Journal of Innovative Optical Health Sciences
Vol. 7, No. 6 (2014) 1450004 (7 pages)

DOI: 10.1142/S1793545814500047

World Scientific
www.worldscientific.com

Characterization of herbal powder blends homogeneity using near-infrared spectroscopy

Wenlong Li and Haibin Qu*
Pharmaceutical Informatics Institute
Zhejiang University, Hangzhou 310058, P. R. China
**quhb@zju.edu.cn*

Received 10 July 2013
Accepted 29 October 2013
Published 10 December 2013

Homogeneity of powder blend is essential to obtain uniform contents for the tablets and capsules. Near-infrared (NIR) spectroscopy with fiber-optic probe was used as an on-line technique for monitoring the homogeneity of pharmaceutical blend during the blending process instead of the traditional techniques, such as high performance liquid chromatograph (HPLC) method. In this paper NIRS with a SabIR diffuse reflectance fiber-optic probe was used to monitor the blending process of coptis powder and lactose (excipient) with different contents, and further qualitative methods, like similarity, moving block of standard deviation and mean square were used for calculation purposes with the collected spectra after the pretreatment of multiplicative signal correction (MSC) and second derivative. Correlation spectrum was used for the wavelength selection. Four different coptis were blended with lactose separately to validate the proposed method, and the blending process of "liu wei di huang" pill was also simulated in bottles to verify this method on multiple herbal blends. The overall results suggest that NIRS is a simple, effective and noninvasive technique can be successfully applied to the determination of homogeneity in the herbal blend.

Keywords: Near-infrared spectroscopy; traditional Chinese medicine; powder blend; homogeneity.

1. Introduction

Traditional Chinese medicine (TCM) in the forms of tablets, pills and capsules were made from pre-blended powder of natural herbs. Despite the acknowledgment of the importance of the process of powder blending in ensuring the homogeneity and consistent potency of the TCM, no method other than mere experience is available at present to monitor the process in TCM manufacturing.

The traditional methods of determination entail stopping the blender at a pre-determined period of time, removing unit-dosage samples from defined

*Corresponding author.

locations by a sample thief probe, extracting the active component from the sample matrix, and analyzing by high performance liquid chromatograph (HPLC) or ultraviolet (UV) spectroscopy. This conventional method of monitoring blend uniformity involves invasive sampling procedures. Significant sampling error is often associated with the use of thief probes for removal of samples from powder blenders. Such sampling devices disturb the powder bed and compromise the validity of collected samples. If the individual components differ in particle size, segregation can be a significant concern during powder flow into the sample compartment of the thief probe. Segregation prevents representative sampling of the blend. Another significant factor adding to the error associated with such invasive sampling is variability between operators during sample collection.[1] Excipients play a variety of roles including dissolving some components, flavoring, accommodating medicinal agents in efficient doses and appealing forms, increasing fluidity and facilitating preparation of the end product.[2] The distribution of excipients is assumed to be homogeneous if the analytical results indicate the uniformity of active component.

The traditional methods, such as HPLC and UV, largely used in synthetic pharmaceutical area to determine homogeneity of powder blending are useless for TCM products, since the products contain multiple active constituents and it is very difficult to extract all active components from sample matrix of natural herbal powders. Therefore, new methods need to be developed.

Near-infrared (NIR) Spectroscopy is a rapid, non-destructive analytical technique. The fiber-optic probe makes remote continuous process monitoring possible. Most active pharmaceutical ingredients (API) and excipients absorb NIR radiation. This method for assessing powder blend homogeneity could be of great value in minimizing the sample preparation and assaying time associated with traditional blend analysis procedures.[3] In recent years, many articles of NIR spectroscopy for the powder blends have been reported. Sekulic and Hailey developed an on-line method of monitoring blend homogeneity with dynamic data collection by fiber-optic and qualitative tools of analyzing homogeneity[4–6]; Berntsson focused on quantitative analysis of the process[7–9]; others were interested in determining the end point of blending.[1–3,10] In these studies, the active components were single species and their pharmacologic effects were clear. It was very different for TCM products which had complex systems with multiple active components and synergistic effects.

