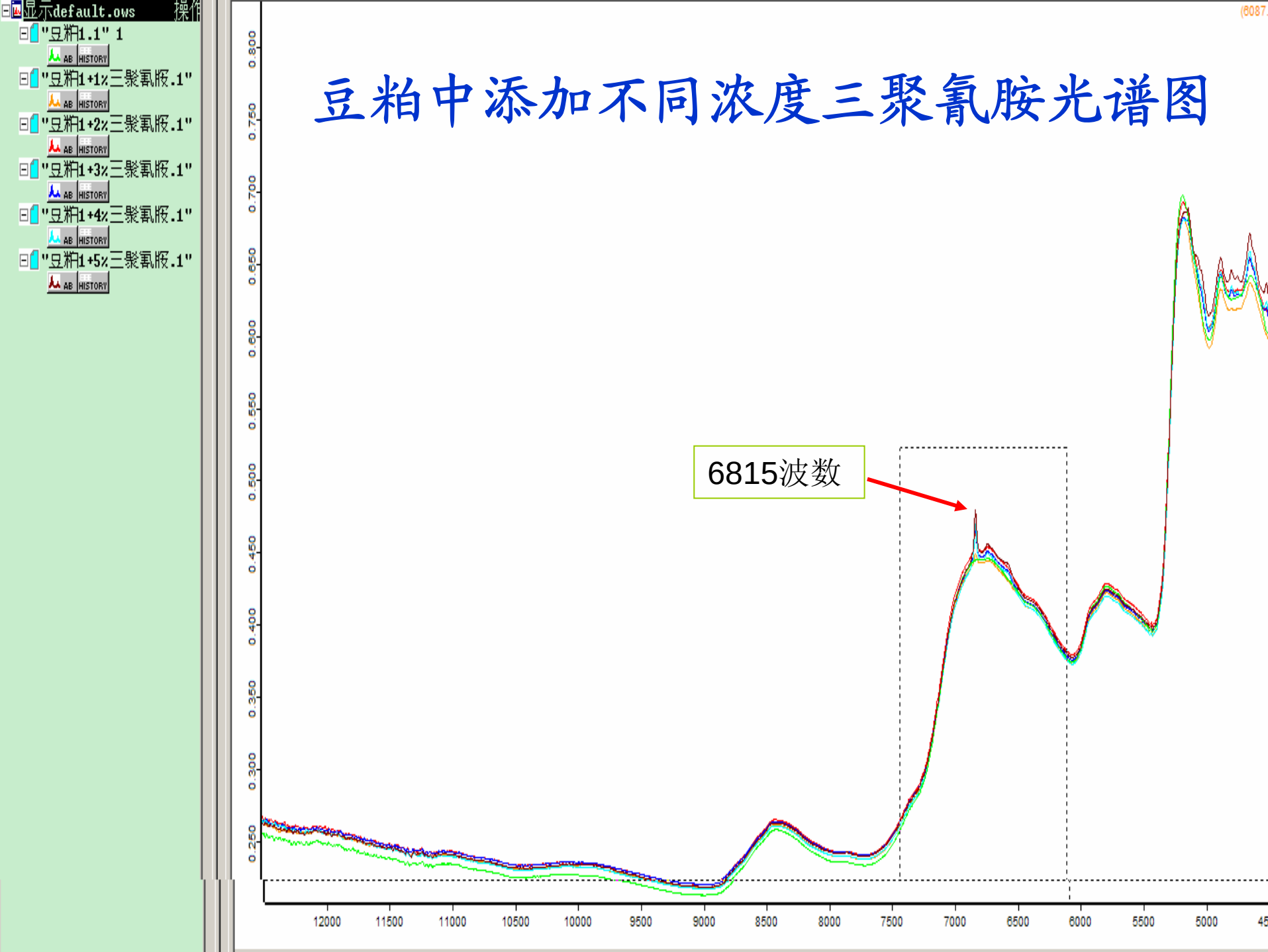


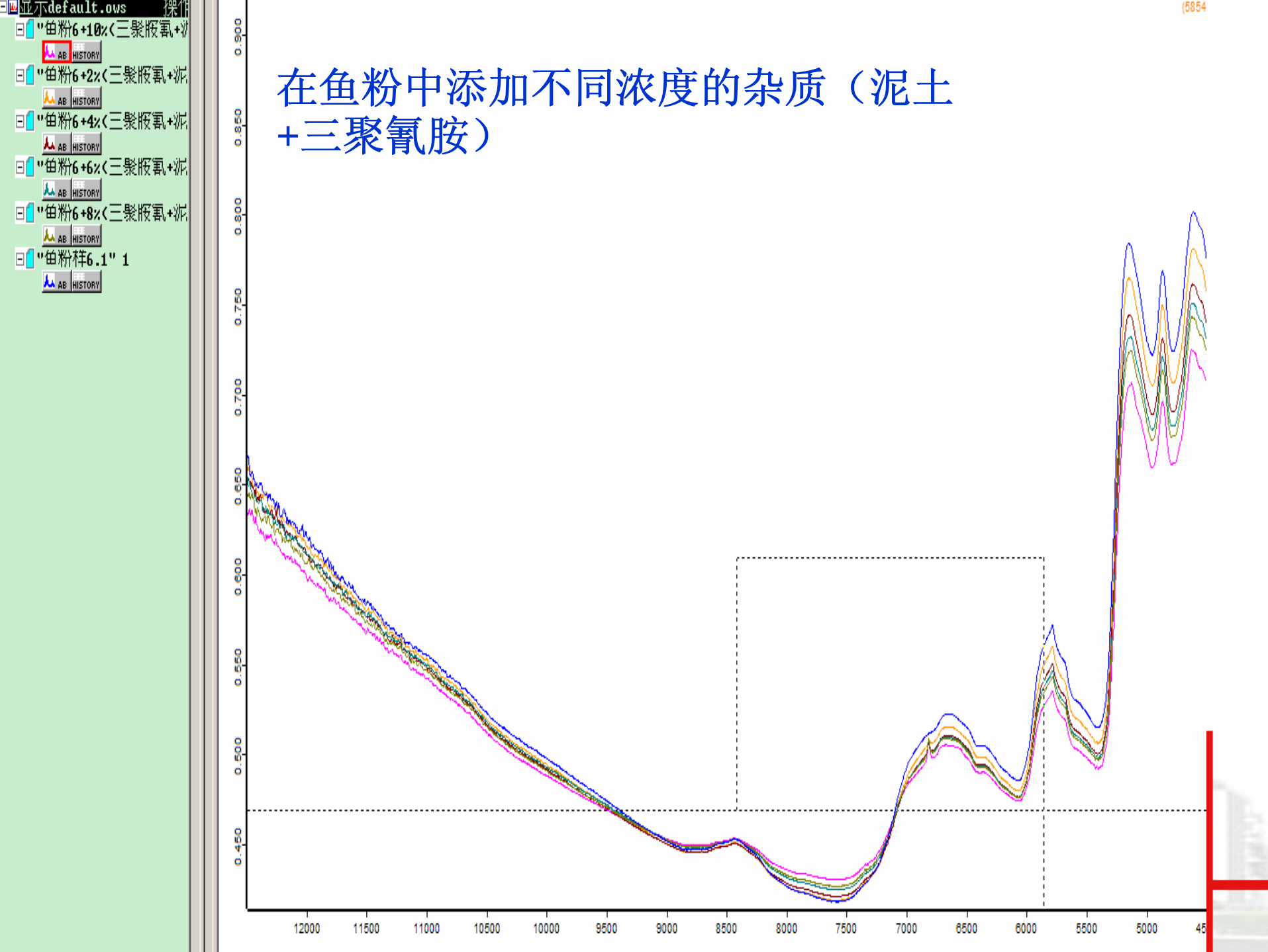
鱼粉中添加不同浓度的三聚氰胺光谱图

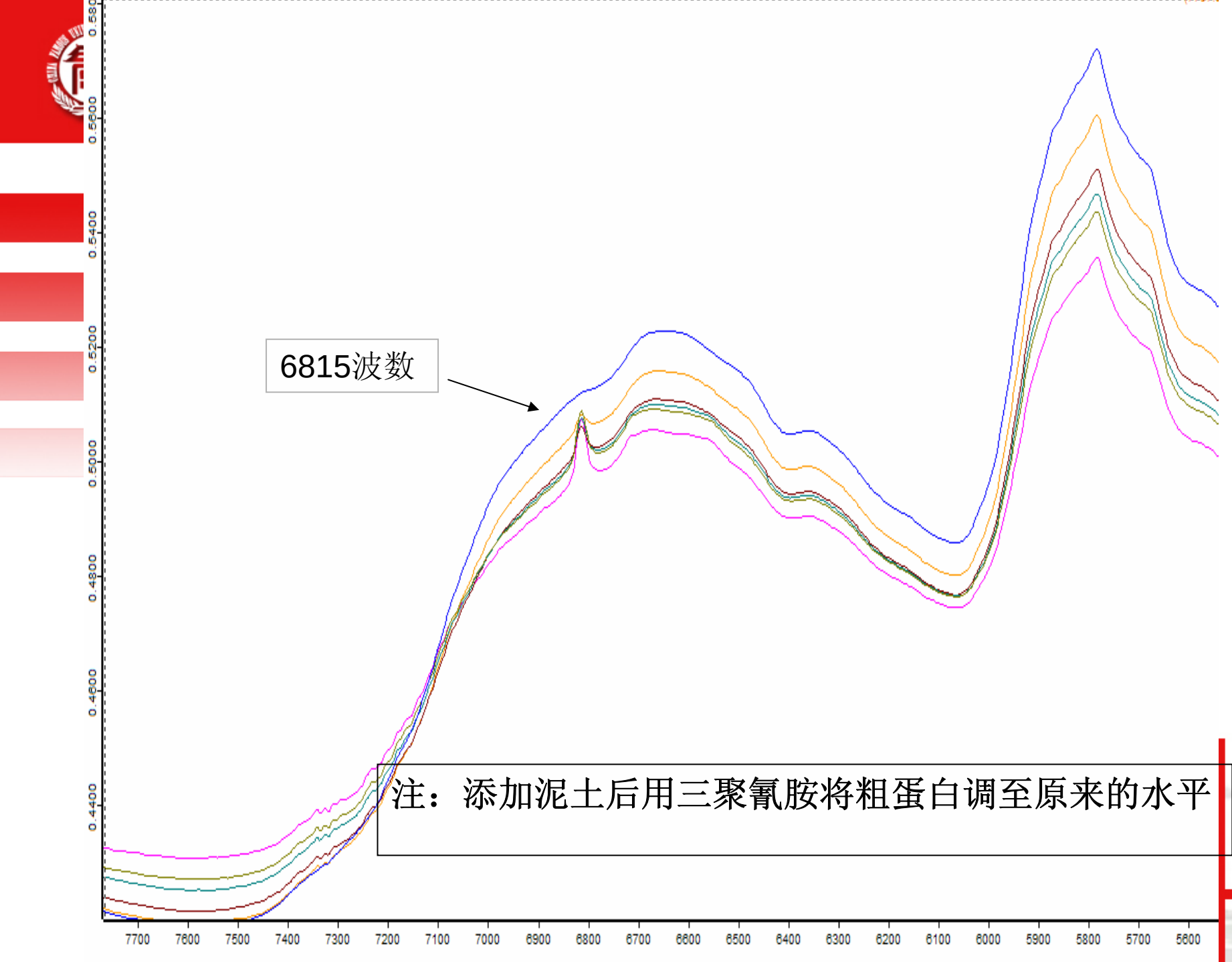


在鱼粉中分别添加1%－5%的三聚氰胺的近红外光谱图




显示default.ows
"豆粕1.1" 1
"豆粕1+1%三聚氰胺.1"
"豆粕1+2%三聚氰胺.1"
"豆粕1+3%三聚氰胺.1"
"豆粕1+4%三聚氰胺.1"
"豆粕1+5%三聚氰胺.1"
豆粕中添加不同浓度三聚氰胺光谱图
6815波数
0.800
0.750
0.700
0.650
0.600
0.550
0.500
0.450
0.400
0.350
0.300
0.250
12000
11500
11000
10500
10000
9500
9000
8500
8000
7500
7000
6500
6000
5500
5000


显示default.ows
"鱼粉6+10%(三聚胺氰+
"鱼粉6+2%(三聚胺氰+泥
"鱼粉6+4%(三聚胺氰+泥
"鱼粉6+6%(三聚胺氰+泥
"鱼粉6+8%(三聚胺氰+泥
"鱼粉样6.1" 1
HISTORY
在鱼粉中添加不同浓度的杂质（泥土+三聚氰胺）
0.900
0.850
0.800
0.750
0.700
0.650
0.600
0.550
0.500
0.450
12000
11500
11000
10500
10000
9500
9000
8500
8000
7500
7000
6500
6000
5500
5000


