Monitoring As and Hg variation in An-Gong-Niu-Huang Wan (AGNH) intermediates in a pilot scale blending process using laser-induced breakdown spectroscopy

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Monitoring As and Hg variation in An-Gong-Niu-Huang Wan (AGNH) intermediates in a pilot scale blending process using laser-induced breakdown spectroscopy

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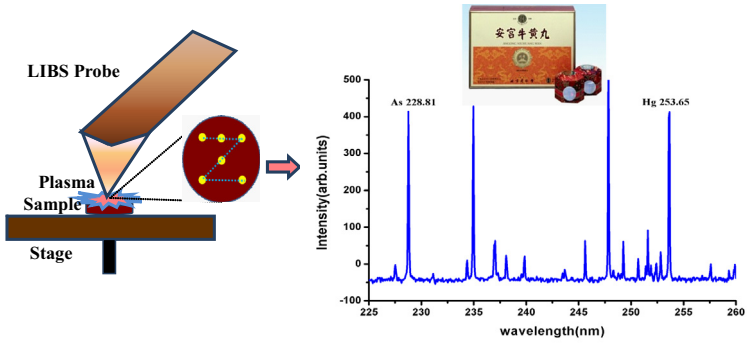
HIGHLIGHTS

- As and Hg emission lines could be determined by LIBS in complex matrix effects.
- Blending end-point was determined by RICR, MWSD and RCCR algorithms.
- LIBS result was consistent with ICP-OES at blending end-point determination.
- LIBS is a viable technique for monitoring the blending process of mineral medicines.

GRAPHICAL ABSTRACT



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ABSTRACT

Laser-induced breakdown spectroscopy (LIBS) was used to assess the cinnabar and realgar blending of An-Gong-Niu-Huang Wan (AGNH) in a pilot-scale experiment, including the blending end-point. The blending variability of two mineral medicines, cinnabar and realgar, were measured by signal relative intensity changing rate (RICR) and moving window standard deviation (MWSD) based on LIBS. Meanwhile, relative concentration changing rate (RCCR) was obtained based on the reference method involving inductively coupled plasma optical emission spectrometry (ICP-OES). The LIBS result was consistent with ICP-OES at blending end-point determinations of both mineral medicines. Unlike the ICP-OES method, LIBS does not have an elaborate digestion procedure. LIBS is a promising and rapid technique to understand the blending process of Chinese Materia Medica (CMM) containing cinnabar and realgar. These results demonstrate the potential of LIBS in monitoring CMM pharmaceutical production.

1. Introduction

An-Gong-Niu-Huang Wan (AGNH) is a famous Chinese Materia Medica (CMM) for brain diseases [1]. It contains herbal, animal and mineral medicines including cinnabar, realgar, *Calculus Bovis*, *Cornu Bubali*, *Margarita*, *Borneolum Syntheticum*, *Rhizoma Coptidis*, *Radix*

Abbreviations: CMM, Chinese material medica; LIBS, laser-induced breakdown spectroscopy; ICP-OES, inductively coupled plasma optical emission spectrometer; PAT, process analytical technology; HPLC, high-pressure liquid chromatography; APOI, active pharmaceutical organic ingredient; AGNH, An-Gong-Niu-Huang Wan; As, arsenic; Hg, mercury; RICR, relative intensity changing rate; MWSD, moving window standard deviation; RCCR, relative concentration changing rate.

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Scutellariae, and other components. [1,2]. Realgar and cinnabar have been used in CMM for thousands of years. Realgar (Xionghuang) contains more than 90% of tetra-arsenic tetra-sulfide and arsenic disulfide (As_4S_4, As_2S_2) [3,4]. Cinnabar (Zhusha) is mercuric sulfide natural ore and the main ingredient is mercury sulfide (96% HgS) [1,3]. Inhomogeneous blending of cinnabar and realgar could lead to poor properties in the final pharmaceutical product.

