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Development of personalized health monitoring using ultra-weak photon emission based on systems medicine concepts

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Chapter 4

Delayed luminescence: an experimental protocol for Chinese herbal medicines

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Abstract

In traditional Chinese medicine, raw herbal materials are used in processed and unprocessed form aiming to meet the different requirements of clinical practice. To assure the chemical quality and therapeutic properties of the herbs, fast and integrated systematic assays are required. So far, such assays have not been established. Delayed luminescence (DL) refers to a decaying long-term ultra-weak photon emission after exposure to light. Its decay kinetics under certain conditions may be a sensitive indicator reflecting the internal structural and chemical/physiological state of a biological system. DL measurements have been used in many applications for quality control. However, relatively little research has been reported on dried plant material such as Chinese herbs. The objective of the present study is to establish a protocol for direct and rapid DL measurements of dried Chinese herbal materials, including the determination of the dependence on: a.) the optimal excitation time utilizing a white light source; b.) the optimal size of the grinded herbal particle; and c.) the humidity conditions before and during measurement. Results indicate that stable and reproducible curves of DL photon emission depend mainly on the water content of herbal materials. To investigate the application of the established DL measurement protocol, non-processed and processed *Aconitum* (*Aconitum carmichaelii* Debx.), wild and cultivated rhubarb (*Rheum palmatum* L.) and ginseng (*Panax ginseng* C.A. Mey.) of different ages were measured using DL. The results suggest that DL technology is a potential tool for assessment of dried Chinese herb qualities. The results warrant a further exploration of this technique in relation to therapeutic properties of the herbs.

Keywords: delayed luminescence (DL), Chinese herbal medicine, quality control, excitation time, particle size, water content.

1. Introduction

Delayed luminescence (DL) refers to a long-term, decaying, weak photon emission shown by various materials after exposure to light. It is a characteristic property of some inorganic and organic materials and has also been described for living organisms.¹⁻⁶ The difference between DL and fluorescence lies in the time of decay of the excited state. Fluorescence usually refers to photon emission that fades in nanoseconds or picoseconds. Light that fades in milliseconds or more is called DL.⁷ The kinetics of the DL decay depend on the excitation conditions including excitation energy and excitation time.^{8,9}

DL has been utilized as a tool for rapid examination of a biological system and found to be a sensitive indicator for its chemical/physiological state.¹⁰⁻¹² A typical application is in the field of food quality control.¹³ Estimations of DL from various water-rich vegetables and fruits showed relationship of DL to development and maturity (ripeness).¹⁴⁻¹⁶ Another DL application is in the field of germination capacity of seeds.¹⁷⁻²⁰ DL measurement may be able to correlate to the functional state of biological system in living materials.¹⁸ Therefore, we ask whether DL could also represent specific properties of dried plant materials such as Chinese herbal medicines.

A few recent studies have focused on the DL signatures of dry powders of Chinese herbal medicines.^{21,22} They focused on therapeutic properties (“heat” and “cold”) of herbal medicines and demonstrated that DL dynamics differ between “heat” and “cold” herbal medicines.²² Chemical analysis (LC/MS, GC/MS and NMR) has also been used to study the different properties of herbal medicines. However, it is still not evident why herbs with different therapeutic properties can have similar chemical constituents, while herbs with the same therapeutic effect have completely different chemical constituents.²³⁻²⁵ Therefore, DL may be a candidate technology to indicate additional, unknown properties of Chinese herbal medicines.

At present, there is no detailed experimental protocol described the use of DL technology in dried herbal materials. The aim of this paper is to present such protocol. A description of the DL signal from dry powders of *Aconitum* root is presented, focusing on how DL depends on a.) time of excitation; b.) the size of grain in powdered herbal materials; c.) moisture; as well as d.) reproducibility of the signal when samples are stored under specific dry conditions. Based on an established protocol, materials from dried roots were compared: a.) in case of *Aconitum* the effect of processing; b.) in rhubarb wild and cultivated materials from different areas; c.) in ginseng, different ages were compared. The results show that the different DL dynamics appear between processed and unprocessed herbs, as well as the herbs with different environmental factors and ages.

2. Materials and methods

2.1 Herbal materials

Raw roots of *Aconitum* (*Aconitum carmichaelii* Debx.) were collected by Sichuan new Lotus Pharmaceutical Co. Ltd. in the Jiangyou district, Sichuan province, P. R. China. Other processed root samples of *Aconitum* with white (salt processed method) and black (black bean processed method) colors were obtained from Xiansheng Company, Nanjing city, the P. R. China.

Raw root samples of rhubarb (*Rheum palmatum* L.) were supplied by the China Academy of Chinese Medical Sciences, which harvested in three locations of Gansu province and Sichuan province, the P. R. China. Samples of raw ginseng roots (*Panax ginseng* C.A. Mey.) were provided by Changchun University of Chinese Medicine, which were obtained in Fusong city, Jilin province, the P. R. China.

2.2 Sample preparation

Samples were prepared prior to the measurements by crushing the herbal materials using a grinder (Yili Company, Zhejiang province, the P. R. China, type QE-100).

