

Potential Uses of NIR and IR Spectroscopy in Quality Control of Allergy Vaccine Production

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Summary

Background: Compared to conventional methods, infrared spectroscopy offers many potential uses in quality control of vaccine production. A major advantage is that the technique is non-invasive and non-destructive. In this study several possible uses of infrared spectroscopy in the control of allergy vaccines were investigated.

Methods: Near infrared spectroscopy (NIR) was applied to determine the aluminium content in aluminium hydroxide formulations, the particle size of aluminium hydroxide, the protein adsorption level on aluminium adjuvant and the moisture content of lyophilized vaccines. Mid infrared spectroscopy (IR) was used to investigate protein structural changes during adsorption and release from aluminium hydroxide and the stability of the complex.

Results: Based on the calibration models of NIR in different applications, it was demonstrated that NIR had great advantages for application in vaccine production. IR also showed advantages on structural analysis of adsorbed proteins.

Discussion: Infrared spectroscopy as a physical, non-invasive, non-destructive method should receive more attention in vaccine research and production.

Introduction

Many vaccines are produced by adsorption of antigens onto aluminium hydroxide adjuvants. Some parameters are of great importance in vaccine production, such as aluminium content in aluminium hydroxide formulations, the particle size of aluminium hydroxide, protein concentrations adsorbed onto aluminium adjuvant, protein structural changes due to adsorption onto or release from aluminium adjuvant, as well as moisture content of lyophilized vaccines. However, the conventional meth-

ods for the assessment of these parameters are expensive, time-consuming, sample destructive and may require environment-polluting reagents.

NIR (780– 2500 nm) and IR (2500– 25000 nm) offer many advantages over traditional methods. Infrared spectroscopy is a physical, non-invasive, non-destructive method. The analyses are typically rapid and the spectra provide information on both, physical and chemical properties, such as sample identity, concentration, particle size, hardness or even taste. Moreover, infrared spectroscopy is easy and safe to use.

Results and discussion

1. Determination of aluminium content in aluminium hydroxide formulations by FT-NIR transmittance spectroscopy [1]

One of the most common methods used for aluminium determination is inductively coupled plasma-atomic emission spectroscopy (ICP-AES). This method is a trace and ultra-trace multi-elemental analysis which requires extensive dilution of aluminium hydroxide. In this study, near infrared spectroscopy was investigated to determine the aluminium content. The major variations, e.g. sample containers, sample sedimentation, day-to-day variation, and batch-to-batch variation, etc., which may influence the NIR spectra, were studied. Two calibration models which covered the major variations were constructed. The precision and accuracy of the NIR methods were evaluated by comparison to ICP-AES data.

A comprehensive variation study (data not shown) demonstrated that the day-to-day variation, which was mainly caused by temperature variation, and the batch-to-batch variation were the major variations. Sample sedimentation, sample preparation and cuvette influences were minor variations that could be ignored. A 1-cm cuvette was chosen as sample container. NIR spectra of seven different aluminium concentra-

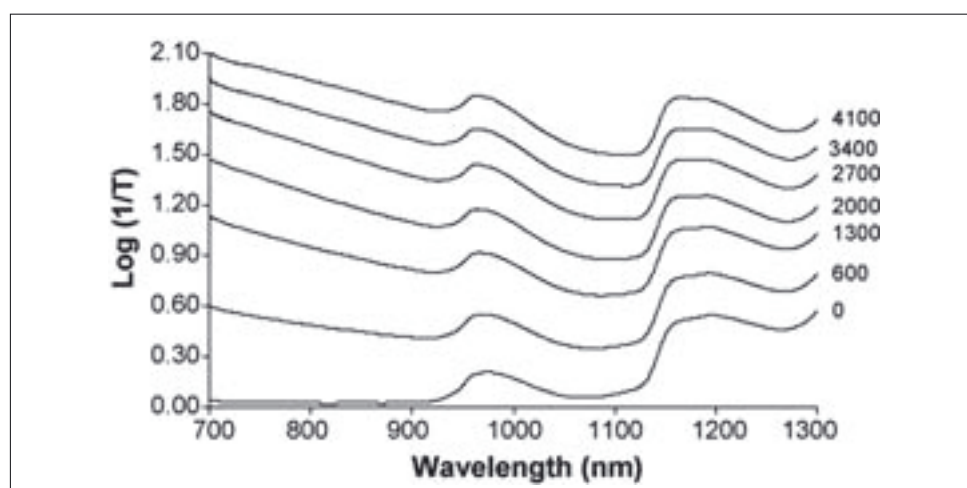


Fig. 1. NIR spectra of different aluminium concentrations at 700–1300 nm: from bottom to top are 0, 600, 1300, 2000, 2700, 3400 and 4100 mg/L, respectively.

