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# Determination of the immunoglobulin G precipitation end-point by an intelligent near-infrared spectroscopy system

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Precipitation is a key manufacturing unit during the immunoglobulin G (IgG) production, which guarantees the quality of the final product. Ethanol is usually used to purify IgG during the precipitation process, so it is important to monitor the ethanol concentration online. Near-infrared (NIR) spectroscopy is a powerful process analytical technology (PAT) which has been proved to be feasible to determine the ethanol concentration during the precipitation process. However, the NIR model is usually established based on the specific process, so a universal model is needed. And the clarity degree of solution will affect the quality of the spectra. Therefore, in this study an integrated NIR system was introduced to establish a universal NIR model which could predict the ethanol concentration online and determine the end-point of the whole process. First, a spectra acquisition device was designed and established in order to get high-quality NIR spectra. Then, a simple prepared ethanol NIR model was constructed to predict the actual

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manufacturing process. Finally, the end-point was determined to stop the peristaltic pump when the ethanol concentration reached 20%. The results showed that the spectra quality was good, model prediction was accurate, and process monitoring was accurate. In conclusion, all results indicated that the integrated NIR system could be used to monitor the biopharmaceutical process to help us understand the pharmaceutical process.

*Keywords*: Ethanol precipitation; near-infrared spectroscopy; blood product; partial least square.

## 1. Introduction

Blood products are the proteins purified from human plasma, which include human serum albumin, immune globulin, blood coagulation factors, etc. They have great physiological and clinical potentials,[1–3] and the demand for blood products is increasing dramatically especially in the face of COVID-19 infection.[4–7] However, inconsistent with the higher clinical requirement, a quality monitoring technology in the manufactory is lacking which causes the whole production to be like a black box. And we could not even understand the process and the critical process parameters. The main method used until now is the cold ethanol precipitation method proposed by Prof. Cohn in 1964.[8,9] However, due to the differences in total protein content from different stations for the sole collection of plasma, the cold ethanol used may differ slightly. Therefore, how to monitor the ethanol concentration online is of great importance.

Process analytical technology (PAT) was introduced in 2004 to realize the process optimization and quality assurance to ensure public safety and product efficacy.[10] In order to achieve the goal of PAT in practice, measurement, analysis, control, and optimization are fundamentally required. Process analyzers, measurement systems, and sensors used for real-time monitoring of the processes are called the PAT tools. PAT tools have evolved from single-variable measurement systems such as for measuring pH, temperature, pressure, and conductivity to multi-variable measurement systems such as UV–Vis spectroscopy, Raman infrared spectroscopy, and near-infrared (NIR) spectroscopy.[11–13] And NIR spectroscopy is a powerful PAT tool which has been proved to be feasible to monitor the