Different diameter sizes of herbal particles were selected by different-sized sieves (Tongrentang Company, Beijing, the P. R. China, Standard sieve with the size of 74 μ m, 150 μ m, 355 μ m and 600 μ m). Thereafter, herbal samples were stored in a dark box together with silica gel (Boom BV, Netherlands, 3-5mm) at room temperature for at least 16h before the DL measurements started. Self-made processed *Aconitum* samples were prepared from powders of raw *Aconitum* roots by sterilization at 127°C for 60 minutes under high pressure,²⁶ using an autoclave device (Prestige Medical, UK, Series 2100 Classic). After processing the powder was stored in a silica environment.

2.3 Delayed luminescence technology, measurement procedure

Fig.1 illustrates schematically the instrumental set-up for DL measurements. The device includes a dark sample chamber (9.5cm $\times$ 15cm $\times$ 16cm) with a vertically positioned photo-multiplier tube (PMT) (Electron Tubes Enterprises Ltd, Ruislip, UK, type 9558QB). The sample chamber was kept at 22°C. The cathode opening of the PMT has a diameter of 44 mm. The sensitivity of the PMT is in the range between 160 and 870nm. The PMT was cooled to -25°C for reducing the dark count rate to 10 counts per second. A fast preamplifier (ORTEC, U.S.A., type 9301) was used to enlarge the signal of photon emission. A PC with a counting card (National instruments, U.S.A., type 6602) was used for data acquisition.

Samples were measured utilizing a petri dish (diameter: 35mm) placed in the dark chamber at 12cm from the shutter of PMT. One-gram herbal material was used to cover the bottom of the Petri dish. For excitation of the powder, a white halogen excitation source (PHILIPS, German, NO. 284-2812) was used at 10 cm distance from the Petri dish.

The protocol for sample preparation immediately prior to DL measurement included that each herbal batch was used to prepare five samples of 1 g. The DLs of such samples were consecutively measured three times. The total number of 15

measurements of each herb batch was used for analyzing the DL of the particular herb. DL kinetics were obtained by recording the number of counts over consecutive periods of 0.05 sec for a total period of 5min resulting in 6000 data points.

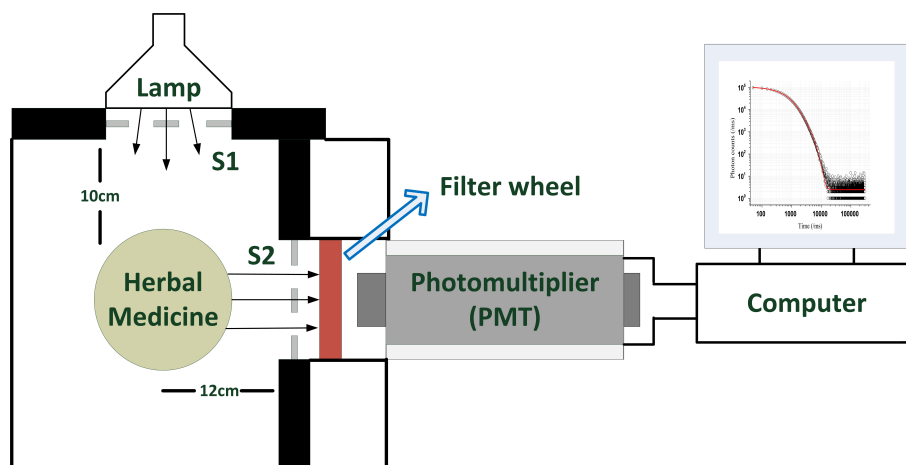


Fig. 1 Schematic diagram of delayed luminescence set-up. Excitation stage: When the excited light irradiated the herbal sample, shutter 1 (S1, shutter between excitation source and Petri dish) was opened and shutter 2 (S2, shutter between Petri dish and photomultiplier) was closed. Emission measurement stage: It begins immediately after the end of the light exposure period. When photon emission from sample radiated into the photomultiplier, S2 was opened and S1 was closed. The response time of S2 was 0.1s.

2.4 Data analysis and statistics

The photon counts during the 5min of each decay curve were used to calculate the parameters of the two-exponential decay function:²⁷

$$y = y_0 + A_1 e^{-\frac{x}{t_1}} + A_2 e^{-\frac{x}{t_2}}$$

where A_1 and A_2 are the amplitudes of photon emission of exponential decay components and t_1 and t_2 are time constants in those exponential decays, while y_0 represents the final value of photon emission in DL decay curve. Curves fitting was performed by using ExpDecay 2 (<http://www.originlab.com/doc/Origin-Help/ExpDec2-FitFunc>) of Origin software (OriginLab Corporation, Northampton, MA, United States, Version 9.0).²⁸ Statistical analysis was carried out using SPSS

Statistics software (IBM, Armonk, New York, United States, Version 23.0).²⁹ Unpaired two-tailed tests were performed to estimate differences between conditions (e.g., *Aconitum* samples with different water contents etc.). Paired two-tailed tests were conducted to estimate differences in repeated samples (e.g., *Aconitum* samples at different times). The significant difference was estimated when p -value < 0.05 .

3. Results

For calculating parameters of herbal DL kinetics, a two-exponential decay function was used to fit the observed decay curve. Fig. 2 illustrates a fitting result taking the *Aconitum* root sample as an example. The raw data measured in consecutive 0.05 seconds periods were too scattered for the visualization of the DL decay kinetics. Therefore, 20 consecutive data points were accumulated, resulting in the decay curves represented in Fig. 3 for the three Chinese herbal medicines after the excitation. Data show that both the background noise and the empty Petri dish noise were far lower than the herbal DL signal.