6815波数
注：添加泥土后用三聚氰胺将粗蛋白调至原来的水平
0.5800
0.5600
0.5400
0.5200
0.5000
0.4800
0.4600
0.4400
7700
7600
7500
7400
7300
7200
7100
7000
6900
6800
6700
6600
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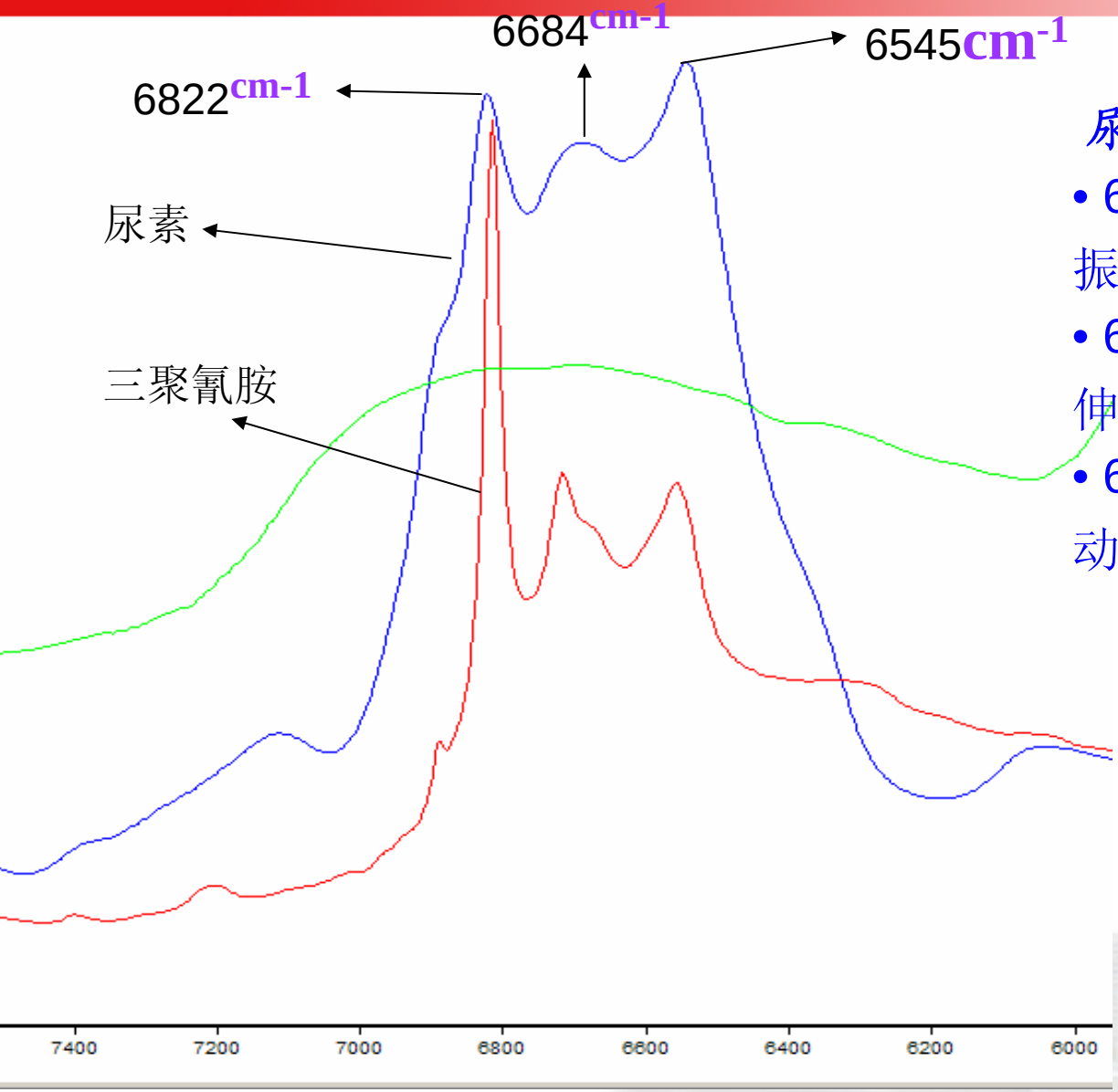