Blending is thus crucial in the production of CMM [5]. Powder homogeneity is essential to guarantee good bioactivity of solid dosage forms (tablets, pills, and capsules) [6]. Currently, the main method to assess the uniformity of powder mixture is to detect the active pharmaceutical organic ingredient (APOI) in blending intermediates using near-infrared (NIR) spectroscopy, high-pressure liquid chromatography (HPLC) and UV–Vis spectroscopy [7]. However, the evaluation of mineral medicine blending remains unexplored.

Laser-induced breakdown spectroscopy (LIBS) is a direct spectroscopic technique for multi-element analysis of diverse materials including solids, liquids, gases and aerosols with minimal sample preparation [8]. It offers elemental information through the analysis of microplasma induced by a high energetic laser pulse.

Several traditional techniques have been successfully used for elemental analysis including flame atomic absorption spectrometry (FAAS), inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled plasma-mass spectrometry (ICP-MS). The ICP-OES method is a routine analytical technique used for metal determination. It is relatively sensitive and reproducible [9,10]. Because it incorporates charge coupled devices (CCD) as solid-state detectors, ICP-OES can reach high spectral resolution and has an excellent linear response range. The ICP-OES method enables the determination of the selected metals in aqueous and dry samples. However, these advantages are always associated with tedious and time-consuming sample pretreatment, *i.e.* digestion, prior to ICP-OES [11,12].

LIBS is faster than these other methods. It provides remote, real-time, *in-situ* sensing analysis [13]. LIBS can also perform elemental chemical imaging of surfaces with a high spatial resolution [14]. These features have drawn more attention to LIBS' applications [8,15] especially in the analysis of chemicals and hazardous samples [16,17]. Recently, LIBS has been used to detect toxic metals and hazardous contaminants in hair dyes and lipsticks [18,19].

Because it does not require extensive sample preparation, LIBS is highly suitable for on-line analysis. The Food and Drug Administration (FDA) has advocated innovative process analytical technology (PAT) to improve pharmaceutical quality [20]. Thus, researchers have shown great interest in LIBS for rapid detection in the pharmaceutical industry. Mowery et al. investigated the coating thickness and uniformity of tablets using LIBS [21]. St-Onge et al. [22,23] and Yokoyama et al. [24] evaluated LIBS as a rapid and direct method for quantitative analysis of pharmaceutical products [22]. Myakalwar et al. used LIBS to classify pharmaceutical tablets [25], and Mukherjee et al. characterized the carbon-containing aerosolized drugs via LIBS [26].

This study focused on the rapid monitoring realgar and cinnabar in AGNH blending intermediates by LIBS on a pilot scale. Qualitative spectral analysis methods, moving-window standard deviation (MWSD) and relative intensity changing rate (RICR), are based on calculations of spectral variance. Relative concentration changing rate (RCCR) is a quantitative approach. It can describe the blending process in terms of variations in concentration. Versus quantitative approaches, qualitative approaches require less data and are therefore more suitable for early process development stages. In this work, the blending end-point was determined based on qualitative algorithms via LIBS. This was verified by ICP-OES as a reference method. Three methods, *i.e.* RICR, MWSD and RCCR, were used to determine whether the end-point of blending process was appropriate.

2. Materials and methods

2.1. Materials

Pharmaceutical samples (silybum cinnabar, silybum realgar, *Moschus*, *C. Bubali*, *Margarita*, *B. Syntheticum*, *R. Coptidis*, *R. Scutellariae*, and other components) were provided by Beijing Tong Ren Tang Group Co., Ltd (Beijing, China). Concentrated nitric and hydrochloric acids were purchased from Beijing Chemical Works (Beijing, China). Element standard solutions for arsenic (As) and mercury (Hg) were purchased from National Standards of China (1000 mg mL^{-1}, Beijing, China). Deionized water was purified by Milli-Q water system (Millipore Corp., Bedford, MA, USA). All powdered materials were crushed using agate mortars with a maximum particle size of 75 μm before blending.

2.2. Blending process

A stainless-steel blender (Yarong Company, Inc., Shanghai, China) was used for blending. The total weight was 20 kg. Rotation velocity of the blender was 40 rpm. The blending time ranged from 0 min to 43 min. The intermediates were collected at 1.5 min, 2 min, and then every minute later. A total of 44 samples were acquired during the blending process.