tions from five different batches samples measured on five different days are shown in Fig. 1. Two calibration models were constructed based on the wavelength ranges 700–1300 nm (model 1) and 1100–1300 nm (model 2), respectively. NIR models were validated using six batch samples; one batch from calibration batches and five from new batches, which were not previously tested in calibration. The precision and accuracy of the two NIR models are shown in Table 1 and Table 2. It was demonstrated that the repeatability and intermediate precision of NIR were identical. The method was stable. The intermediate precision of NIR was comparable to the ICP-AES.

In conclusion, NIR transmittance spectroscopy can be used as an alternative method for aluminium content determination. The only sample preparation necessary is dilution of the sample.

2. Particle size determination in aluminium hydroxide suspensions using near-infrared transmittance spectroscopy [2]

The particle size of aluminium hydroxide is an important factor, which strongly affects the absorption capacity of antigenic protein. The most common method for particle size determination is laser diffraction (LD). In most cases, knowledge of the precise refractive index of the suspended material and the medium is required

Table 1. Precision results of batch #1 compared to ICP-AES

Method	Expected Al (mg/L)	Measured Al (Y_{mean}) (mg/L)	Upper 95% confidence level (mg/L)	Lower 95% confidence level (mg/L)	S_{rep}^a	%CV $_{rep}^b$	S_{ip}^c	%CV $_{ip}^d$
ICP-AES	1130	1190.2	1277.5	1102.8	8.5	0.71	29.1	2.58
NIR (700–1300 nm)	600	587.2	598.7	575.8	27.15	4.62	27.15	4.62
	1140	1154.4	1116.6	1095.7	24.82	2.15	24.82	2.15
	3400	3487.8	3498.6	3477.0	26.27	0.75	26.27	0.75
	4100	4136.4	4153.0	4119.8	36.17	0.87	36.17	0.87
NIR (1100–1300 nm)	600	618.6	628.1	609.1	22.52	3.64	22.52	3.64
	1140	1307.0	1315.7	1298.3	20.99	1.61	20.99	1.61
	3400	3442.3	3453.1	3431.4	27.13	0.79	27.13	0.79
	4100	3936.0	3949.8	3922.2	28.91	0.73	28.91	0.73

$$^a S_{rep} = \sqrt{(S_1^2 + S_2^2 + S_3^2 + S_4^2 + S_5^2)/5}$$

$$^b \%CV_{rep} = S_{rep} * 100/Y_{mean}$$

$$^c S_{ip} = \sqrt{(S_{ba}^2 + S_{rep}^2)}; S_{ba} \text{ (between assay)} = \sqrt{(S_{bm}^2 - S_{rep}^2/n)}; S_{bm} \text{ (between mean)} = \text{standard deviation between means per assay. For NIR method, } S_{bm}^2 < S_{rep}^2/n, \text{ then } S_{ba} = 0, \text{ and then } S_{ip} = S_{rep}$$

$$^d \%CV_{ip} = S_{ip} * 100/Y_{mean}$$

Table 2. Accuracy comparison of 6 batches Aluminum hydroxide using two NIR calibration models (Model I: 700–1300 nm; Model II: 1100–1300 nm)

Batch no.	Reference Al (mg/L)	700–1300 nm Predicted Al (mg/L)	1100–1300 nm Predicted Al (mg/L)	700–1300 nm Average recovery $\pm$ SD (%)	1100–1300 nm Average recovery $\pm$ SD (%)
1	6848.0	7024.8	6933.0	102.6 $\pm$ 0.7	101.2 $\pm$ 0.7
6	6950.0	7151.2	7042.4	102.9 $\pm$ 1.1	101.3 $\pm$ 1.0
7	6430.0	6005.5	6331.4	93.4 $\pm$ 2.7	98.5 $\pm$ 1.8
8	6302.0	5671.5	6035.7	90.0 $\pm$ 2.0	95.8 $\pm$ 1.6
9	6596.0	6319.2	6395.6	95.8 $\pm$ 1.4	97.0 $\pm$ 0.9
10	6794.7	7291.7	7024.0	107.3 $\pm$ 0.3	103.4 $\pm$ 0.4

in advance. For emulsion samples, cleaning of the measurement window is difficult, which leads to laborious procedures. NIR spectra also give physical information, e.g. particle size. In this study, NIR spectroscopy was investigated as an alternative method for prediction of the particle size of aluminium hydroxide.