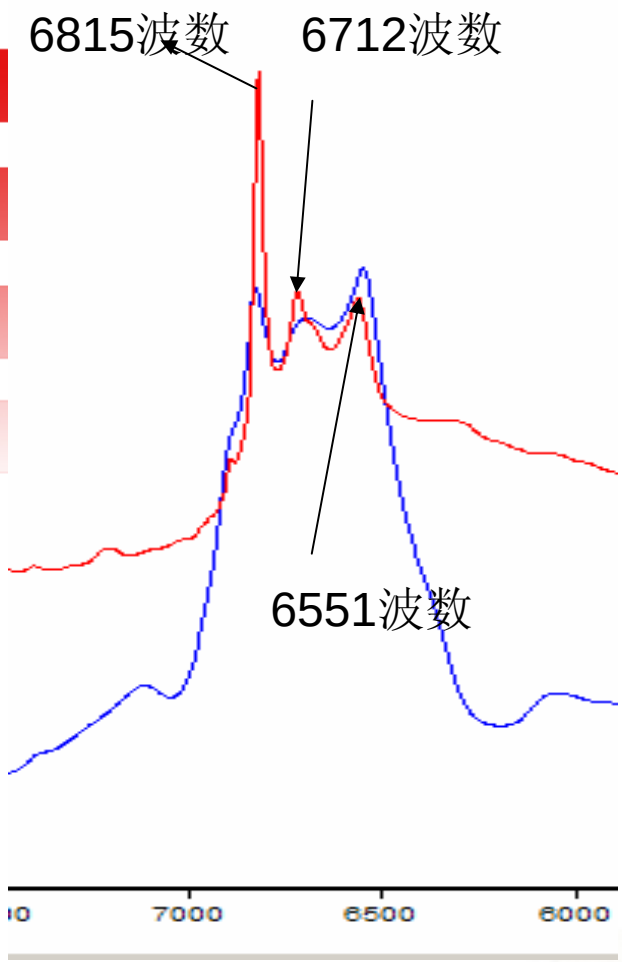
6300
6200
6100
6000
5900
5800
5700
5600



尿素的基准振动、倍频及合频

- 6803cm^{-1}是NH的非对称伸缩振动的第一倍频；
- 6667cm^{-1}是NH的对称-非对称伸缩振动的组合频；
- 6536cm^{-1}是NH的对称伸缩振动的第一倍频



