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A PORTABLE MICRO-VOLUME COLORIMETRIC ASSAY FOR ALUMINIUM DETERMINATION USING NATURAL REAGENT EXTRACTS FROM SAPPAN HEARTWOOD BY ULTRASOUND-ASSISTED EXTRACTION

(Satu Kaedah Uji Warna Isipadu-Mikro yang Mudah Alih untuk Penentuan Aluminium Menggunakan Ekstrak Bahan Semulajadi dari Teras Kayu Sappan Melalui Pengekstrakan Dibantu Ultrasonik)

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Abstract

An environmentally friendly small-scale ultrasound-assisted extraction and portable micro-volume colorimetry was proposed for the determination of aluminium in pharmaceutical formulations by extraction of Sappan heartwood in acetate buffer pH 5.5. Sonication parameters influencing the extraction of non-synthetic reagents from Sappan heartwood for quantification of aluminium including type of solvent, mass/volume ratio of solvent, extraction time and temperature were investigated. Efficiency of the ultrasound-assisted extraction procedure of aluminium from aluminium hydroxide gel was evaluated by comparing label values and the official method for pharmaceutical formulations. Under optimal conditions, a linear calibration graph ranging from 0.5 to 10 mg L^{-1} was obtained from micro-volume colorimetric determinations (at 530 nm) with limits of detection and quantification of 0.07 and 0.37 mg L^{-1}, respectively. Relative standard deviations were 2.3 and 1.3% for 1 and 3 mg L^{-1}, respectively. The developed system was successfully applied for the analysis of various forms of aluminium in pharmaceutical formulations (suspension and tablet). Good recoveries between 91 and 107% were obtained.

Keywords: Micro-volume colorimetry, ultrasound-assisted extraction, natural reagent, Sappan heartwood, aluminium

Abstrak

Satu kaedah pengekstrakan dibantu ultrasonik berskala kecil yang mesra alam dan uji warna isipadu-mikro mudah alih telah dicadangkan untuk penentuan aluminium dalam formulasi farmaseutikal dengan mengekstrak teras kayu Sappan dalam penimbal asetat pH 5.5. Parameter sonikasi yang mempengaruhi pengeluaran bahan kimia bukan sintetik dari teras kayu Sappan untuk pengukuran aluminium termasuk jenis pelarut, nisbah jisim/isipadu pelarut, masa pengekstrakan, dan suhu telah disiasat. Kecekapan prosedur pengekstrakan bantuan ultrasonik aluminium dari gel hidroksida aluminium di nilai dengan membandingkan

nilai label dan kaedah rasmi untuk formulasi farmaseutikal. Di bawah keadaan optimum, graf pemilihan linear dengan julat dari 0.5 hingga 10 mg L^{-1} diperolehi dari penentuan warna isipadu-mikro (pada 530 nm) dengan had pengesanan dan pengukuran masing-masing adalah 0.07 dan 0.37 mg L^{-1}. Sisihan piawai relatif adalah 2.3 dan 1.3% untuk 1 dan 3 mg L^{-1}, secara berturut-turut. Sistem yang dibangunkan telah berjaya digunakan untuk analisis pelbagai bentuk aluminium dalam formulasi farmaseutikal (ampaian dan tablet). Perolehan semula yang baik antara 91 dan 107% telah diperolehi.

Kata kunci: Uji warna isipadu-mikro, pengekstrakan dibantu ultrasonik, bahan semulajadi, teras kayu Sappan, aluminium

Introduction

Aluminium (Al), an abundant metal in the earth's crust, is a non-essential element commonly used to make aluminium-containing packaging for food/drink and cooking utensils [1]. It is also used for cosmetics production and pharmaceutical products [2]. Aluminium compounds are less toxic than heavy metals but excessive intake of Al has been associated with various diseases such as Alzheimer's, anaemia, osteomalacia and neurological syndrome [3-4]. Therefore, it is important to develop detection methodologies to monitor and quantify different matrices containing this metal.

Conventional methods for the determination of aluminium include flame atomic absorption spectrometry (FAAS) [5], graphite furnace atomic absorption spectrometry (GFAAS) [6], inductively coupled plasma-optical emission spectrometry (ICP-OES) [7], inductively coupled plasma-mass spectrometry (ICP-MS) [8], spectrophotometry [9-12], and fluorometry [13]. However, these methods require tedious pre-treatment as time-consuming processes that require professional operation with high instrumentation and maintenance costs. The development of green analytical techniques or procedures which provide economical and environmentally friendly advantages has now become an important aspect of sustainable analytical chemistry [14]. The use of a portable miniaturized system with green analytical methods involves stocking fewer samples, with reduced transport and analytical risks of contamination or analyte losses, together with positive environmental benefits [15].