2.3. Sample preparation and data acquisition using LIBS technology

2.3.1. Sample preparation

To avoid splashing, the blended intermediates were pressed into 13 mm diameter pellets using a hydraulic press. The press was sustained for 3 min at 20 tons. The spectra were collected on the surface of each pellet by LIBS.

2.3.2. LIBS instrument and parameters

The LIBS instrument integrates a Q-switched Nd:YAG laser (Dawa200 Series, Beamtech Optronics Ltd., Beijing, China) with a maximum of 200 mJ per pulse and 3–5 ns pulse duration (FWHM) at 1064 nm. The repetition rate and emergence angle are 20 Hz and 1 mrad, respectively. Plasma light was collected by a focal lens at a 45° angle through the optical fibers, which were connected to a spectrometer (HR 2000+ Ocean Optics, USA). The continuous wavelength ranged from 200 nm to 900 nm with a nominal resolution of about 0.1 nm. The spectra were recorded with a channel-charge-coupled device (CCD) detector and analyzed by OOILIBS software (Ocean Optics, USA). In this study, spectra were acquired at 140 mJ pulses energy, 2 μs delay time and 2 ms gate width. Each set of spectral data was the average of 6 ablation pulses at different locations on the sample surface. To ensure a fresh spot, pellets were placed on the 3D motion platform. Each LIBS spectrum provided more than 13,600 data points. Data analysis was performed with OriginPro 8 software.

2.4. Reference method using ICP-OES

To compare the results of LIBS, As and Hg concentrations of blending intermediates were measured simultaneously by ICP-OES. A 1600 W microwave oven (MARS-X, CEM Microwave Accelerated Reaction System, ANALYX CORP, USA) was used for sample digestion. The 100 mg of each sample was placed in a closed Teflon tank with 4 mL of concentrated nitric acid (HNO_3) and 3 mL of hydrochloric acid (HCl). The pressure of the oven was controlled at 0.5 MPa for 1 min and then at 1.0 MPa for

3 min. The solution was transferred to a 25 mL volumetric flask using deionized water. The microwave-assisted acid digestion conditions are listed in Table 1.

The ICP-OES measurements were carried out using an Optima 2000 DV (Perkin-Elmer Instruments Co., Ltd., USA) with the following instrument conditions. The RF power was 1.5 kW, the plasma gas flow was 15 L min^{-1}, the auxiliary flow was 0.2 L min^{-1}, the nebulizer gas flow was 0.65 L min^{-1}, and the solution uptake was 1 mL min^{-1}. Mono-elemental standard solution was used. The analytical lines of As and Hg elements were 193.696 nm and 253.652 nm. Regressions were calculated across the linear range of 0–1000 ppm to be $y = 770.2x + 1475.6$, $R^2 = 0.9999$ (Hg) and $y = 26.1x + 93.3$, $R^2 = 0.9999$ (As).

2.5. Data analysis

2.5.1. Data analysis for LIBS

2.5.1.1. End-point determined by peak relative intensity changing rate (RICR). The RICR was applied to monitor the blending process and determine the blending end-point. RICR refers to the signal intensity variation between two consecutive time points at a selected wavelength which can be calculated from Eq. (1):

$$\mathrm{RICR} = \frac{A_{t+1} - A_t}{A_t} \times 100\% \quad (1)$$

where A_t is the elemental intensity recorded at a given time t, and A_{t+1} is the elemental intensity recorded at a given time $(t + 1)$.

2.5.1.2. End-point determined by moving window standard deviation (MWSD). The other qualitative method, MWSD, was used to determine the blending end-point. For each formulation, a plot of the moving standard deviation (S.D.) versus blending time at selected windows (a set of n consecutive spectra) was made to examine the process trend. In this study, three consecutive spectra ($n = 3$) were selected as an appropriate window size [13]. The MWSD values were calculated according to the following Eq. (2):

$$\mathrm{MWSD} = \sqrt{\frac{\sum_{i=1}^{n} A_{ij-} \bar{A}_i}{n-1}} \quad (2)$$

where A_{ij} is the intensity at selected wavelength i at blending time j, and $\bar{A}_i$ is the mean intensity of n consecutive spectra. Term n is the window size.