Polystyrene particles were used as particle standards to construct a calibration model with NIR spectra and to predict the particle size of aluminium hydroxide samples. There was a linear relationship between the spectra and the particle size in the wavelength region 700–900 nm, when the spectra of different particle sizes were normalized at 700 nm (data not shown). Particle size standards (from 0.1–1.5 μm) were applied for model construction. A new particle (0.8 μm), which was not included in the calibration dataset, was used for method validation by comparison to the LD method. Table 3 shows the validation results. The particle size predicted by NIR fits well to that predicted by the surface weighted mean and d_{50} (volume %). It

Table 3. Accuracy and precision results of both, NIR and laser diffraction, in the measurements of 0.8 μm polystyrene standard

Method	Reported size (μm)	Mean $\pm$ SD (μm)	Mean % Recovery ^a	%CV ^b
NIR	0.78	0.813 $\pm$ 0.008	104.21	0.94
LD Surface weighted mean	0.78	0.811 $\pm$ 0.009	103.85	1.14
LD d_{50} (surface%)	0.78	0.793 $\pm$ 0.011	101.71	1.35
LD Volume weighted mean	0.78	0.832 $\pm$ 0.010	106.61	1.17
LD d_{50} (volumne%)	0.78	0.816 $\pm$ 0.010	104.57	1.27

^a %Recovery = Mean / Reference $\times 100$

^b %CV = SD / Mean $\times 100$

was also demonstrated that the NIR method had the same accuracy and precision as the LD. In addition, the NIR method showed the possibility to detect particle size changes during the storage of aluminium hydroxide (data not shown).

3. Determination of adsorbed protein concentration in aluminium hydroxide suspensions by near-infrared transmittance spectroscopy [3]

The quantification of protein concentration is important for biological and biomedical research. Classical and widely used methods, e.g. ultraviolet (UV)-visible spectroscopy, can only be applied to transparent solutions. Turbid samples containing particles produce large interferences in these methods due to the light scattering effect. Furthermore, when proteins are adsorbed onto aluminium adjuvants, traditional methods can only determine the free protein concentration, while the total protein concentration is required for calculating the fraction of protein adsorbed. In this study, the NIR method was used to demonstrate the possibility of direct determination of adsorbed protein concentration.

Table 4. The accuracy of NIR predicted results of three batches of aluminium hydroxide by the two calibration models

Adsorbed BSA (mg/ml)	Batches	N	Predicted Recovery (%)			
			Model 1 ^c		Model 2 ^d	
			Mean	SD	Mean	SD
0.5	Batch 1 ^a	5	98.0	6.2	100.0	8.2
	Batch 2 ^a	5	94.1	6.2	96.2	7.9
	Mix Batches ^b	7	99.3	9.3	98.1	5.6
1.0	Batch1	5	107.2	4.7	106.7	5.5
	Batch2	5	102.9	4.9	103.4	5.8
	Mix Batches	7	92.8	7.3	96.7	3.1
0.25	Mix Batches	7	91.9	9.9	101.9	6.3
0.8	Mix Batches	7	96.8	5.8	99.5	6.1
1.5	Mix Batches	7	90.2	9.6	100.0	5.2

^a Batch 1 and Batch 2: The BSA adsorption samples on one batch of aluminum hydroxide were prepared and measured on five different days

^b Mix batches: BSA adsorption on seven batches of aluminum hydroxide with single preparation, were measured on the same day. The two old batches and three new batches were included in this group

^c The predicted results by Model 1 were corrected by subtracting the predicted result of the related BSA blank control, in order to compensate the batches variation. The corrected predicted results were used to calculate the recovery