Natural reagent extracts from various sources including vegetables, plant-based, animals, or bacteria require minimal treatment [16]. Natural reagents may be used instead of high purity chemical reagents when a reagent is only required in excess, or a crude extract contains the necessary active compound [17]. The use of crude plant extracts such as Indian mulberry (*Morinda citrifolia*) root extract [18], Indian almond (*Terminalia catappa* L.) leaf extract [19] and heartwood of Sappan wood (*Ceasalpinia sappan* L.) [20, 21] as chromogenic agents for analysis of Al in combination with flow-based analysis techniques [18-20] and paper-based analytical devices [21] have been reported as green analytical approaches. Contained plant compounds can be extracted by classical solvent extraction processes that include maceration with an organic solvent or boiling with water; however, these methods are time consuming and generate high waste production.

Sappan heartwood extracts are popular used for Al determination due to their high sensitivity and selectivity. The red homoisoflavonoid, brazilein is one of the main compounds found in the extracts of Sappan heartwood. In aqueous solution at pH 4.5, aluminium and pure brazilein extracted from Sappan heartwood form a 1:2 ratio complex via the ionized 10-hydroxyl group and 9-carbonyl oxygen of two molecules of brazilein bidentate ligands as shown in Figure 1 [22]. Traditional extraction methods of red dye from Sappan wood involve boiling the wood in water [23]. Various extraction methods have been proposed for the extraction of brazilein from Sappan heartwood such as maceration with ethanol [24], methanol [25], microwave-assisted extraction (MAE) [26-28] and ultrasound-assisted extraction (UAE) [29-30]. Among these, the ultrasound-extraction method is popular as a green analytical technique with clean protocol, safety, short operation time (less than 1 h) and moderate volume/concentration consumption of solvents and energy [31].

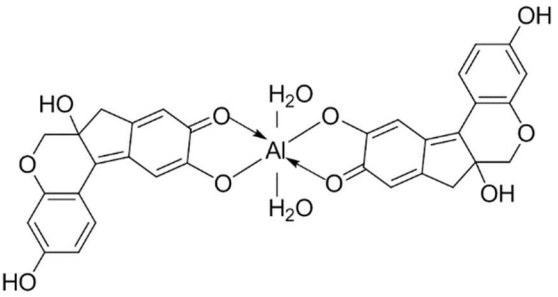

Figure 1. The proposed chemical structure of the Al(brazilein)$_2$ complex at pH 4.5

This study presented a new green analytical approach for Al determination based on simple small-scale ultrasound-assisted treatment procedures for (1) extraction of natural reagent from Sappan heartwood for complexation with Al, and (2) dissolution of Al in pharmaceutical products (suspension and tablet). A portable micro-volume colorimeter with an LED light was utilized to minimize the consumption of chemicals/reagents as a cost-effective device.

Materials and Methods

Chemicals and reagents

All chemicals used were of analytical reagent grade. Deionized water from Simplicity 185 (Millipore, Billerica, MA, USA) with resistivity 18.2 MΩ cm was used throughout the experiments. A 1000 mg L^{-1} aluminium (Al) standard solution (Merck, Darmstadt, Germany) was used in all the experiments. Working standard solutions of Al with different concentrations were prepared by appropriately diluting the stock solution. Hydrochloric acid (HCl, 37%) (Univar, Ingleburn, New South Wales, Australia) was used to digest the samples. Acetate buffer solutions at different pH were prepared from sodium acetate and acetic acid (Carlo Erba, Milano, Italy).

Instrumentation

A photo absorbance sensor, PiCOEXPLORER™ Model PAS-110 (Ushio Inc., Japan), controlled by a PAS-110 application via a smart phone/tablet mobile device was used for micro-scale colorimetric determination. A visible spectrophotometer, Genesys™ 20 (Thermo Fisher Scientific, USA) with Thermo Scientific VISIONlite 5 software was used for scanning the absorbance wavelength. Ultrasound-assisted extraction was carried out using a high power ultrasonic cleaning unit, Sonorex Digitec DT 100/H (Bandelin Electric GmbH & Co. KG, Berlin, Germany) with technical specifications: timer 0-30 min, 230 V, 50/60 Hz, frequency 35 kHz, and built-in heater 20 - 80 °C. All pH measurements were conducted using a pH meter (Model 713, Metrohm, Switzerland).