3.1 Procedure for obtaining reproducible DL measurements of dried herbs.

Two types of explorative experiments were performed to establish the measurement protocol. These experiments were carried out with the powders of *Aconitum* root samples. These powders were divided into five independent samples. These samples were stored in separate Petri dishes under dry conditions in the presence of silica gel (500 g). The measurements were made with the same amount (1.0 g) of herb powder.

The first explorative study focused on the effect of excitation time on the kinetics of the delayed emission of the sample. Different excitation times were applied ranging from 1 sec to 60 sec. The DL curves of the five independent samples were highly similar under each excitation time. Fig. 4 shows the DL behavior after illumination. Additionally, Table 1 presents the DL parameters for different excitation times. Based on the data in Fig. 4 and Table 1, the excitation time resulting in the highest total photon counts (i.e. 10 sec) was selected for further studies.

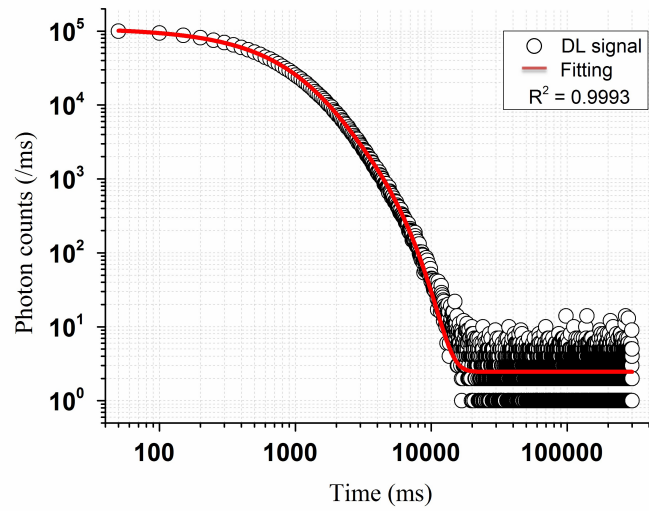


Fig. 2 Fitting effect of the DL decay curve of an *Aconitum* root sample.

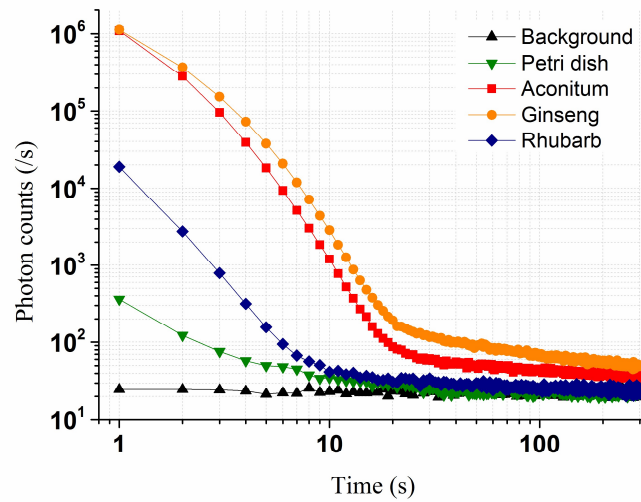


Fig. 3 Comparison of herbal DL signal and the noise from an empty Petri dish and background. Each herbal sample was grinded to approximate 150 μm , and saved with silica gel (500 g) for 16h before measurement. The excitation time was 10 s.

Table 1 DL parameters of *Aconitum* root samples under different excitation time

Excitation time (s)	Photon counts (Mean $\pm$ SD)	A1 (Mean $\pm$ SD)	A2 (Mean $\pm$ SD)	t1 (Mean $\pm$ SD)	t2 (Mean $\pm$ SD)	y0 (Mean $\pm$ SD)
1	1256463.33 $\pm$ 91231.79	96614.67 $\pm$ 8763.66	11648.81 $\pm$ 3820.62	508.67 $\pm$ 29.30	1398.16 $\pm$ 186.34	1.49 $\pm$ 0.24
3	1545725.47 $\pm$ 106928.89	103114.50 $\pm$ 5024.72	10388.50 $\pm$ 2407.08	604.72 $\pm$ 33.82	1674.23 $\pm$ 127.87	1.51 $\pm$ 0.23
5	1621452.20 $\pm$ 70209.91	100366.40 $\pm$ 6125.15	13067.23 $\pm$ 3856.46	616.72 $\pm$ 36.31	1668.37 $\pm$ 156.14	1.74 $\pm$ 0.27
10	1642700.67 $\pm$ 62888.33	99917.40 $\pm$ 4313.14	13155.96 $\pm$ 1919.77	623.01 $\pm$ 24.13	1688.84 $\pm$ 99.30	1.85 $\pm$ 0.27
30	1587917.93 $\pm$ 89464.48	95751.15 $\pm$ 3785.44	14634.53 $\pm$ 1345.91	604.34 $\pm$ 30.84	1611.06 $\pm$ 95.25	2.31 $\pm$ 0.30
60	1542705.53 $\pm$ 120026.23	94100.47 $\pm$ 4122.80	15185.18 $\pm$ 1490.20	587.85 $\pm$ 41.86	1564.39 $\pm$ 126.88	2.56 $\pm$ 0.44