2.5.2. End-point determination method for ICP-OES

The similar end-point determination protocol was employed for ICP with the following modifications. Similar to the Eq. (1), the value of relative concentration changing rate (RCCR) reflects the concentration variations between two adjacent spectra. The RCCR values were calculated using the following equation:

$$\mathrm{RCCR} = \frac{C_{t+1} - C_t}{C_t} \times 100\% \quad (3)$$

where C_t is the concentration of a measured element at a given time t, and C_{t+1} is the concentration recorded at a given time $(t + 1)$.

Table 1
Heating program parameters of closed-vessel microwave-assisted acid digestions.

Step	Ramp (min)	Power (W)	Control (°C)	Hold (min)
1	08:00	1600	120	01:00
2	06:00	1600	160	05:00
3	04:00	1600	180	20:00

3. Results and discussion

3.1. Raw spectra of LIBS

Representative LIBS spectra of AGNH intermediates at different time points (0 min, 15 min, 30 min and 43 min) are shown in Fig. 1. Obvious differences in the spectra could be observed. In addition, matrix effects appeared at 590–610 nm, but did not interfere with the signals of As and Hg.

3.2. Determination of As and Hg emission lines using LIBS

The optimal elemental lines were selected for further analysis. The emission lines of 228.81 nm (As) and 253.63 nm (Hg) were assigned according to the NIST spectral database and previous works [27–29]. From 225 to 260 nm, the two selected emission lines were absolutely isolated with no overlapping peaks (Fig. 2).

3.3. Time profile during blending process using ICP-OES and LIBS

Fig. 3 shows visual blending trends on the concentration and signal intensities of As and Hg recorded using ICP-OES and LIBS. During the blending process, a dramatic trend difference was observed at the initial stage. This indicated a high mixing efficiency and a fast dynamic blending stage. The plots gradually became flat with further blending, which illustrated that the blending process reached a stable stage. With ICP-OES, the concentrations varied dramatically during the first 20 min for As and the first 12 min for Hg before becoming steady (Fig. 3a). Similarly, the signal intensities changed dramatically during the first 35 min in LIBS (Fig. 3b). Importantly, it is difficult to determine the end-point through visual inspection alone.

3.4. Blending investigation of the end-point

3.4.1. Homogeneity analysis of As

Figs. 4 and 5 show the results of As analysis using LIBS and ICP-OES, respectively. The homogeneity analysis of As with LIBS exhibited four different phases (Fig. 4b). First, the RICR and MWSD values showed a high variability in phase A (0–27 min). In addition, the RICR and MWSD values were small and stable in phase B (28–32 min). With further blending, these values varied dramatically in phase C (32–38 min). The steady state then appeared in phase D (38–43 min). Meanwhile, the RICR result was coincident with MWSD in phase D indicating that the blending was homogeneous for As. Clearly, the blending process was unstable at phase A and C as shown by RICR and MWSD. Large variations in RICR and MWSD data in phase C demonstrated that back-mixing occurred similar to prior reports [30]. Generally, the terminal blending stage of As was phase D (38–43 min), which was approximately consistent to ICP-OES (stage 4, Fig. 5b). Thus, the blending end-point of As was 38 min.

3.4.2. Homogeneity analysis of Hg

Figs. 6 and 7 show the Hg results using LIBS and ICP-OES, respectively. The Hg also had four different phases by LIBS (Fig. 6). First, phase A showed an initial decline in the RICR and MWSD values within 3 min. After that, both values varied from 3 min to 20 min. Then, there was a trend of uniformity in phase B from 20 min to 27 min (Fig. 6b). However, the mixture in phase B was not homogeneous because of the higher values of RCCR from 17 min to 33 min (stages 3, Fig. 7b). Similarly, de-mixing was observed during phase C (27–37 min). Finally, a minimum value occurred from 37 min to 43 min (phase D, Fig. 6b), which indicated a general terminal phase of Hg. This trend was further verified by