Preparation of natural reagent: Plant materials

Sappan heartwood powder (> 5 kg) was purchased from a local retail shop and dried in an oven at 50 °C for 24 h, storing in a dry place until use.

Ultrasound-assisted extraction method

Plant powder (0.5g) was accurately weighed into centrifuge tubes with caps (15 mL capacity) and 10 mL of 1 M acetate buffer solution pH 5.5 was added. The resulting solution were then mixed by a vortex and allowed to stand for 10 min at room temperature, termed as presonication time. The tubes were then immersed in an ultrasonic water bath and subjected to ultrasonic energy at 35 kHz for 30 min, termed as sonication time. The ultrasonic bath was set at 80 °C using a built-in heater. After sonification, the extract was centrifuged at 6000 rpm for 5 min, then passed through a filter paper (Whatman® No.1, UK) and made up to a volume of 10 mL with 1 M acetate buffer solution pH 5.5.

Conventional extraction method

Plant powder (5.0g) and deionized water (100 mL) were mixed in an extraction flask and heated on a hotplate until boiling. The extract was boiled for 10 min. After cooling, the extract was passed through a filter paper (Whatman® No.4, UK) and made up to a volume of 100 mL with deionized water.

Sample preparation: Pharmaceutical samples

Twelve aluminium hydroxide ($Al(OH)_3$) gel samples as suspensions (S1 – S6) and tablets (T1 – T6) were bought from local drug stores in Maha Sarakham Province, Thailand. Before analysis, 20 tablets were manually ground to powder and homogenized with an agate mortar and pestle.

Ultrasound-assisted extraction method

An accurately measured quantity of gel or fine powder (in case of tablet) as 0.024 to 0.030g of aluminium hydroxide (0.4-0.8 mL of aluminium hydroxide gel suspension, 0.010-0.020 g of aluminium hydroxide gel tablet) was transferred to a glass test tube. Then, 0.3 mL of hydrochloric acid was added and the mixture was immersed in an ultrasonic water bath for 10 min at 40 °C using a built-in heater. After sonification, the solution was cooled, filtered (in case of tablet) and diluted with 10 mL of deionized water.

Official method

A stock solution of the suspension was prepared following the pharmacopoeia procedures [32]. Briefly, the method involved the transfer of an accurately measured quantity of gel or fine powder (in case of tablet), equivalent to 1.2 to 1.5 g of aluminium hydroxide into a beaker. Then, 15 mL of hydrochloric acid was added and the mixture was heated gently until completely dissolved. The solution was then cooled, filtered (in case of tablet) and diluted with deionized water in a 500 mL volumetric flask.

Micro-volume colorimetric assay

This assay was performed in a micro-centrifuge tube (200 µL capacity), with reactive solutions measured by a portable colorimeter. For the construction of standard curves, 90 µL of natural reagent was added to each micro-centrifuge tube, followed by aliquots of eight different concentrations (0, 0.5, 1, 2, 4, 6, 8, and 10 mg/L) of aluminium standard solutions. Deionized water was used to make up the volume to 150 µL per tube. The contents were then mixed by shaking and kept at room temperature for 20 min, followed by reading at 530 nm (green LED) using PiCOEXPLORER™.

Results and Discussion

Ultrasound-assisted extraction of natural reagent from Sappan heartwood: Type of solvent

Normally, the extraction solvent has a strong impact on extraction yield by the solubility of the target analytes and also by physical parameters such as viscosity, vapor pressure and surface tension of the solvent [33]. From our previous study [20], the extraction solvent types (boiling with water, and maceration with different polar solvents, such as methanol, ethanol, and acetone) were optimized. An aqueous extract by boiling Sappan heartwood with water was chosen due to its simplicity and reduced chemical usage. After aqueous extraction, the extracts were mixed with sodium acetate and acetic acid to make a buffer solution of pH 5.5. This pH was chosen from its high sensitivity when comparing to other studied pH ranges from 3.0-6.0 [20]. However, the disadvantages of this method include tedious preparation steps and high consumption of water and chemicals (≈100 mL per batch per extraction). Therefore, ultrasound-assisted extraction (sonication time 30 min, temperature 80 °C) using 0.5 g of plant powder with water and acetate buffer pH 5.5 (10 mL) as extraction solvent was investigated for extraction efficiency compared to the conventional extraction method at the same mass ratio per extractant volume. Absorption spectra at 400-700 nm were recorded for the obtained solutions as shown in Figure 2. The conventional extraction method by boiling with water gave the highest absorbance of reagent extracts and reagent-Al complex. The decrease in absorption of the reagent and reagent-Al complex spectra obtained from ultrasound-assisted extraction was due to mass transfer limitation of soluble constituents from the material to the solvent by diffusion [34]. The absorption spectra obtained from ultrasonic energy with buffer solution were higher than with water. Therefore, acetate buffer pH 5.5 was chosen as the solvent for ultrasound-assisted extraction.