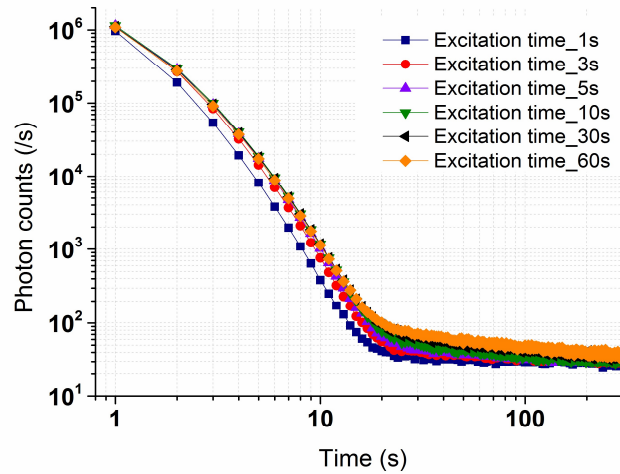


Fig. 4 Relationship between *Aconitum* DL-dynamics and excitation time. In total, 15 measurements of five independent *Aconitum* samples were averaged to obtain the DL dynamics under each excitation time.

The second type of explorative experiments was related to test reproducibility as well as stability of samples over a period of 3 weeks. For this purpose, the aforementioned five independent *Aconitum* root samples were used. Each sample was repeatedly excited and analyzed for its DL within 2 h utilizing a protocol including four periods, with each period including again three consecutive tests within 17min, and an interval of 17min between each two periods. In order to maintain the same excitation conditions in consecutive exposures, samples were kept

in the dark chamber before the next measurement period took place. The 12 curves of each sample were highly similar within 2 h suggesting that excitation has no influence on the DL properties of the sample. Therefore, the 12 DL curves of the same sample were averaged in order to represent the DL of that sample. To study the stability of the samples, this procedure was repeated with the same samples over a period of 3 weeks. Fig. 5A shows the results from the five independent samples measured with this protocol in the first week, and the DL decay curves are similar. Similar DL curves were also obtained for these five samples in the second and third weeks (data not shown). Therefore, the estimated differences of DL parameters between the 3 weeks were estimated using paired two-tailed tests (Fig. 5B). The results showed that most DL parameters could significantly differ (p -value < 0.05) between weeks, except y_0 between week 1 and week 3 (p -value = 0.067) and y_0 between week 2 and week 3 (p -value = 0.140). The differences were not unidirectional and must be attributed to yet unknown storage conditions.

3.2 Effects of powder grain size on DL

In the previous section, the DL decay kinetics was estimated using a 150 μ m grain size of the powdered *Aconitum* root. In studies utilizing organic materials, it has been reported that grain size has influence on DL features.^{30,31} In the present study the effect of grain size of *Aconitum* powder on DL was estimated using different particle sizes, ranging from 74 μ m to 600 μ m. Herbal particle size larger than 600 μ m has also been explored. However, with a powder size larger than 600 μ m, the surface of the Petri dish could not be completely covered with the standard 1.0 g of herbal material. Each measurement of a specific particle size involved five independent *Aconitum* samples. Fig. 6 illustrates the DL curves of the *Aconitum* powder samples with particles of different size after 10 sec excitation time. The data demonstrate that initial values of emission differ for the various grain sizes. Table 2 demonstrates the relationship between DL parameters and powder size. The maximum of total DL was observed with grain size of 150 μ m. The parameters reflecting DL dynamics are