^d The predicted results were used directly to calculate the recovery

Batch variation of aluminium hydroxide was the major influence factor. Therefore, the calibration dataset contained eight batches of aluminium hydroxide. The adsorption samples were prepared by adding BSA with final concentrations of 0, 0.1, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5 and 1.75 mg/mL. Two models were built: model I with raw spectral data and model II with pre-treated data by subtracting the BSA blank control. Validation of the models was carried out with an independent dataset. Table 4 shows the validation results. The results show that it is possible to directly determine adsorbed protein concentrations in aluminium hydroxide suspensions using NIR spectroscopy. NIR may be the only non-destructive method for the direct determination of protein concentration in suspension so far. It has great advantages for application in vaccine production.

4. Determination of moisture content of lyophilized allergen vaccines by NIR spectroscopy [4]

Moisture content (MC) is an important parameter for lyophilized vaccines. Currently, Karl Fischer titration (KF) is widely used for moisture determination in routine analysis. However, the method is time-consuming, sample destructive, requires environment polluting reagents and the results rely on random samplings.

In this study, NIR as an alternative method was applied to test moisture content. Five lyophilized allergy vaccines, a total of 95 vials, were investigated. Water content was in the range of 0.17– 1.51% (w/w, KF). Two calibration models were constructed with 1) an original dataset (one sample with four spectra at different angles by rotating the vial) and 2) a reduced dataset (one sample with one spectrum, i.e. the mean of the spectra representing the four different angles). The MC correlation between NIR and KF is shown in Fig. 2. The MC predicted by NIR showed acceptable agreement with KF results. The preliminary data demonstrate that NIR spectroscopy can be used to assess MC in lyophilized vaccines.

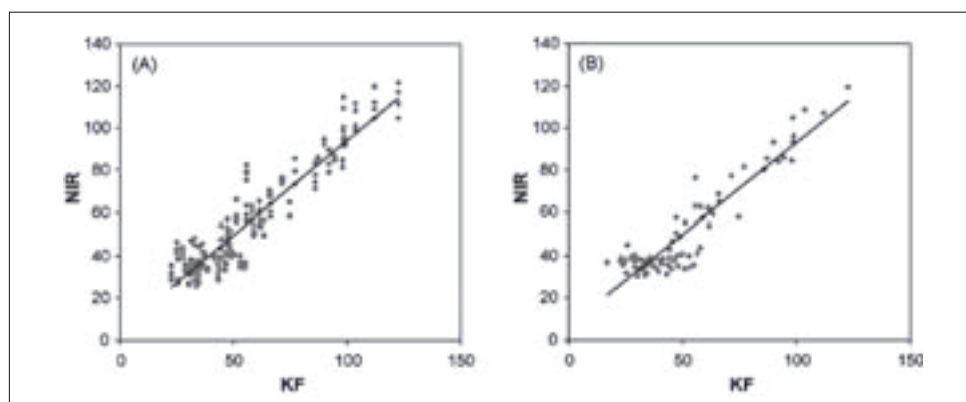


Fig. 2. The MC correlation plot between NIR prediction and KF results for multi-products models: A– model based on original data set (RMSEP 10.20 $\mu\text{g H}_2\text{O}$ /vial and 2 PLS factors): slope is 0.82; offset is 8.95; correlation is 0.90 and RMSED is 9.94 $\mu\text{g H}_2\text{O}$ /vial. B– model based on reduced data set (RMSEP 8.99 $\mu\text{g H}_2\text{O}$ /vial and 2 PLS factors): slope is 0.86; offset is 6.92; correlation is 0.93 and RMSED is 8.64 $\mu\text{g H}_2\text{O}$ /vial.