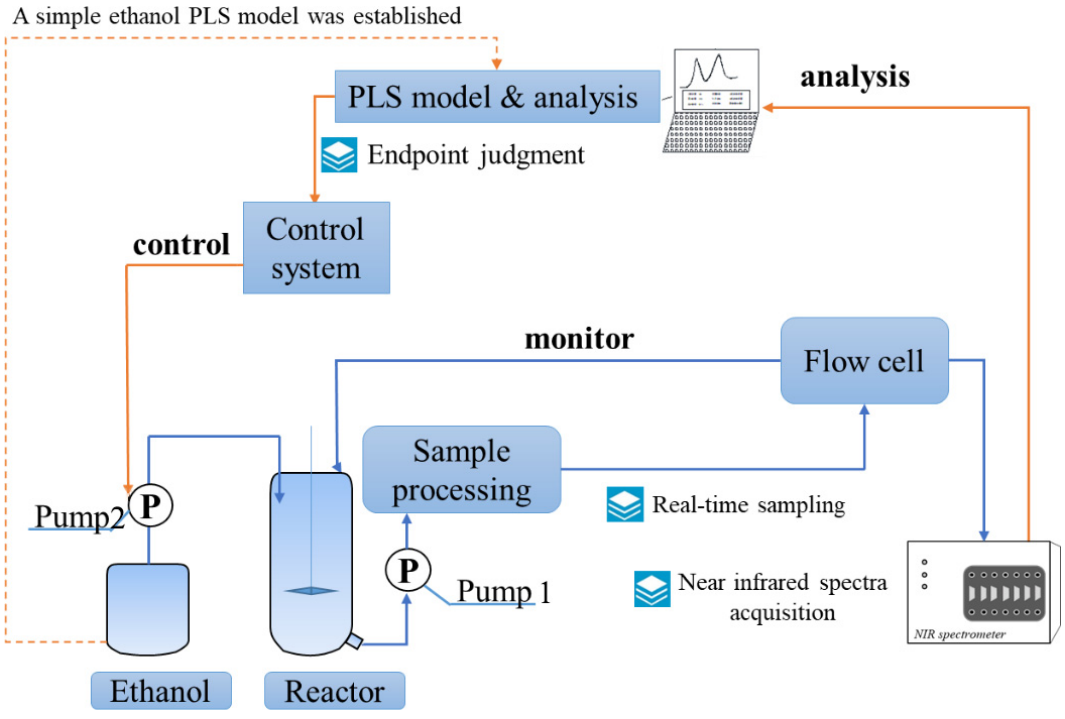



Fig. 1. Flowchart of an integrated NIR system which is used for end-point determination.

precipitation process.[14,15] However, these NIR models are usually established based on a specific process which means that they could only be used in one manufactory or product line, making it difficult to be popularized. Hence, a universal model is needed. In addition, the sampling system is also very important in NIR spectroscopy analysis.[16] The solution during precipitation is normally turbid which makes it difficult to acquire an NIR spectrum with high quality, and some pretreatment is essential. Nevertheless, to the best of our knowledge, there is no relative online pretreatment for blood product filtration.

Therefore, in this study an integrated NIR system was introduced to establish a universal NIR model which could predict the ethanol concentration online and determine the end-point of the whole process which is shown in Fig. 1. First a single-use filtration was designed for sample processing. Then the spectra were acquired by an NIR spectrometer. Meanwhile, a simple ethanol partial least square (PLS) model was established. Finally, a control system was designed and used to stop the pump based on the end-point data from the PLS model.

## 2. Materials and Methods

### 2.1. *Materials*

A flow cell with 2-mm path length was purchased from Ocean Optics, Inc. And the fiber was obtained from LEONI company. Programmable peristaltic pumps were from Ditron Technology Co., Ltd. The STC 12 core board, PCB, buttons, copper pillar, heat shrink tube, led, and pins were bought from Youxin Electronics Co., Ltd. Capsule filters with different sizes were obtained from Yuanwang Co., Ltd., Sanglian Co., Ltd., and General Electric company. Luer fittings and four-way valves were from Sanglian Co., Ltd. Human serums were provided by China Biologic Products Holdings, Inc. Ethanol and acetic acid were from China National Pharmaceutical Group Co., Ltd.

Fourier transform near-infrared spectrometer (Bruker, Germany) was used to collect the NIR spectra. OPUS software was introduced to establish the PLS models and predict the online data. A homemade software provided by Dr. Zhang was applied to grab the predictive data from the PLS models and control the pumps.

### 2.2. *Methods*

#### 2.2.1. *Sampling system*

A filtration system was designed to get qualified NIR spectra as shown in Fig. 2. The human plasma was first filtrated by a primary filter which was composed of two kinds of colatoriums with pore sizes of 0.8–1.2 mm and 90–110 $\mu$m, respectively. Then a second filter was introduced to clarify the liquid further. During this step, the materials and filter element were investigated. Polypropylene, polyamide, and polyethersulfone were compared to select one suitable material. Also, different filter diameters including 80, 30, 5, and 1 $\mu$m were investigated to choose the best one. A regulator marked No. 5 was applied to eliminate bubbles and steady the pressure. Finally, the flow cell was used to collect the NIR spectra as shown in Nos. 8 and 9. The reference analysis was carried out by using the sample collected from the sample vial (marked 10).