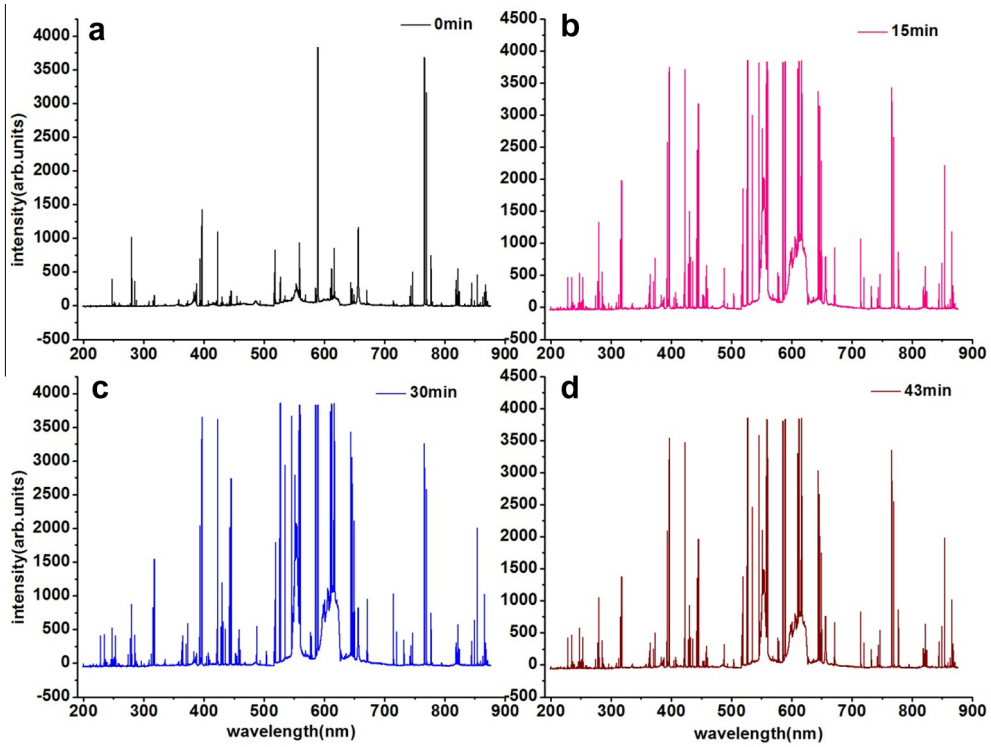

Fig. 1. Representative LIBS spectra of AGNH intermediates during blending process. (a) 0 min, (b) 15 min, (c) 30 min, and (d) 43 min.

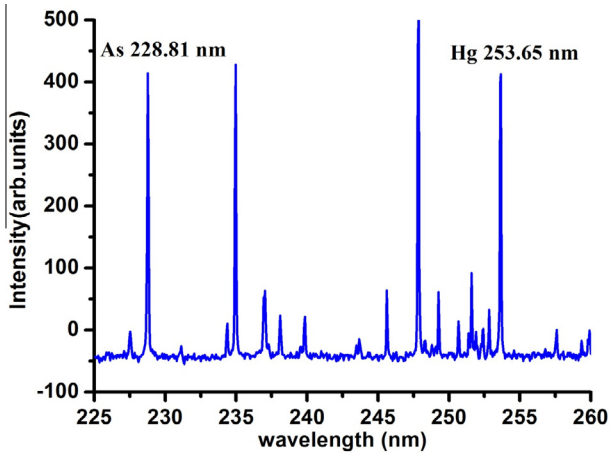

Fig. 2. LIBS spectrum of AGNH with the 228.81 nm As(I) and 253.65 nm Hg(I) lines.

ICP-OES (stage 4, Fig. 7b). Hence, the blending end-point of Hg was estimated to be 37 min.

3.5. Comparison of end-point identification

LIBS and ICP-OES techniques performed comparably during the blending process analysis, especially for measuring the end-point of cinnabar and realgar mixing. Results of both techniques showed a 4-stage mixing profile. Generally, both independent techniques verified each other and the intermediates were homogenous at the fourth stage. The results indicated that the end-point for both minerals in the AGNH blending process was 38 min.