However, NIR allows the analysis of complex material to be performed rapidly, non-destructively, and organic solvent-free. NIR was therefore receiving a great deal of attention for its potential application in herbal medicines analysis.[11,12] It is a vital technique for the mixture uniformity determination of herbal powder blends. In this paper, a typical traditional herb coptis and one excipient lactose were chosen to examine the application of NIR in assessing the homogeneity of binary blends by simulating the process in bottles. The contents of herbal materials varied according to different geographic origins, climate and age, etc. Calibration set for quantitative methods should be extremely large to represent all spectral variations, which cost lots of money and time. Hence, qualitative methods like spectral similarity,[13] moving block of standard deviation[6] and the mean square[2] were used to determine the end point of blending process for herbal medicines.

Four different coptis samples were used to verify the application of proposed methods on diverse samples. In the manufacturing of "liu wei di huang" pill, powders of Radix Rehmanniae Preparata, Fructus Corni, Cortex Paeoniae, Rhizoma dioscoreae, Poria and Rhizome Alismatis were blended together till homogeneity. This blending process was simulated in bottles to validate the presented methods on multiple blends of herbal medicines.

2. Materials and Methods

2.1. *Materials*

Coptis samples of four different geographic origins, Radix Rehmanniae Preparata, Fructus Corni, Cortex Paeoniae, Rhizoma dioscoreae, Poria and Rhizome Alismatis were bought from drugstores in Hangzhou. Powders of these herbal medicines were used after milling and thieving through 100 meshes. Lactose powder ($<150\,\mu$m, Hengxin Chemical Reagent Co. Ltd., Shanghai) was used as supplied without any further treatment.

2.2. *NIR instrumentation*

The FT-NIR spectrometer used for all measurements was an Antaris NIR Analyzer (Thermo

Nicolet Corp., Madison, WI), equipped with a SabIR fiber-optic probe (Thermo Nicolet Corp., Madison, WI). The collection and processing were controlled via the RESULT software v.1.2.

2.3. *Mixing of powders and recording of NIR spectra*

The reflective NIR spectra were obtained as $\log(1/R)$ in the 1000–2500 nm (10,000–4000 cm^{-1}) range with a resolution of 8 cm^{-1}, empty Attenuator and each spectrum were acquired as an average of 16 subsequent spectral scans by the SabIR fiber-optic probe. About 750 data points were obtained for each spectrum. 125 mL wide-mouth bottles ($\phi 50 \times 80$ mm) were used in this study. One of the four coptis samples and lactose were weighed to bottles at a total weight of 20 g at different coptis contents of 10, 20, 30, 40, 50% (w/w), and labeled 1 to 5. Coptis powder was first loaded. The samples were mixed in the bottles by manual tumbling for 25 times. After per tumbling, the probe was inserted into the mixture around the center of the bottle at different depth, then the NIR spectrum was recorded. Totally, 25 NIR spectra were collected for each batch. All four coptis samples were mixed with lactose at 50% (w/w) contents.

Radix Rehmanniae Preparata, Fructus Corni, Cortex Paeoniae, Rhizoma dioscoreae, Poria and Rhizome Alismatis were blended at the manufacturing ratio (8:4:3:4:3:4, w/w), and three simulated processes were recorded.

2.4. *Wavelength selection*

The correlation spectrum was used for the wavelength selection. Through calculating the correlation coefficients at each wavelength, the correlation spectrum, which shows the correlation between the spectral information and component concentrations of a single standard[13] was obtained. The wavebands with higher correlation coefficients were selected for the modeling.

2.5. *Data analysis*

Diffuse reflection NIR spectra had scattering variation due to particle size or shape or powder density. Multiplicative signal correction (MSC) method was used to remove additive and multiplicative spectral differences. Second derivative was usually applied to minimize both the offset and drift components of the spectra, due to the physical nature of the sample. A Savitzky–Golay[14] filter was employed in the second derivative calculation to smoothen spectra data. The spectral contributions resulting from the physical properties of the samples could be effectively handled after these pretreatments. In this research, preprocessing of MSC followed by second derivative was used to minimize variations from physical factors. All preprocessing were done in TQ Analyst v.6.2 (Thermo Nicolet Corp., Madison, WI).