When the sampling system was established, the spectra were collected to evaluate the system. First, the deionized water was pumped through the sampling system. When the water reaches the flow cell, the NIR spectra were collected. The spectral range was set to 12,000–4000 $cm^{-1}$, and the resolution was 8 $cm^{-1}$. Every sample was scanned for 32 times and the averaged spectrum was used as the final spectrum. Then, ethanol with different concentrations

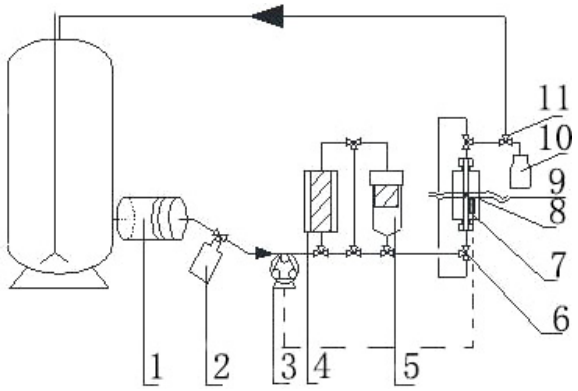



Fig. 2. The sampling system schematic diagram. No. 1 is the primary filter which is used to eliminate the large particles. No. 2 is the relief valve which is used to adjust the pressure during the sampling system. No. 3 is the peristaltic pump which is controlled by home-made software. No. 4 is the second filter which is used to get satisfied NIR spectra. No. 5 is used to eliminate bubbles and steady the pressure. Nos. 6 and 11 are the three-way valves. Nos. 8 and 9 are the optical flow cell devices used to collect the NIR spectra. No. 10 is sample vial which is used to collect samples for the reference analysis.

was pumped through the sampling system and the NIR spectra were acquired. We compared the spectral difference between adjacent concentrations collected within 1 min to twice the standard deviation (STD) of deionized water and the ratio ($R$) was calculated [as shown in Eq. (1)]. If $R > 1$, it indicates that the spectrum is reliable and reproducible,

$$R = \frac{|X_{c_{i,A}} - X_{c_{i-1,A}}|}{2 * \mathrm{STD}(X_{c_{0,A}})}, \quad (1)$$

where $c_{i,A}$ was the absorbance of ethanol–water liquid at a concentration $i$. $c_{0,A}$ was the absorbance of deionized water.

#### 2.2.2. *Process analytical system*

Process analytical system was introduced to realize the online analysis. First, a simple ethanol prediction model was constructed. A total of four repeated batches of 100-mL ethanol–water solutions ranging from 0% to 25% with 1% intervals (except for the solutions at 0.5% and 1% concentrations) were prepared. Then the ethanol–water solution was pumped through the sampling system and when it reached the flow cell the NIR spectra were collected as described previously in Sec. 2.2.1. Finally, 108 samples were obtained in total.

Three batches were selected as the calibration set and the remaining one was used as the validation set. The PLS regression method was applied to establish the prediction model. And different spectral pretreatment methods and spectral ranges were investigated.

#### 2.2.3. *Process control system*

Three batches of human plasma precipitation processes were simulated in lab scale. First, 250-mL plasma was centrifuged for 20 min at 4000 rpm, then the supernatant pH was adjusted to $5.95 \pm 0.05$ with the acetic acid buffer. The plasma was then transferred into a reactor with the temperature of $-4°C$ and rotational speed of 190 rpm. A homemade software was used to control pump 1 (P1) and pump 2 (P2). Ethanol was pumped into the reactor at a speed of $0.5\,\mathrm{mL} \cdot \mathrm{min}^{-1}$ by P1. P2 was introduced to transfer samples to the flow cell and back to the reactor finally. NIR was used to monitor the ethanol concentration. When it reached about 15%, the speed of P2 would be decreased in order to investigate the immunoglobulin G (IgG) content variation. When the concentration got to 20%, P2 would automatically stop.