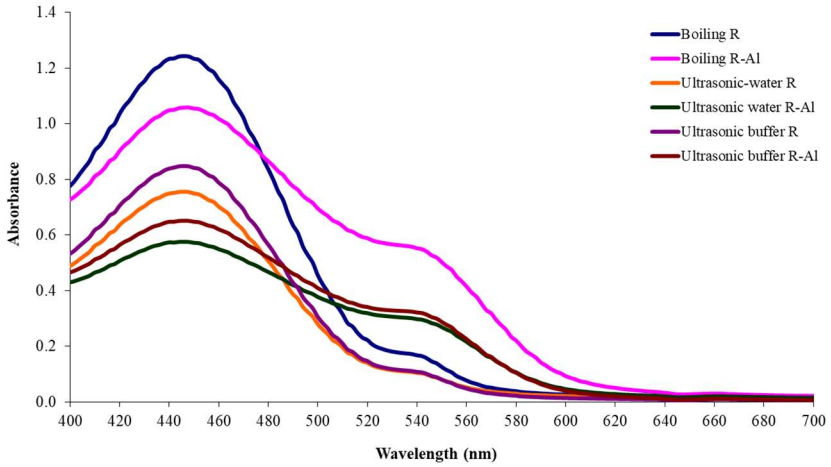

Figure 2. Absorption spectra of extracts from Sappan heartwood using different extraction procedures (boiling with water and ultrasonic-assisted extraction) and different extraction solvents (water and acetate buffer pH 5.5)

Effect of Sappan heartwood mass

In general, an increase in the solid sample mass improves sensitivity due to the transfer of higher amounts of analyte to the liquid phase. In this experiment, the volume of the extractant solvent was kept constant at 10 mL, while the mass of Sappan heartwood powder utilized in the extraction was varied from 0.1 to 1g. All other conditions were set according to the previous optimization experiments, with results shown in Figure 3. The absorbance of the reagent (at 446 nm) and reagent-Al complex (at 530 nm) increased with increasing amounts of plant powder from 0.1-0.5g, then slightly increased before remaining constant. A ratio of 0.5g of plant material per 10 mL of acetate buffer was selected as suitable for the extraction procedure from its high extraction reproducibility.

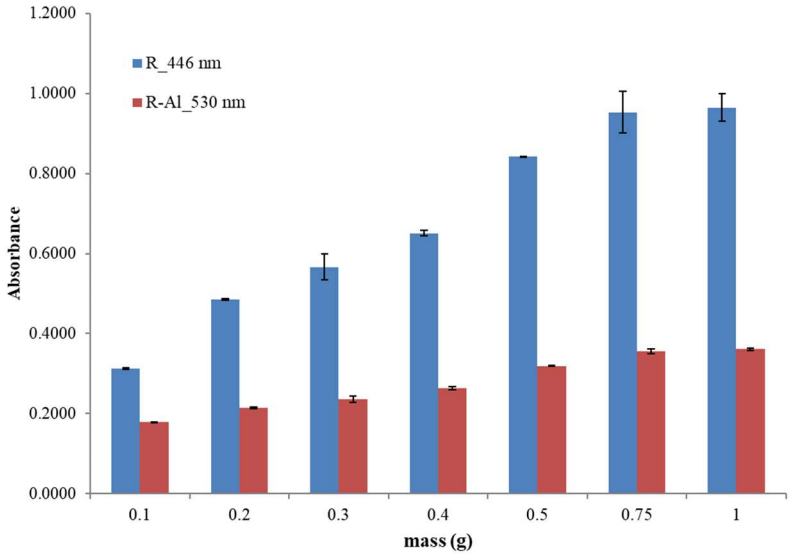

Figure 3. Effect of plant powder mass on the extraction of natural reagent performed in 10 mL of acetate buffer pH 5.5 with sonication time 30 min and temperature 80 °C