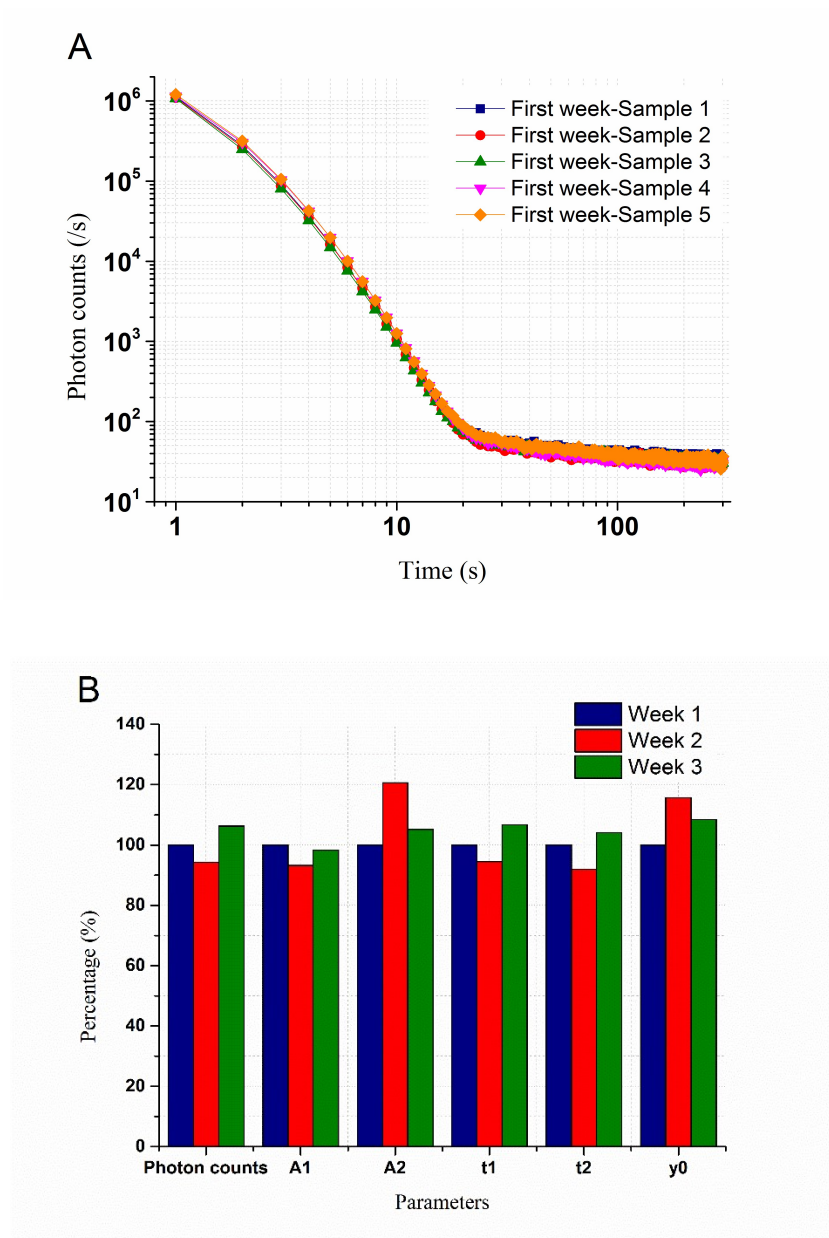


Fig. 5 Stability and repeatability tests of *Aconitum* root samples. (A) DL dynamics of five independent *Aconitum* samples in the first week. (B) Variations of DL parameters of *Aconitum* root samples in 3 weeks. The value of each parameter in the first week was fixed as 100%. The relative percentage of each parameter in both second and third week was compared with that in the first week.

shown in Table 2. They also demonstrate that DL dynamics varies with grain size. Interestingly, A1 and A2 show opposite behavior. The size of 150 μm was selected for standardizing the grain size in further studies.

Table 2 DL parameters of *Aconitum* root samples under different powder size

Size (μm)	Photon counts (Mean $\pm$ SD)	A1 (Mean $\pm$ SD)	A2 (Mean $\pm$ SD)	t1 (Mean $\pm$ SD)	t2 (Mean $\pm$ SD)	y0 (Mean $\pm$ SD)
74	788134.00 $\pm$ 45833.48	62255.12 $\pm$ 6167.34	14930.44 $\pm$ 5572.62	375.69 $\pm$ 15.86	1280.78 $\pm$ 174.00	2.16 $\pm$ 0.52
150	1642700.67 $\pm$ 62888.33	99917.40 $\pm$ 4313.14	13155.96 $\pm$ 1919.77	623.01 $\pm$ 24.13	1688.84 $\pm$ 99.30	1.85 $\pm$ 0.27
355	984808.60 $\pm$ 57288.06	67765.75 $\pm$ 3675.07	20335.40 $\pm$ 2555.35	397.93 $\pm$ 14.43	1160.66 $\pm$ 41.07	2.94 $\pm$ 0.25
600	777038.60 $\pm$ 95133.52	52951.56 $\pm$ 3610.42	20726.16 $\pm$ 1597.95	344.95 $\pm$ 22.35	1030.68 $\pm$ 57.45	3.02 $\pm$ 0.16

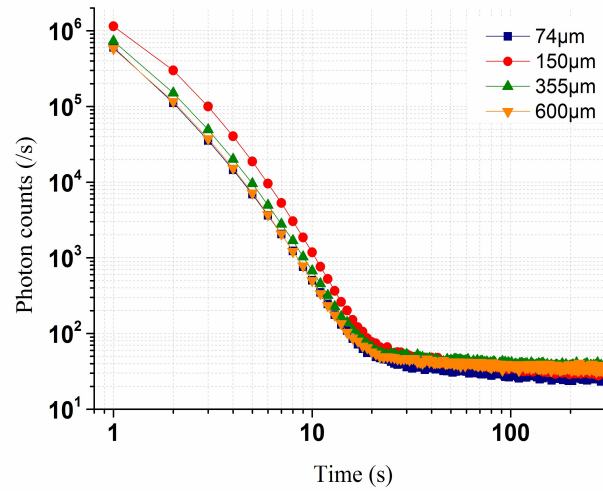


Fig. 6 Effects of powder size on DL of *Aconitum* root samples. In total, 15 measurements of five independent *Aconitum* samples were averaged to obtain the DL dynamics under each powder size.

3.3 Effects of water content on DL

As described in literature, there is a strong influence of the water content of the samples on the DL characteristics.^{32–34} For that reason the herbal samples for research were usually stored in a silica-dried environment. However, to study the effect of silica to decrease humidity, the DL of *Aconitum* was estimated from silica-dried samples and samples that were measured without silica for storage. The

condition without silica-dried storage resulted in a sample water content of 9.4% as compared to 6.6% with silica-dried conditions. The measurement protocol was the same as that used in previous tests of reproducibility and stability. Fig. 7 illustrates the changes of photon emission of those two *Aconitum* samples during the four measurement periods. For each measurement period the difference in response between the two water content conditions were significant (p -value < 0.05). The variations of four periods were evaluated using relative standard deviation (RSD). The variations of silica-dried samples (RSD:1.55%) were far lower than that of non-silica-dried samples (RSD: 9.26%) in all 12 measurements. Table 3 represents the data of other parameters over the four periods. The differences of parameters were significant (p -value < 0.05) between the two humidity conditions. In addition, the RSD values of all parameters were smaller than 5% with the silica-dried condition, which indicated relatively small variations over the four measurement periods. In contrast, four parameters (A1, t1, t2 and photon counts) showed RSD values higher than 5% without silica-dried condition, which reflected relatively large variations. In conclusion, whereas DL of silica-dried *Aconitum* was stable over time, the *Aconitum* samples without silica, show relatively strong changes which can be related to an increasing water content. It is obvious that non-controlled humidity affects DL, hence the strictly control of humidity is important and necessary.