## 3. Results and Discussion

### 3.1. *Selection of the filter*

Three different filter materials including PP, PA, and PES were investigated and the results are shown in Fig. 3. PP-1, PA-1, and PES-1 had similar filtration effect, and considering the cost performance PP was selected as the filter material. Then different filter diameters were tested, and PP-30 was used finally because the filtration time was acceptable and it did not get easily blocked during the whole process.

### 3.2. *Evaluation of the sampling system*

Figure 4 shows the results of evaluation of the sampling system. The spectral range of 6100–5446 $\mathrm{cm}^{-1}$ was used as this range mainly reflected

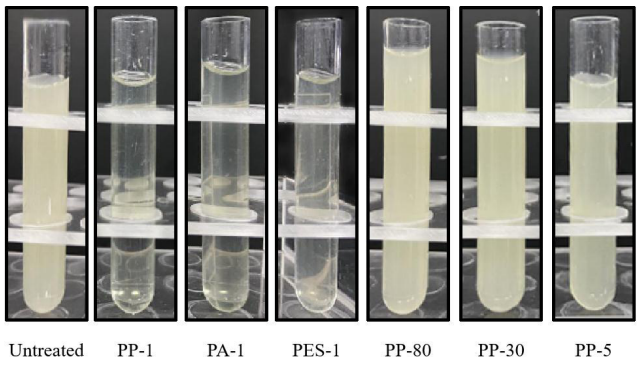



Fig. 3. Investigation of different filter materials and diameters.

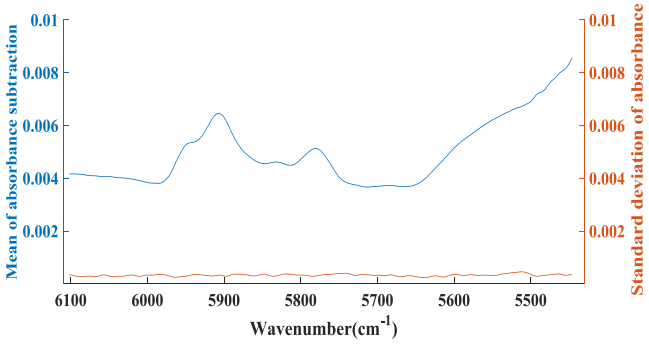
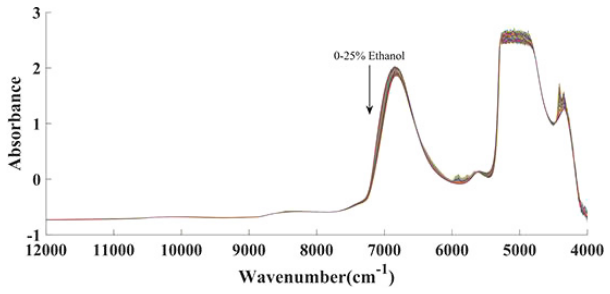



Fig. 4. The results of evaluation of the sampling system.

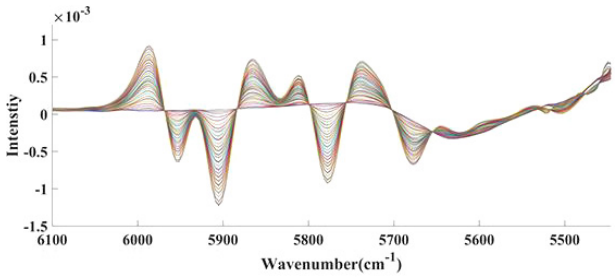
the C–H overtone of ethanol. It could be found that the difference between two adjacent concentrations was significantly higher than the STD of pure water spectra. It indicated that the spectral fluctuation caused by the sampling system or NIR spectrometer could be omitted.

### 3.3. *Process analytical model*

Eighty-one samples from three batches were used to construct the PLS model. First, Fig. 5(a) shows the original spectra and the spectral range is investigated as shown in Fig. 5(b). It could be found that the absorbance at 6100–5446 $cm^{-1}$ was between 0.7 and 0.9, which indicated that it was suitable for establishing the PLS model. Also, this range reflected the C–H overtone of ethanol. Then different pretreatment methods were investigated and the second derivative with SG smoothing (polynomial order: 2, points: 17) method was adopted [shown in Fig. 5(b)] to improve the spectral resolution.[17] More peaks could be identified, and as the concentration increased the positive peak intensity increased while the negative peak intensity decreased. The peaks at 5880 $cm^{-1}$ and 5773 $cm^{-1}$ indicated the absorption of $CH_2$–OH, and the one at 5905 $cm^{-1}$ indicated the absorption of $CH_3$.