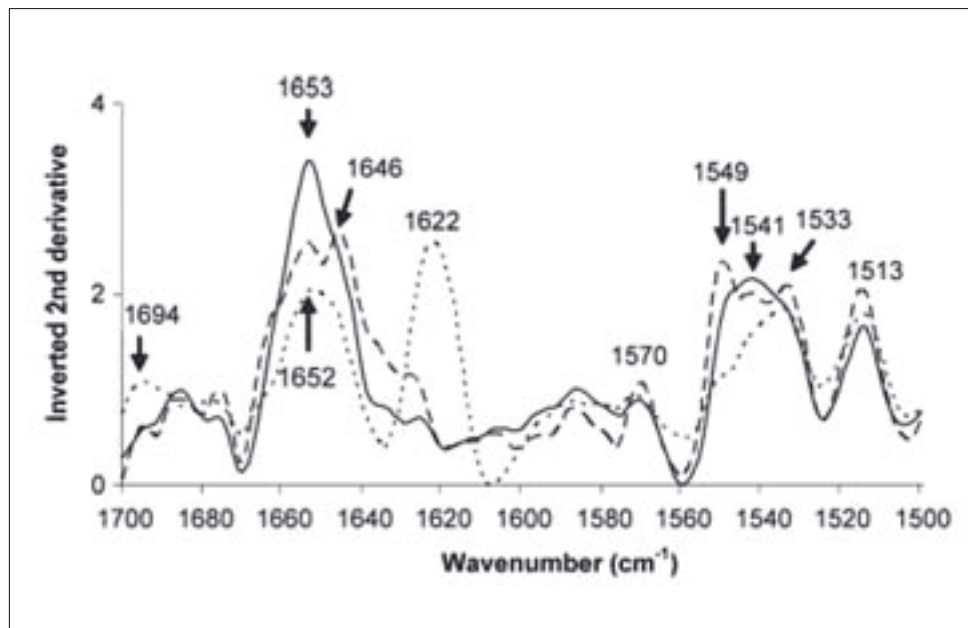


Fig. 3. Inverted 2nd derivative spectra of BSA in amide I and amide II region: free BSA (BSA, solid line), adsorbed BSA (onto aluminium hydroxide, dashed line) and aggregated BSA (BSA solution preheated at 90 °C for 30 min, dotted line).

5. Structural changes of protein antigens during adsorption and release from aluminium hydroxide using FTIR-ATR [5]

Structural integrity of antigens upon adsorption and release is not only important for the immunogenicity of the vaccine, but also for the epitope specificity of the resulting immune response and hence the therapeutic efficacy of allergy vaccination. Antigen structural information is also important for understanding the mechanism of how adjuvants enhance the immune response. However, little is known about the structure of an antigen when it is adsorbed on and subsequently released from aluminium adjuvants. Traditional methods for determination of protein structure have limitations in the analysis of aluminium hydroxide complexes. Fourier transformed infrared-attenuated total reflection (FTIR-ATR) spectroscopy is a method well suited for the determination of the secondary structure of globular proteins. It offers many advantages over other spectroscopic techniques. The most important is that the structure of adsorbed proteins can be analysed.

Two model proteins, bovine serum albumin (BSA) and β -lactoglobulin were investigated during adsorption and release from aluminium hydroxide. Fig. 3 shows the inverted 2nd derivative FTIR spectra of BSA. The significant difference in the spectra of adsorbed and free BSA indicated that the secondary structure of BSA changed due to adsorption. However, the adsorption-induced secondary structural changes were not caused by protein aggregation (analysed after heating to 90°C for 30 min). The structural changes were dependent on how much protein was

adsorbed by aluminium hydroxide. A high ratio of adsorption gave a more native-like structure (data not shown). Approximately 80% of the adsorbed protein could be released by phosphate buffer (pH 7.4). BSA regained its native structure upon release as analysed by FTIR-ATR and circular dichroism. Similar results were found for β -lactoglobulin.

In conclusion, FTIR-ATR spectroscopy provided direct information on changes of the secondary structure of allergens during adsorption and release from aluminium hydroxide.

6. The structural stability of protein antigens adsorbed by aluminium hydroxide in comparison to the antigens in solutions [6]

In order to investigate whether the structure of the protein antigen is stabilized by the aluminium hydroxide complex, FTIR-ATR was used to investigate the structural stability of two model proteins when they adsorbed onto aluminium hydroxide and dissolved in solution. Data (not shown) indicated that the secondary structure was stabilized by adsorption onto aluminium hydroxide compared to protein in solution.

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Discussion

Vieths: Just for curiosity: have the methods already been implemented in controlling the production at ALK?

Zheng: No, not yet. I mean, the method is almost ready there, but this is for the management to decide. That is another case.

Chapman: I am curious why you did all this work with BSA or HSA when you have all those purified allergens available at ALK.

Zheng: Okay, we used model proteins just because the allergen proteins are so expensive.