Extraction time

Long extraction time enhances extraction yields, but long extraction time may induce undesirable changes in the extracted compounds [35]. The influence of sonication time (10, 20, 30, 40, 50, and 60 min) on the yield of natural reagent extracts from Sappan heartwood using buffer pH 5.5 as extraction solvent was investigated with results shown in Figure 4. The yields of brazilein gradual increased from 10 to 30 min. After 30 min, there was a slight decrease in the yield, possibly due to degradation of the extracts at longer extraction times. This result indicated that ultrasonic irradiation played an important role in the extraction process. The increasing of the absorbance at 60 min might be due to the absorption of other species during degradation or the low reproducibility of the extraction process. An extraction time of 30 min was selected as suitable.

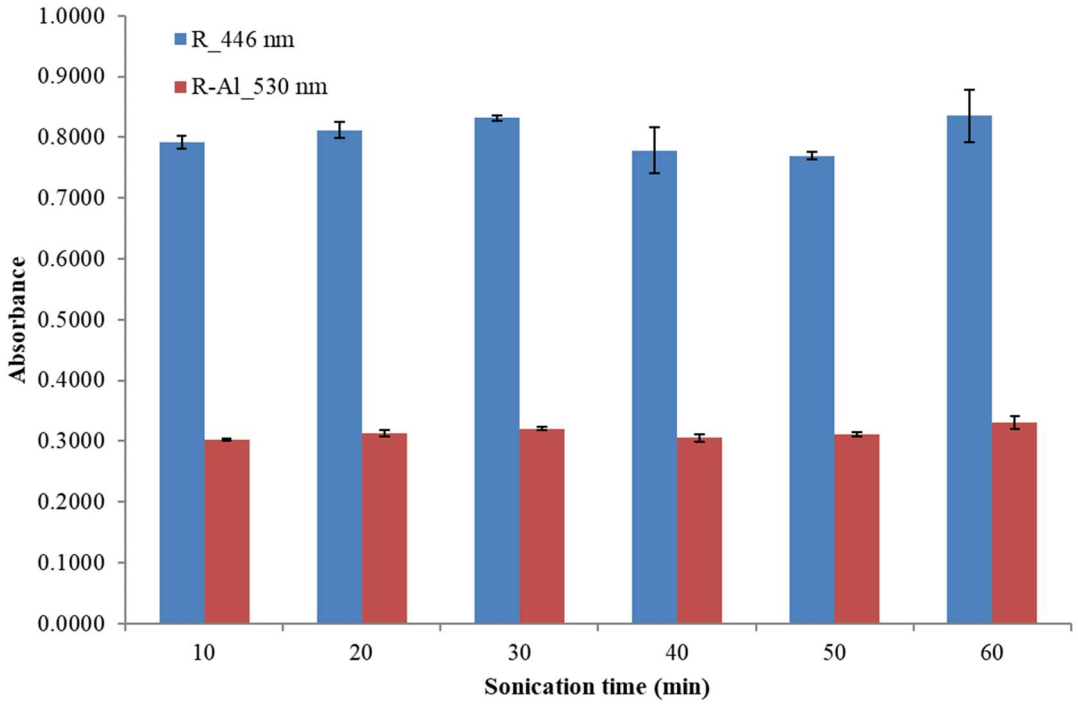

Figure 4. Effect of sonication time on the extraction of natural reagent performed with 0.5g of plant powder and 10 mL of acetate buffer pH 5.5 at 80 °C

Temperature

The extraction temperature is also important. Temperature contributes to efficiency of extraction. Usually, increase of temperature leads to an increase of extraction yield [33]. In this study, using an ultrasonic cleaning bath with built-in heater from 20-80 °C, the effect of different temperatures (30, 40, 50, 60, 70, and 80 °C) on the extraction yield was investigated. All other conditions were set according to the optimal parameters obtained above. Results are displayed in Figure 5. Extraction yield increased as temperature increased from 30 to 80 °C, showing that sonication temperature had a profound influence on the extraction of active ingredients from plant materials. Extraction temperature at 80 °C was selected as suitable.