One of the widely used procedures to assess mixture uniformity is the calculation of spectral dissimilarity.[2,6] In this paper, similarity calculation was used instead. The procedure compares spectra recorded after per tumbling with sample spectra assumed to be homogeneous as standards. The algorithm performs a Gram–Schmidt analysis of the standards and the resulting orthogonal model represents the spectral information provided by all the data points in all of the standards. The residual spectrum of new sample was calculated by removing any spectral information represented by the model from the new sample spectrathen compares the information in the sample spectrum with the information in the residual spectrum and calculates a "match value".[13] The match value represents the unexplained variation in the spectrum of the new sample, which is expected to be stable as the homogeneity approached.

Another popular and simple procedure used to determine mixture uniformity is the "moving block of standard deviation", reported by Sekulic, which involves selecting a window size to encompass a sufficient amount of information without diminishing the information content by making the window too large and calculating the standard deviation at each wavelength for the "n" spectra selected. The pooled standard deviation (over all wavelengths) of the standard deviation spectrum is reported as a single value, the mean standard deviation is plotted against the tumbling times. It is expected to approach zero as blend homogeneity is approached.[6]

The mean square, reported by Blanco, is a new, faster method for assessing mixture uniformity that requires no prior knowledge of the spectrum for a homogeneous sample and relies on the difference between two spectra recorded at two consecutive times. When the mean square levels off, the spectral

difference can be assumed to be minimal, the mixture was uniform.[2]

The spectral variation values provided by the moving block and mean square methods were normalized by dividing them into the vector norm in order to facilitate comparison of the respective profiles. The FT-NIR spectral data obtained from the Result software were imported to the TQ Analyst software and exported to Matlab v.6.5 (The Mathworks Inc., Natick, MA) after the preprocessing. Similarity was calculated in the TQ Analyst software, others were performed in Matlab with written routines.

3. Results and Discussion

Typical traditional herb coptis was used as active component and lactose was used as excipient. Spectral features (the mean of three recordings) of the two components were distinct and the variation of coptis from different geographic origin was obvious, as shown in Fig. 1.

3.1. *Spectral pretreatment and region selection*

Preprocessing of MSC followed by 13 points Savitzky–Golay smooth second derivative was applied to all spectra. The 25 spectra for batch 5 before and after preprocessing are shown in Fig. 2. Both offset and drift components of spectra in Fig. 2(a) are minimized in Fig. 2(b). The first spectrum collected after the first tumbling was largely due to lactose, which was the latter added to the bottle. The spectra obtained after the fourth tumbling seemed similar, chemometric methods were needed to ensure uniformity.

The average spectrum of the last five spectra, which achieved uniformity after previous tumbling at each coptis contents, was used in the coefficient calculation. Three spectral regions (10,000–6985, 6728–5559 and 5317–4577 cm^{-1}) were chosen by the

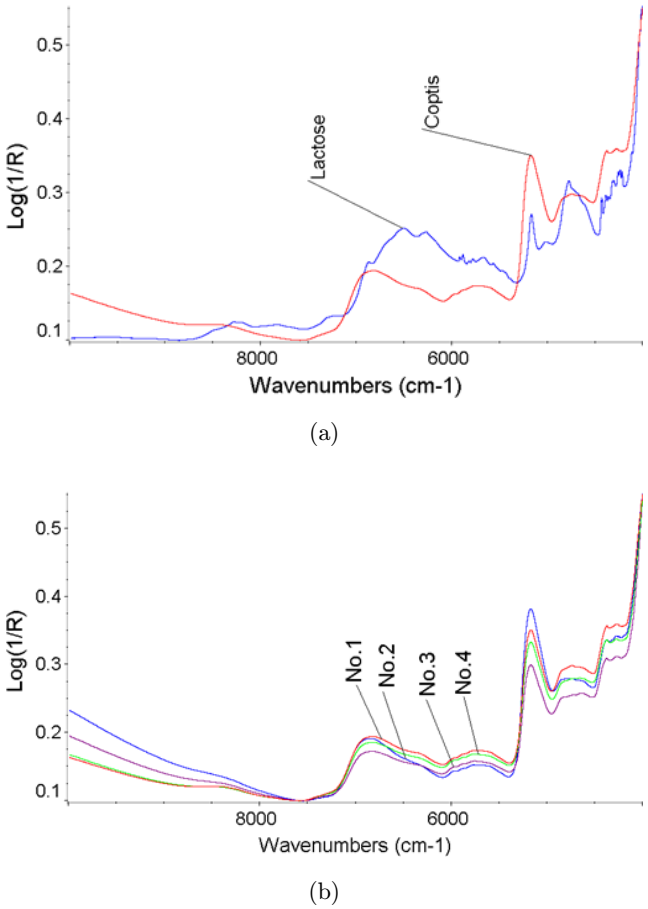

Fig. 1. NIR spectra of coptis and lactose (a) and four different coptis (b).