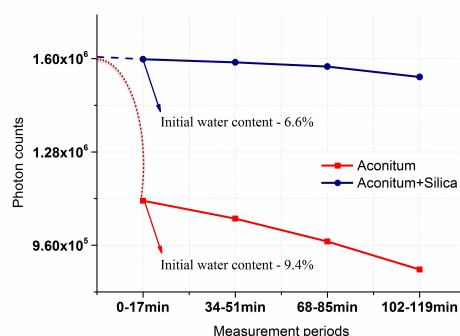


Fig. 7 Effects of water content on photon counts of *Aconitum* root samples. Solid lines present the real variations of photon counts in the measurements of the four periods. Dotted lines show possible variations of photon counts with changes in water content.

Table 3 DL parameters of *Aconitum* root samples under different stored conditions

Initial water content	Periods (Mean $\pm$ SD)	Photon counts (Mean $\pm$ SD)	A1 (Mean $\pm$ SD)	A2 (Mean $\pm$ SD)	t1 (Mean $\pm$ SD)	t2 (Mean $\pm$ SD)	y0 (Mean $\pm$ SD)
6.60%	0-17min	1597930.67 $\pm$ 1422.59	101174.11 $\pm$ 397.45	602.15 $\pm$ 3.31	12835.77 $\pm$ 499.19	1648.63 $\pm$ 24.73	2.11 $\pm$ 0.02
	34-51min	1587248.33 $\pm$ 5192.25	100722.81 $\pm$ 179.98	599.33 $\pm$ 0.53	12917.13 $\pm$ 319.59	1640.59 $\pm$ 11.27	2.04 $\pm$ 0.02
	68-85min	1572848.33 $\pm$ 3105.36	100221.05 $\pm$ 302.59	598.67 $\pm$ 1.74	12634.88 $\pm$ 329.98	1648.26 $\pm$ 16.99	2.02 $\pm$ 0.03
	102-119min	1537294.00 $\pm$ 8357.14	97800.83 $\pm$ 238.82	582.25 $\pm$ 2.71	14048.24 $\pm$ 89.75	1568.78 $\pm$ 5.29	2.14 $\pm$ 0.02
9.40%	0-17min	1112804.33 $\pm$ 8656.56	71425.34 $\pm$ 184.69	439.41 $\pm$ 0.71	21483.04 $\pm$ 397.43	1198.79 $\pm$ 5.30	2.12 $\pm$ 0.07
	34-51min	1051625.33 $\pm$ 18365.84	68117.43 $\pm$ 1293.34	424.23 $\pm$ 9.73	21550.34 $\pm$ 1122.90	1166.43 $\pm$ 27.71	2.54 $\pm$ 0.10
	68-85min	972914.67 $\pm$ 16900.15	63106.75 $\pm$ 417.84	397.71 $\pm$ 2.93	22524.24 $\pm$ 746.20	1104.48 $\pm$ 7.46	2.59 $\pm$ 0.08
	102-119min	877027.33 $\pm$ 4906.83	57554.02 $\pm$ 987.62	373.95 $\pm$ 4.10	22186.04 $\pm$ 880.45	1060.28 $\pm$ 17.69	2.56 $\pm$ 0.09

3.4 Effects of *Aconitum* processing on DL

In Chinese herbal medicine, “processing” refers to a pharmaceutical technique that alters the properties of herbal materials to meet the requirements of therapeutic application. It includes methods such as steaming, boiling and stir frying etc. of herbal materials to reduce toxicity, decrease side-effects and enhance the pharmacological efficacy and stability etc..^{35,36} For instance, a raw root of *Aconitum* requires specific processing in order to reduce the content of toxic components. In this section, we studied the effect of processing of herbal materials in relation to DL dynamics. For this purpose, *Aconitum* roots powder (150 μ m) was processed by autoclaving (127°C, 60 minutes). A further comparison was made with two other commercially processed *Aconitum* samples resulting in white and black colored *Aconitum* material.³⁶ Fig. 8 shows that DL drastically decreased as a result of these processing methods. The differences in DL parameters between the raw *Aconitum* samples and processed *Aconitum* samples are demonstrated in Fig. 9. Only parameter A2 does not show significant difference (p -value = 0.102) between raw *Aconitum* and processed *Aconitum* by autoclaving (Fig. 9C). The other parameters of processed *Aconitum* samples show significant differences compared with the raw *Aconitum* sample (p -value <0.05) (Fig. 9).