Although direct comparison between two techniques did not produce completely consistent results, it has more practical merits. With regard to two elements, the shapes of mixing profiles also did not completely match. There are many factors to which these differences could be attributed, *i.e.* different components have different physical properties such as particle size, particle shape, and

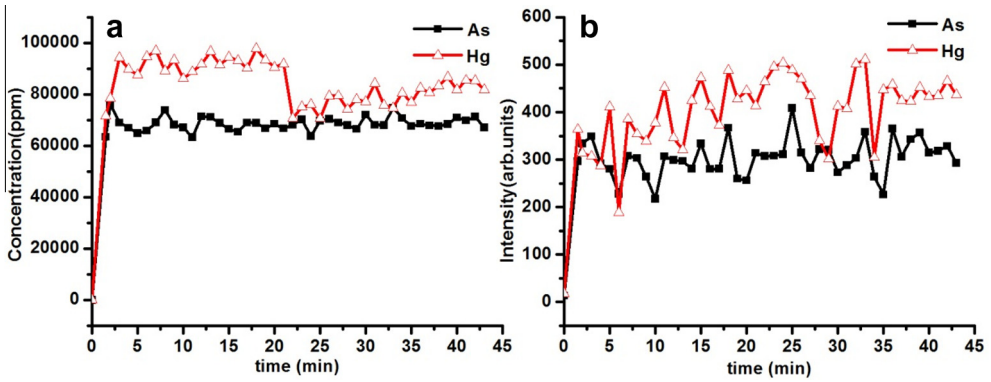

Fig. 3. As and Hg concentration/intensity profiles during blending process. (a) ICP-OES, (b) LIBS.

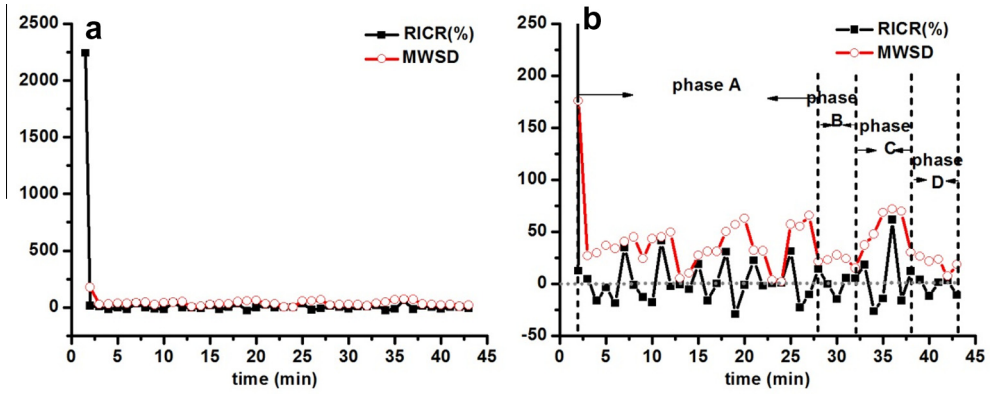

Fig. 4. Homogeneity analysis of As during blending process by LIBS. (a) Denoted the RICR/MWSD profiles, (b) the enlargement of (a).

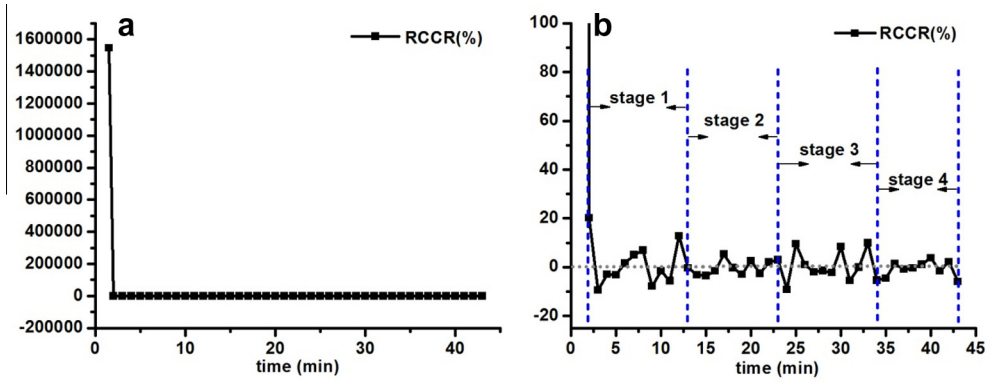

Fig. 5. Homogeneity analysis of As during blending process by ICP-OES. (a) Denoted the RCCR profile, (b) the enlargement of (a).