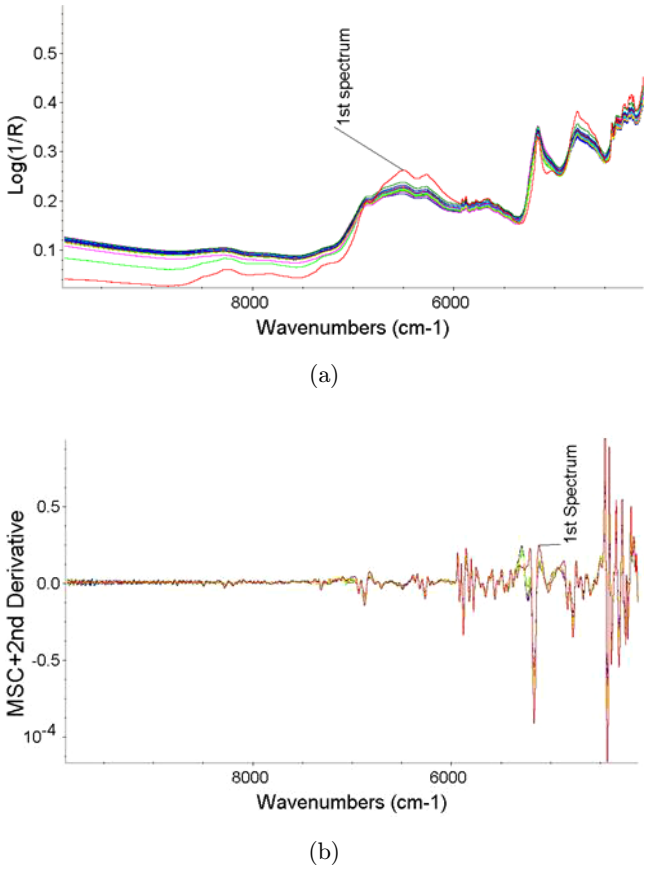

Fig. 2. NIR spectra collected of the 50% (w/w) blend batch 1 of 25 tumbling before (a) and after preprocessing (b).

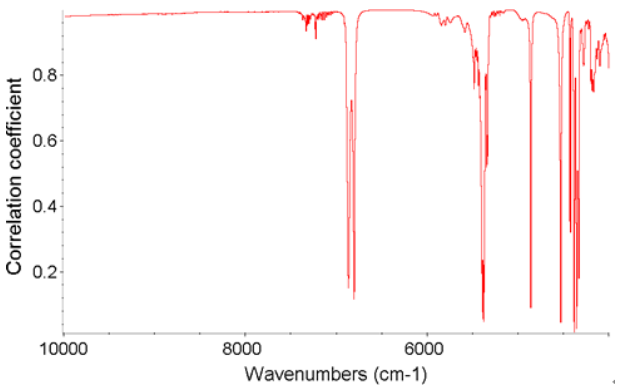

Fig. 3. Correlation spectrum of coptis and lactose binary mixture.

correlation spectrum in Fig. 3 for further calculation where the coefficients were above 0.9.

3.2. *Data analysis*

Qualification methods like spectral similarity, moving block of standard deviation and the mean square were applied to determine the uniformity of the five blending experiments at different coptis contents. Results of these algorithms were plotted against tumbling times in Fig. 4. All five batches were shown the same conclusion: varied values obtained at the beginning of blending process eventually fluctuated in a small range around a stable value (100 is perfect for similarity method, 0 is perfect for moving block and mean square methods), which suggest high homogeneity of the mixture.

In the similarity calculation, the last five spectra of each batch were used as reference instead of one mean spectrum (generally used). The total sample spectra involved in the calculation of the moving block method was small, so that a window size of three spectra was chosen. Mean square methods calculated the differences between two consecutive spectra. Hence, similarity, moving block and mean square methods had 20, 23 and 24 point separately for each batch.