3.5 DL of other herbs (rhubarb and ginseng)

In the present research, we also included rhubarb root and ginseng root samples,

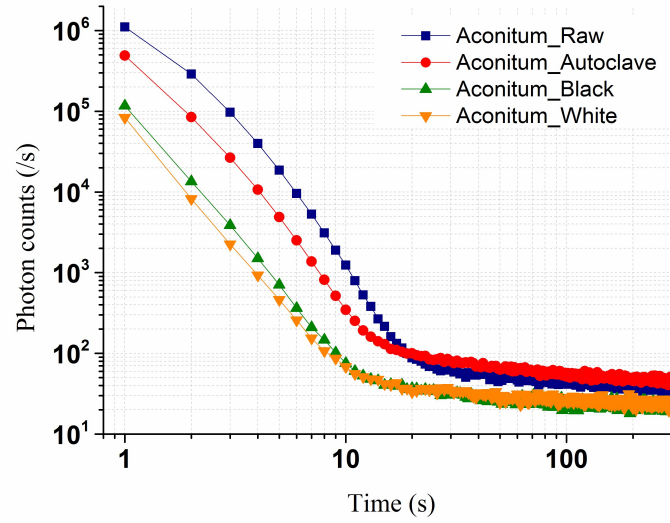


Fig. 8 Effects of *Aconitum* processing on DL. In total, 15 measurements of each sample were averaged to obtain the DL dynamics for different *Aconitum* samples.

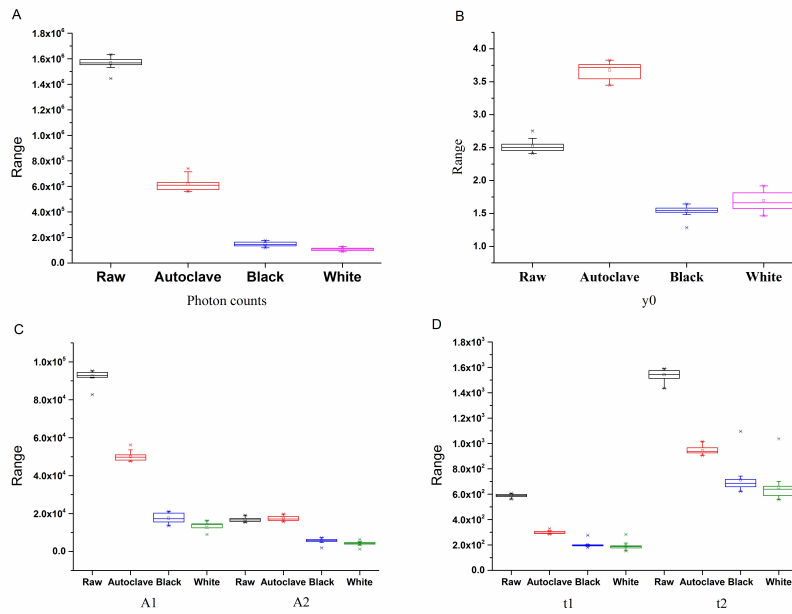


Fig. 9 DL parameters of *Aconitum* root samples and processed *Aconitum* root samples. (A-D) Show the value of DL parameters of raw and processed *Aconitum* samples.

because these two herbal materials are widely used and easily collected. In the case of rhubarb roots we compared roots from plants, grown under different conditions. Fig. 10A illustrates the DL curves of two wild and two cultivated rhubarb samples respectively. It is obvious that one wild rhubarb samples (grown in Longdeng village) demonstrate different decay behaviors compared with other rhubarb samples. The DL parameters show significant differences (p -value <0.05) between two wild rhubarb samples (grown in different location) except the parameter of photon counts (p -value $=0.19$). Two cultivated rhubarb samples grown in the same location and with the same age demonstrated only two parameters (t_1 and t_2) with significant differences (p -value <0.05). These results may indicate that different growth condition may be reflected in DL differences.

In the case of ginseng, two samples were studied. The two samples were grown in the same location but had different growth ages (10 years and 16 years). Fig. 10B illustrates the different shapes of two ginseng sample curves. In addition, significant differences in all DL parameters (p -value <0.05) were found. Therefore, herbal age may be also reflected in the DL dynamics.

4. Discussion

A DL measurement protocol of dried herbal materials (*Aconitum*, ginseng and rhubarb) has been developed in which the particle size of dry powder is defined as well as the excitation and measurement conditions. Utilizing this protocol, the effects of different processing conditions as well as differences in growth conditions of the herbs (locations, growth age) were evident. DL measurements show typical long-term kinetic patterns depending on the duration of excitation. A long measurement time (5 min) led to a long DL tail (Fig. 3). As these tails do not completely overlap with the DL signal of the empty Petri dish, these tails may represent specific features of different herbal medicines. DL curves of the same sample measured at weekly intervals demonstrated small differences. Further investigation on the reproducibility