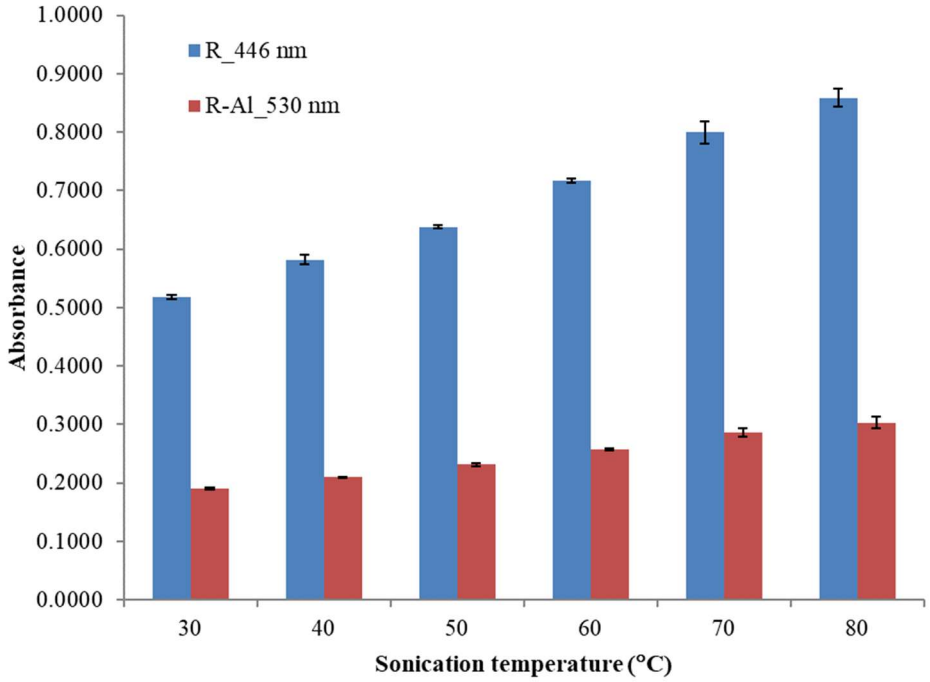

Figure 5. Effect of sonication temperature on the extraction of natural reagent performed with 0.5g of plant powder and 10 mL of acetate buffer pH 5.5 for 30 min

Extraction efficiency and reproducibility of crude plant extracts

After optimization, the extraction efficiency of the proposed small-scale ultrasonic-assisted extraction was lower than the conventional extraction by boiling with water (relative absorbance of 50%). However, the crude plant extracts contained sufficient active compounds to reacts with Al. To ensure the reproducibility of the various batches of natural reagent extracts for future use, the precision of absorbance of 10 batches of extracts prepared independently was evaluated. The relative standard deviation (%RSD) for the 10 batches was 3.8% and lower than the conventional extraction procedure [20]. The proposed small-scale ultrasonic-assisted extraction procedure had advantages over the conventional extraction procedure such as ease of operation, low chemical consumption, and high precision.

Determination of Al by micro-scale colorimetric procedure

Aluminium analysis was conducted using crude plant extracts containing brazilein. The complexation ratio of Al with brazilein in the crude plant extracts was 1:2 [22] with 530 nm the maximum complexation absorption wavelength. A marked change in color of the natural reagent with Al from yellow to reddish was observed.

Effect of mixing volume ratio of sample and reagent

The concentration of natural reagent extract used was 0.5g of heartwood /10 mL of buffer pH 5.5. The effects of sample and reagent volumes on the sensitivity of the system were studied at mixing volume ratios of Al:reagent 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:4, 8:2, and 9:1 for a total volume of 150 µL. As shown in Figure 6, the mixing volume ratio of Al:reagent at 4:6 (60:90 µL) gave the highest sensitivity and was selected as the optimal condition.