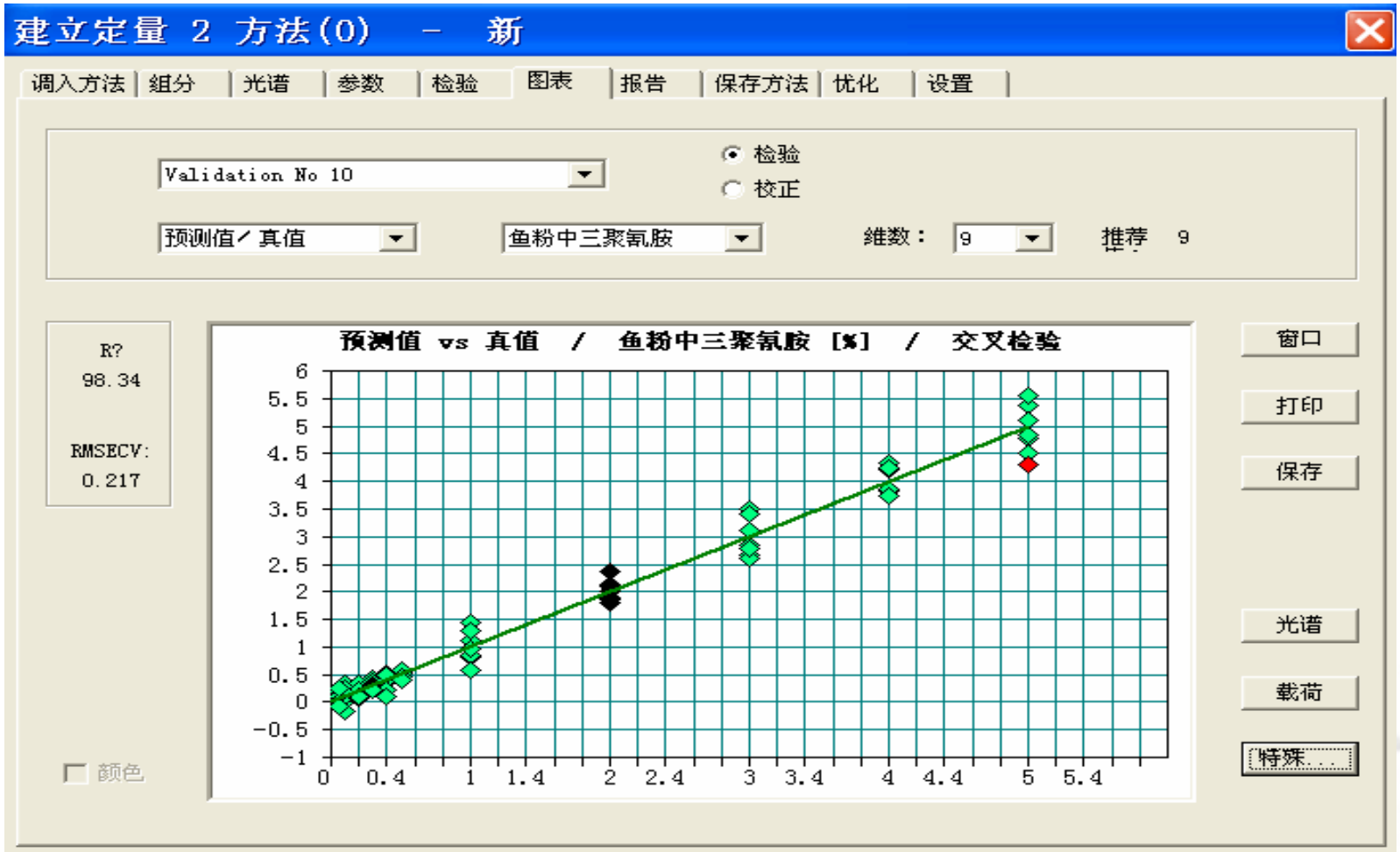
将三聚氰胺光谱向上平衡，在此波段，三聚胺的三个吸收波段与尿素的吸收波段非常接近。