(a)




(b)

Fig. 5. (a) NIR original spectra of 81 samples, from top to bottom, representing 0–25% ethanol solutions. (b) Spectra at 6101.9–5446.2 $cm^{-1}$ after pretreatment.

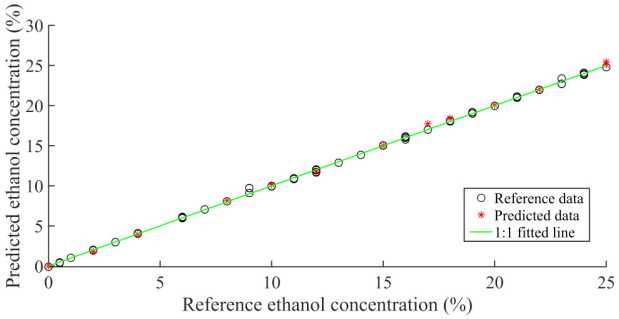
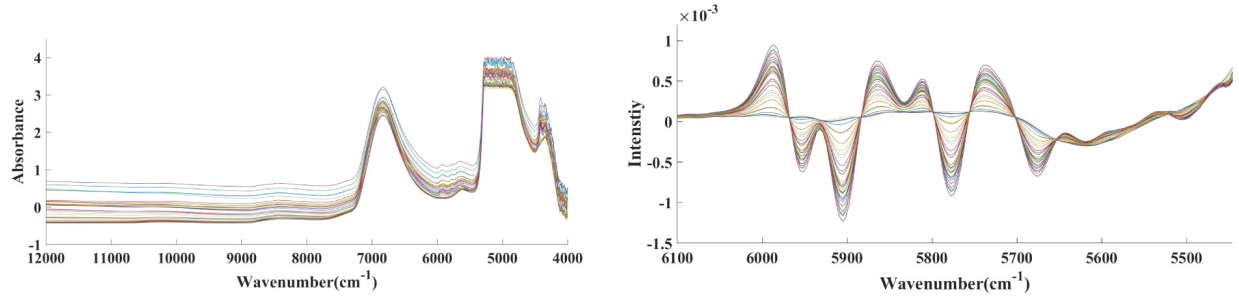



Fig. 6. PLS model of ethanol aqueous solution and external validation results.

Finally, the PLS model was constructed and the results are shown in Fig. 6. Only one principal component was introduced in this model. And the loading mainly reflected the absorption of ethanol. The $R$-value was 0.9997 and the RMSECV was 0.165%, which demonstrated that the PLS model should have good predictive capacity. Twenty seven samples were used to validate the PLS model and the results are shown in Fig. 6. We got the results that the $R$-value was 0.9997 and the RMSEP was 0.22% indicating that the PLS model could be used in real-time monitoring.