3.3. *Validation*

Herbal medicines from different geographic origins had varied contents; the application of proposed method on diverse materials should be validated. The preprocessing methods and analysis regions were the same with previous works. Although the spectral variations of the four coptis samples were obvious, the blending processes monitored by NIR

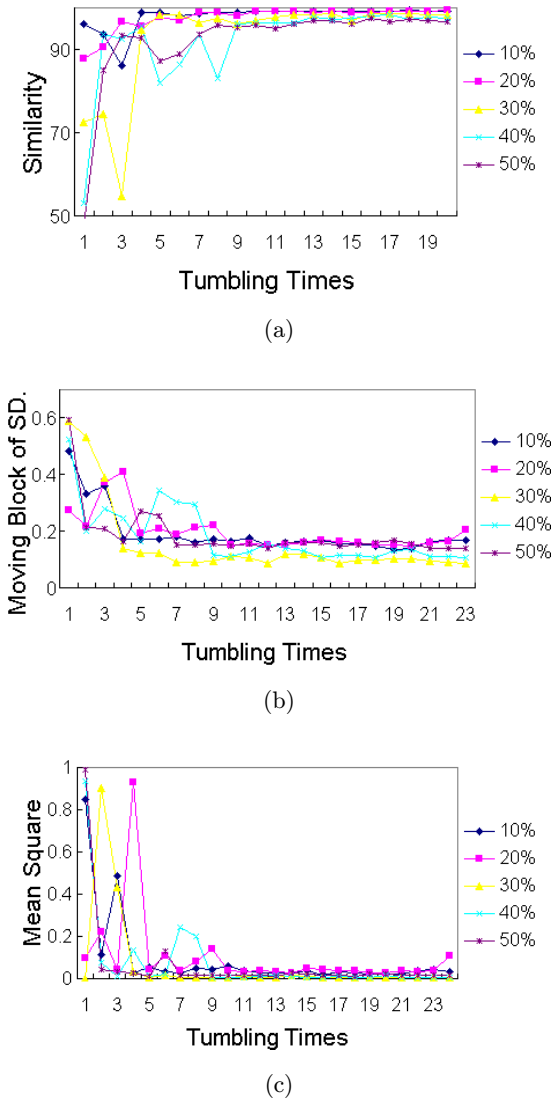

Fig. 4. Similarity (a), moving block values (b) and mean square (c) profiles of all batches at different coptis contents as a function of tumbling times.

technique were similar with previous experiments. As shown in Fig. 5, the trendline of each algorithm became stable after 10 to 15 rotations.

All spectra obtained in the blending simulation of "liu wei di huang" pill were preprocessed as the previous binary blending. After the calculation of correlation spectrum, two regions (9881–7225 and 6117–4161 cm^{-1}) were selected for further analysis. Ten homogeneous mixtures with different contents ratios were made to generate the correlation spectrum. As shown in Fig. 6, the results were same as the binary mixtures.

In the first few rotations, some mixtures in the bottles were near homogeneous but others were