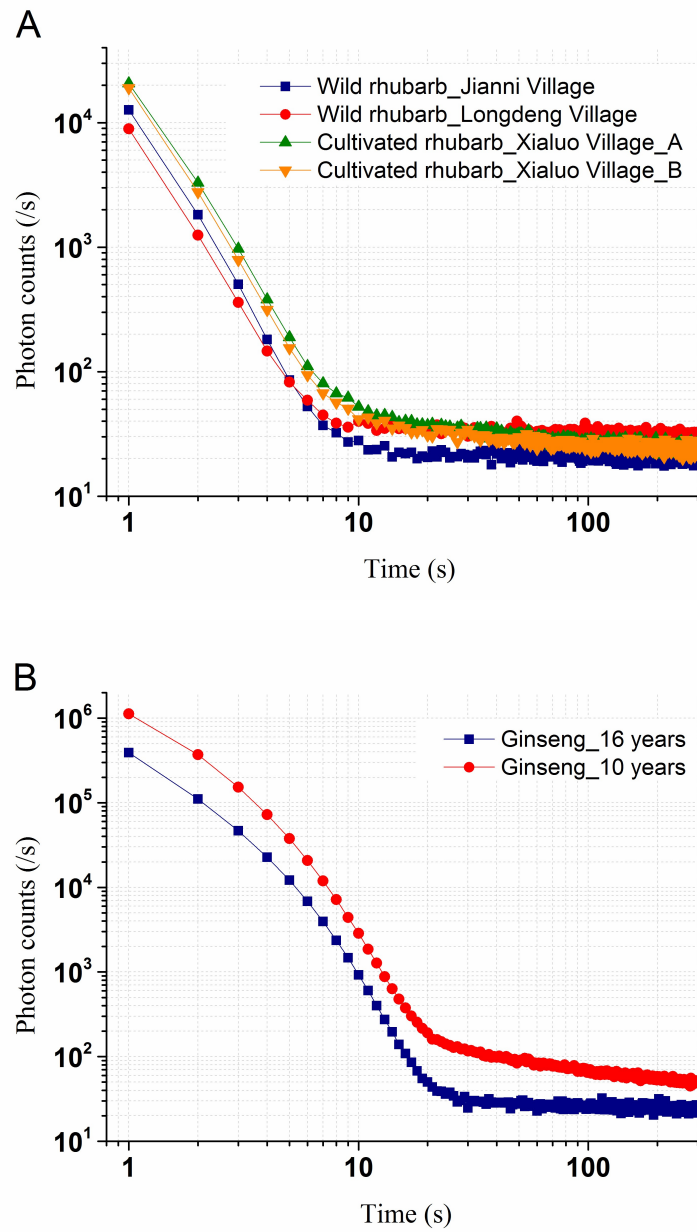


Fig. 10 Effects of growth location and different age on DL. (A) Shows two wild rhubarb samples grown in different location compared with two cultivated rhubarb samples grown in the same location and with the same age. (B) Shows two ginseng samples grown in the same location but different age.

of measurements is needed to elucidate effects of time of the day, season and weather.³⁷ However, compared with the differences that were measured between experimental conditions presented in the processing, location and age sections, the weekly variation was relatively small and thus seems not to be very relevant.

Luminescence properties are influenced by molecular interactions. The main reason for changes in emission may be due to the conformational changes of cell proteins. Such interactions may refer to radiation-less (resonance) transfer of energy from an excited molecule to another molecule. Therefore, DL (similar to fluorescence) depends on a whole series of factors that affects the luminescence properties of the aromatic residues.³⁸ Here hydrogen bonding as a specific interaction needs to be considered. Hydrogen bonding is represented widely between water and macromolecules such as proteins, RNA and DNA. In the literature it is reported that an increasing water content is accompanied with a decreasing DL intensity in collagen and lanthanide samples.^{32–34,39} These data correspond to the experimental results described for *Aconitum* samples. Moreover, the changes of carbohydrates (cellulose) may also affect the molecular structure, thereby influence the DL signatures in herb.

It is well known that the excited energy pattern formed by light exposure depends on the molecular absorption of the excitation energy.³⁸ The changes of chemical composition may change the internal structure of herbal medicines, thus may change the molecular interaction and absorption and storage capacities of the excitation energy leading to different DL dynamics. Chinese herbal medicines of different ages and from different growth locations as well as subjected to different processing procedures may differ in chemical composition. For instance, the contents of major toxic compounds (i.e. aconitine, mesaconitine and hypaconitine) are decreased significantly after processing.⁴⁰ Ginsenosides contents are shifted by the different ages of the ginseng.⁴¹ With the changes in environment factors, the contents of anthraquinone derivatives are also changed on rhubarb.⁴² Importantly, such chemical

compounds may directly influence the pharmacological effects of those herbal medicines, and hence the therapeutic properties.^{43–45} For instance, ginseng roots demonstrate growth age-dependent therapeutic effects on diabetic rats, which may be the result of the variation in both the concentrations and ratios of ginsenosides in ginseng roots of different growth ages.⁴³ The dynamics of DL may not only be an indicator of the changes of herbal age etc. but also may relate to the therapeutic effects of those bioactive compounds. Therefore, if a correlation between DL parameters and active compound contents could be established, DL might become a candidate technology for herbal quality control.

At present, the chemical changes of herbal medicines are typically measured by tools such as liquid chromatography and gas chromatography.^{40,43,46–49} Chemical analysis is the method of choice to estimate the content of specific substances (either as medical or toxic substance) of herbal materials. However, chemical analysis requires isolation and extraction procedures, which may lead to losses of substances and incomplete identifications. In addition, existing chemical analysis platforms are not able to evaluate the entire integral profile of chemical compounds. Moreover, complex structural changes of chemical molecules are not reflected by chemical analysis. While DL measurement does not require isolation and extraction procedures, and DL can reflect the overall properties of a biological system.⁵⁰ Therefore, combining DL and chemical analysis may provide new insight for herbal quality control in the future.

5. Conclusion

The data represented in this article show that DL characteristics from Chinese herbal medicines can be influenced by the excitation time, herbal particle size and herbal water content. The established DL measurement protocol is both stable and reproducible and demonstrates the sensitive response of several herbs under the different conditions. We suggest that the combination of DL and chemical analysis may point to a possible new herbal quality control method in the future.

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