6815波数是**NH**的非对称伸缩振动的第一倍频；
6712波数是**NH**的对称-非对称伸缩振动的组合频；
6551波数是**NH**的对称伸缩振动的第一倍频

定性鉴定结果


显示default.ows:1
"鱼粉样本1+3%三
AB REPORT HISTORY
评价结果 - 显示 -
"鱼粉样本1+3%三
AB REPORT HISTORY
定性评价结果:
样品名称: sample
样品: E:\刘小莉-黄兰\鱼粉+三聚氰胺\鱼粉样本1+3%三聚氰胺.1
日期与时间: 25/11/2008 04:34:19.050 (GMT+1)
方法文件: D:\饲料模型\鱼粉的定性鉴定模型\定性\鱼粉+三聚氰胺\鱼粉+三聚氰胺全部.FAA
匹配数 样品名称 匹配值 阈值 组
1 sample 0.00031 0.94709 三聚氰胺+鱼粉
2 sample 1.41452 0.25450 真鱼粉
IDENTIFIED AS 三聚氰胺+鱼粉
OK

定量分析模型


建立定量 2 方法(0) - 新
调入方法
组分
光谱
参数
检验
图表
报告
保存方法
优化
设置
Validation No 10
检验
校正
预测值/真值
鱼粉中三聚氰胺
维数：
9
推荐 9
R?
98.34
RMSECV:
0.217
预测值 vs 真值 / 鱼粉中三聚氰胺 [%] / 交叉检验
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
0
0.4
1
1.4
2
2.4
3
3.4
4
4.4
5
5.4
颜色
窗口
打印
保存
光谱
载荷
特殊...

结论：

①近红外光谱中6810~6550cm^{-1}处的吸收峰可能是三聚氰胺的吸收峰，而6815cm^{-1}可能是特征吸收峰。

②可以用近红外仪器来鉴别饲料原料是否掺假，并作定量分析。

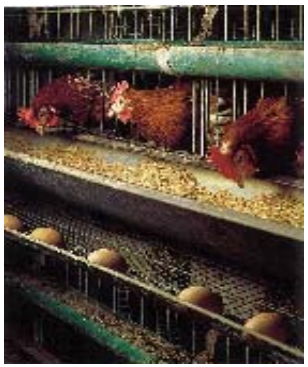
4. MPA™ – 多用途近红外光谱分析仪的使用

完全配置的MPA

- 几乎集成所有采样技术
  - 光纤探头
  - 积分球
  - 液体透射
  - 固体透射
  - 自动进样盘
  - 样品旋转器
- 全部智能控制
- 完全满足GLP、ISO、FDA技术规范

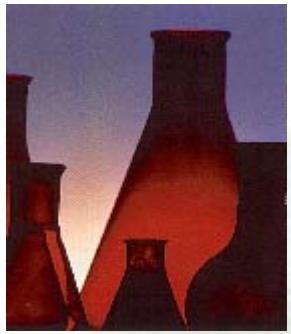
广阔的应用领域

食品与饲料

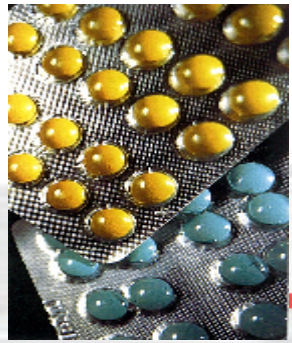



石化与高分子






化妆品与药物

造纸




4. 仪器操作步骤

- 4.1 开机、联机
- 4.2 自检及自检参数、报告
- 4.3 扫描参数的设定
- 4.4 光谱扫描及光谱分析
- 4.5 分析报告
- 4.6 关机
- 4.7 注意事项及日常维护

5. 实验总结及问题

- 5.1 鉴定结论：
  - 掺假鱼粉，掺入物为玉米蛋白粉
- 5.2 思考
  - 模型的建立与维护…
  - 影响分析的因素有哪些？
  - 会误判吗？为什么？
  - …