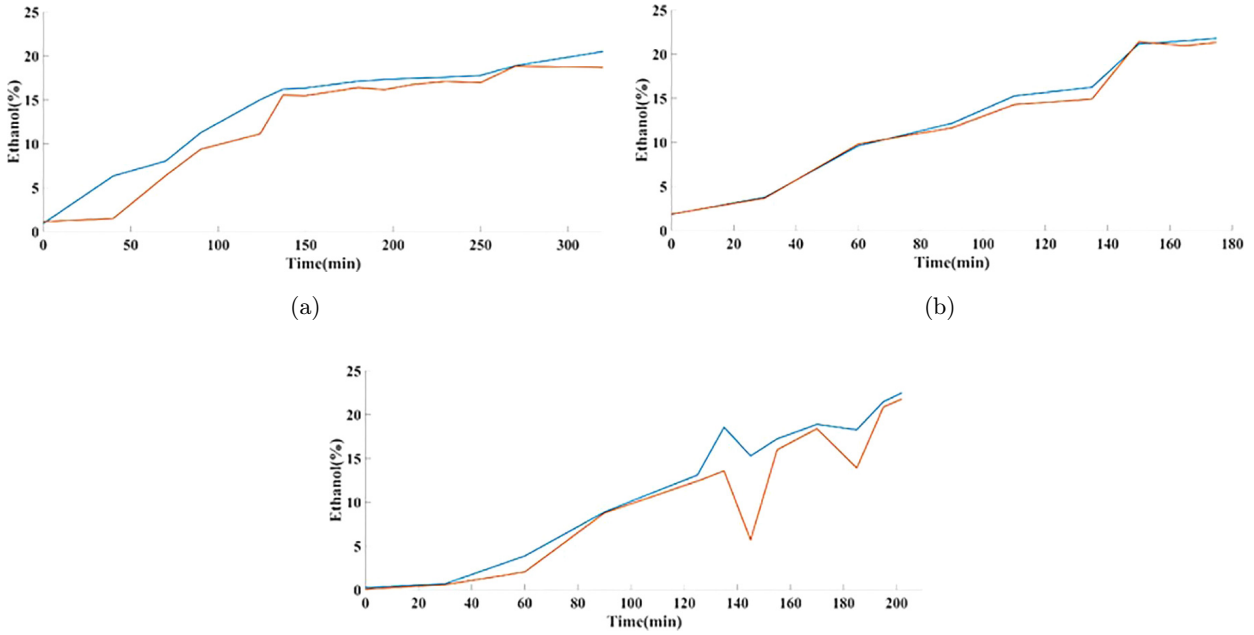
### 3.4. *Process monitoring and control*

The PLS model established by a simple system was introduced to predict the real precipitation process and the results are shown in Fig. 7. Figure 7(a) is a batch of online NIR spectra during the plasma precipitation process. It can be found from Fig. 7(b) that the wavenumbers ranging from 6100 $cm^{-1}$ to 5446 $cm^{-1}$ with second derivative and SG smoothing have the same characteristic peaks which could be found in the simple ethanol–water system (shown in Fig. 5). It could be concluded that this range was mainly composed of the ethanol C–H overtone. Three batches simulated in lab scale were used to evaluate the predictive accuracy using the simple PLS model. Figure 8 shows the predictive ethanol concentration value during precipitation and the reference value determined by GC method. Both lines were similar except at two points which may be caused by the determination errors. When the ethanol concentration reached 15%, the pump speed of P2 was decreased and IgG content was determined to investigate the ethanol effects upon




(a) (b)

Fig. 7. (a) Original NIR spectra of plasma during alcohol precipitation and (b) the spectra at 6101.9–5446.2 $cm^{-1}$ after pretreatment.




(a) (b)

(c)

Fig. 8. Trend figures of changes in ethanol concentration: Panels (a)–(c) correspond to the first, second and third batches of data, respectively.

the IgG content (data was not shown here). When the ethanol concentration reached 20%, the P2 stopped automatically. The whole process was finished. With this method, there is no need for manufacturers to operate in the field, they could monitor the whole precipitation process and determine the end-point.

## 4. Conclusions

Traditional methods for ethanol precipitation monitoring were tedious and time-consuming, which declined the production efficiency and accuracy. In this study, an intelligent integrated end-point determination system was investigated. In order to overcome the shortcomings that the NIR spectra would be influenced by the particles during the precipitation process, a pretreatment system was designed and applied to eliminate the particle and temperature effects to get steady NIR spectra. Then a simple and universal PLS model was established to predict the ethanol concentration during the complicated actual manufacturing process. Finally, a home-made software was used to monitor the process and ethanol addition was stopped when its concentration reached 20%.

However, due to the quality variance of the plasma used for precipitation, the parameter value 20% may be not suitable. And the IgG and total protein concentrations should be taken into consideration in the future research. This study provides a new strategy for biopharmaceutical quality real-time monitoring and control.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## Acknowledgments

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