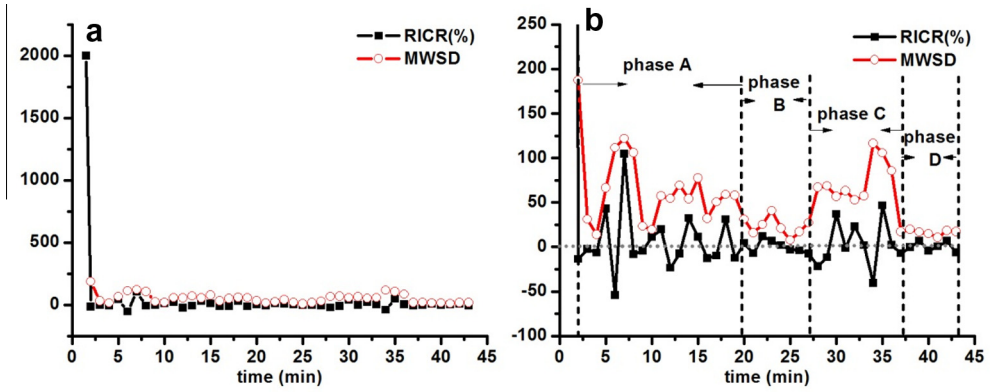

Fig. 6. Homogeneity analysis of Hg during blending process by LIBS. (a) Denoted the RICR/MWSD profiles, (b) the enlargement of (a).

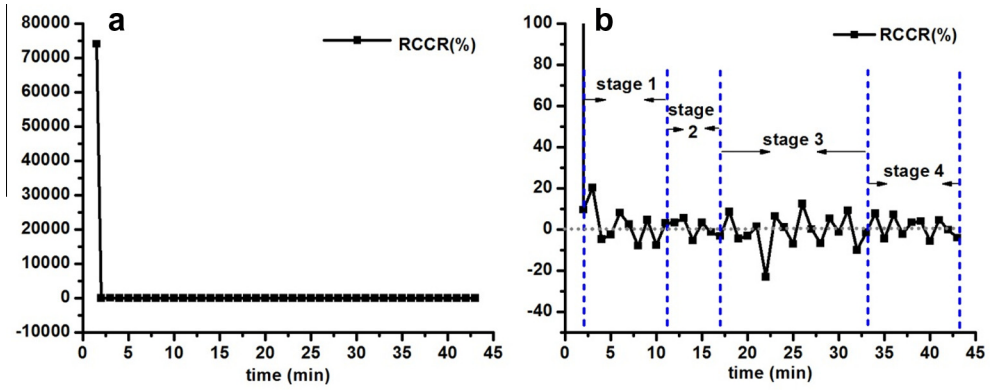

Fig. 7. Homogeneity analysis of Hg during blending process by ICP-OES. (a) Denoted the RCCR profile, (b) the enlargement of (a).

flow properties thus displaying different process behaviors. Hence, the time required to reach blending end-point for different mineral components could differ even for the same formulation.

4. Conclusion

Rapid analysis of mineral medicine blending uniformity is increasingly significant for CMM production. This study explored the feasibility of monitoring cinnabar and realgar powder blending during CMM production in a pilot scale through LIBS. The qualitative spectral analysis based on LIBS and the quantitative based ICP-OES demonstrated good performance for blending end-point determination by accounting for both spectral variances and constituent concentrations. Compared to ICP-OES, LIBS is a promising process analytical technique for rapid monitoring cinnabar and realgar in the pharmaceutical blending of AGNH. This study provided knowledge about the blending process of CMM samples containing minerals.

LIBS has many merits and is an evolving elemental analysis technique with tremendous potential applications in CMM. Further LIBS studies will focus on the quantitative analysis of CMM.

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