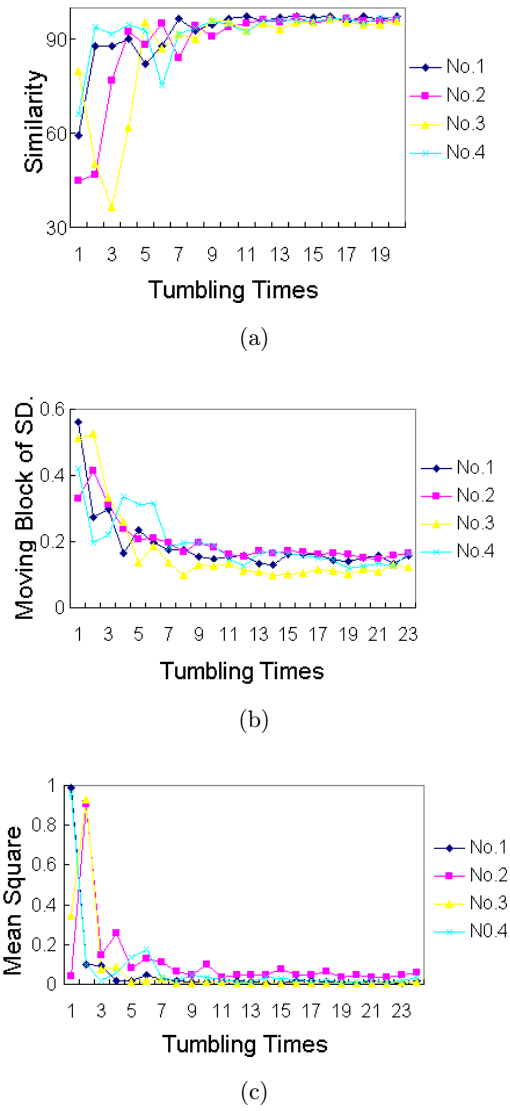

Fig. 5. Similarity (a), moving block values (b) and mean square (c) profiles of four different coptis samples as a function of tumbling times.

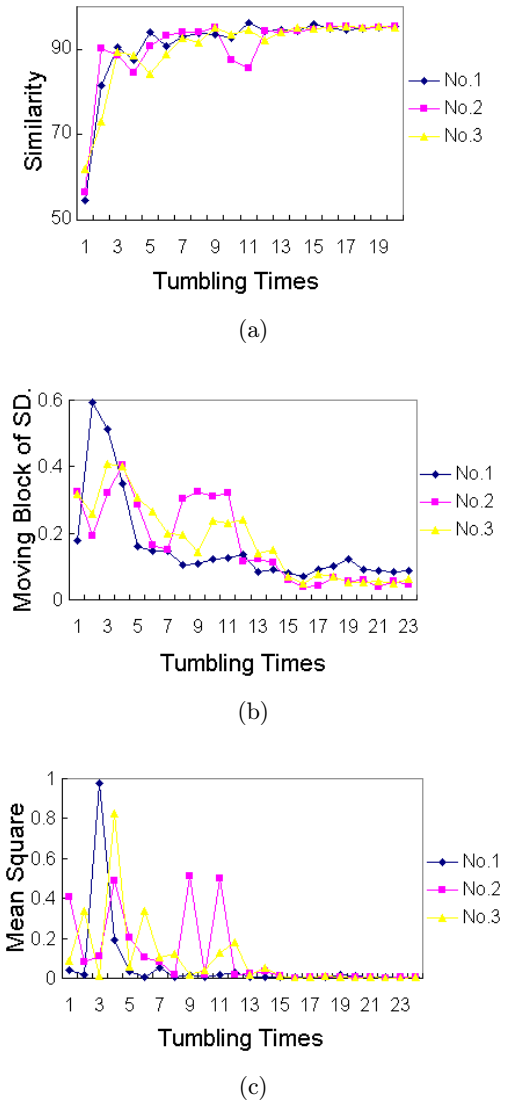

Fig. 6. Similarity (a), moving block values (b) and mean square (c) profiles of the simulated "liu wei di huang" blending as a function of tumbling times.

far from uniformity, these spectra had obvious deviations. After 15 tumbling times, some may be earlier, all mixtures approached uniformity and the NIR spectra were close. Pattern recognition algorithms like SIMCA, etc. had difficulties since spectra of early rotations near homogeneous might be clustered with the latter. Similarity method compared spectra with last five spectra, these reference spectra were recorded from uniform mixtures. Moving block and mean square methods compared with adjacent spectra, which were far from uniformity in early stages of the blending process. Although the curves of these methods were quite different at first, their trends became the same later, which meant the uniformity of mixture was achieved. As shown in the figures, moving block and mean square methods were more sensitive to spectral variations; their values might be significantly affected by spectral outliers. They could be applied in the on-line monitoring of blending process. The similarity method was more robust, anomalous spectra had fewer effects; but it cannot be used on-line since the reference spectra were obtained from uniform mixtures. Therefore, moving block and mean square methods can be used to determine the mixture uniformity in the blending process, and after the certification of end point, similarity method can be applied for further verifications.

4. Conclusion

In China, none of the modern scientific analysis techniques were used in the end-point determination of herbal powder mixtures. This paper is using current NIR spectroscopic technique and chemometric algorithms to determine the homogeneity in blending process of herbal powders, which were difficult to be analyzed by traditional methods like HPLC or UV spectroscopy. After preprocessing methods, the focus was on the chemical composition of the blend components. A correlation spectrum was calculated to choose spectral regions with high coefficients. It was difficult to identify homogeneity from NIR spectra by visual inspection. Calculations, like similarity, moving block and mean square, make the variation in the NIR spectra during the process as a function of tumbling times obvious and precise. NIR technique and qualification methods monitored the blending process of herbal materials despite of various differences according to location of growth, climate and age, etc. Determination of the simulated blending process in manufacturing of TCM was also successful. The criterion used to characterize the blending process of herbal medicines in this paper can be extrapolated to industrial scales.

The proposed NIR methods avoid invasive sampling errors and provide the possibility of on-line determination that can be used in herbal homogenization studies with expeditious and accurate results, and have the potential to be applied as a quality control technique in TCM manufacturing.

Acknowledgments

Financial support from China Postdoctoral Science Foundation Special Funded Project (2013T60604) and Zhejiang Provincial Public Welfare Application Project of China (2012C21102) is gratefully acknowledged.

References

1. A. S. El-hagrasy, H. R. Morris, F. D'amicao, R. A. Lodder, J. K. Drennen, "Near-infrared spectroscopy and imaging for the monitoring of powder blend homogeneity," *J. Pharm. Sci.* **90**, 1298–1307 (2001).
2. M. Blanco, R. G. Bano, E. Bertran, "Monitoring powder blending in pharmaceutical processes by use of near infrared spectroscopy," *Talanta* **56**, 203–212 (2002).
3. D. J. Wargo, J. K. Drennen, "Near-infrared spectroscopic characterization of pharmaceutical powder blends," *J. Pharm. Biomed. Anal.* **14**, 1415–1423 (1996).
4. S. S. Sekulic, H. W. WardII, D. R. Brannegan, E. D. Stanley, C. L. Evans, S. T. Sciavolino, P. A. Hailey, P. K. Aldridge, "On-line monitoring of powder blend homogeneity by near-infrared spectroscopy," *Anal. Chem.* **68**, 509–513 (1996).
5. P. A. Hailey, P. Doherty, P. Tapsell, T. Oliver, P. K. Aldridge, "Automated system for the on-line monitoring of powder blending processes using near-infrared spectroscopy Part I. System development and control," *J. Pharm. Biomed. Anal.* **14**, 551–559 (1996).
6. S. S. Sekulic, J. Wakeman, P. Doherty, P. A. Hailey, "Automated system for the on-line monitoring of powder blending processes using near-infrared spectroscopy Part II. Qualitative approaches to blend evaluation," *J. Pharm. Biomed. Anal.* **17**, 1285–1309 (1998).
7. O. Berntsson, L. G. Danielsson, S. Folestad, "Characterization of diffuse reflectance fiber probe sampling on moving solids using a Fourier transform near-infrared spectrometer," *Anal. Chim. Acta.* **431**, 125–131 (2001).
8. O. Berntsson, L. G. Danielsson, B. Lagerholm, S. Folestad, "Quantitative in-line monitoring of powder blending by near infrared reflection spectroscopy," *Powder Tech.* **123**, 185–193 (2002).
9. H. Martens, J. P. Nielsen, S. B. Engelsen, "Light scattering and light absorbance separated by extended multiplicative signal correction. Application to near-infrared transmission analysis of powder mixtures," *Anal. Chem.* **75**, 394–404 (2003).
10. J. H. Cho, P. J. Gemperline, P. K. Aldridge, S. S. Sekulic, "Effective mass sampled by NIR fiber-optic reflectance probes in blending processes," *Anal. Chim. Acta.* **348**, 303–310 (1997).
11. Y. A. Woo, H. J. Kim, J. H. Cho, H. Chung, "Discrimination of herbal medicines according to geographical origin with near infrared reflectance spectroscopy and pattern recognition techniques," *J. Pharm. Biomed. Anal.* **21**, 407–413 (1999).
12. M. Laasonen, T. Harmia-Pulkkinen, C. L. Simard, E. Michiels, M. Rasanen, H. Vuorela, "Fast identification of *Echinacea purpurea* dried roots using near-infrared spectroscopy," *Anal. Chem.* **74**, 2493–2499 (2002).
13. W. J. McCarthy. *TQ Analyst User's Guide*, Thermo Nicolet Corp., Madison, WI (2000).
14. A. Savitzky, J. E. Golay, "Smoothing and differentiation of data by simplified least squares procedures," *Anal. Chem.* **36**, 1627–1639 (1964).