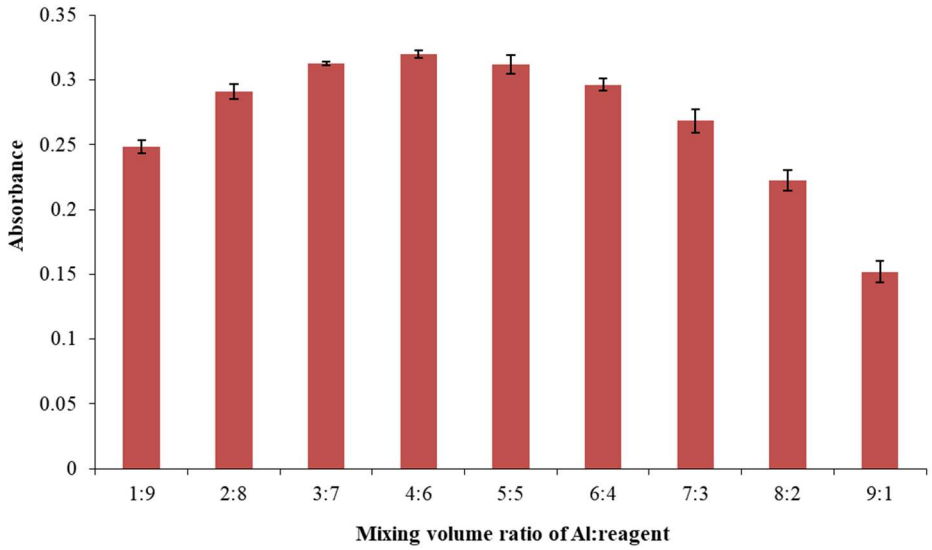

Figure 6. Effects of mixing volume ratios of sample (Al) and natural reagent extracts from Sappan heartwood at pH 5.5 on the sensitivity of the proposed micro-volume colorimetric measurement at 530 nm

Analytical features of the proposed system

The analytical characteristics of the proposed colorimetric assay were investigated. Using the optimal extraction conditions and the mixing volume ratio described above, standard calibration in the range 0.5-10 mg L^{-1} was constructed by plotting the absorbance against Al concentration. A linear graph was obtained with the calibration equation y = (0.0528±0.0024)x + (0.0111±0.0032) and R^2 = 0.9977, as shown in Figure 7. The limits of detection and quantification for Al were 0.07 and 0.37 mg L^{-1}, respectively with relative standard deviations for ten replicate determinations of 1 and 3 mg L^{-1} 2.3 and 1.3%.

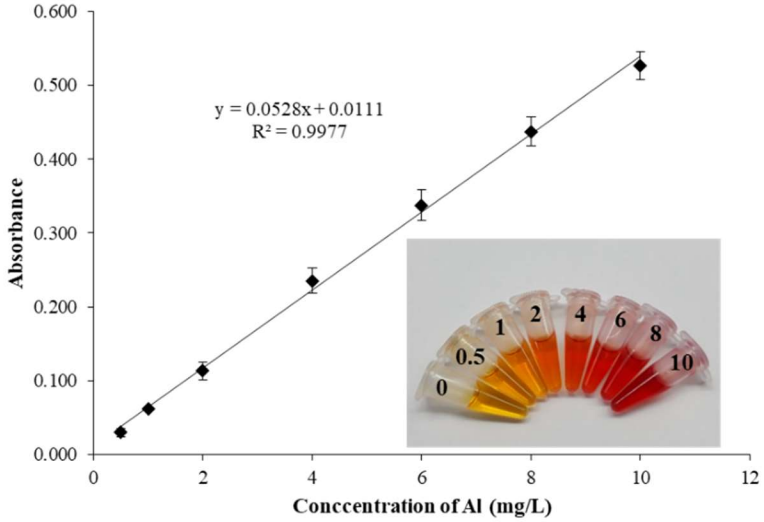

Figure 7. Visual detection and calibration curve of the aluminium standard with natural reagent extracts from Sappan heartwood at pH 5.5 obtained from micro-volume colorimetric measurement at 530 nm

The analytical performance of the proposed method was compared with previous methods using various natural reagents, as summarized in Table 1. The proposed method was not as sensitive as some other flow-based systems but the sensitivity was sufficient to measure Al in pharmaceutical samples (2.4-3.0 g/L). Our proposed micro-volume colorimetric assay consumed only 150 µL of each sample/standard and reagent solution per analysis. This assay also reduced contamination risk and analysis time and allowed portable on-field measurements.

Table 1. Analytical characteristics of the colorimetric determination method for Al using various natural reagents

Method	Natural Reagent	Extraction Method	Linear Range (mg L^{-1})	LOD (mg L^{-1})	Sample Volume (µL)	Ref.
Flow injection	Indian mulberry root extract	Maceration with 80%v/v acetone	0.1 – 1.0	0.05	75[a]	[18]
Flow injection	Indian almond leaf extract	Blending with acetate buffer pH 4.8	0 – 100	0.8	100[a]	[19]
Sequential injection	Sappan heartwood extract	Boiling with water	0.075 – 1.0	0.021	300[b]	[20]
Naked-eye detection	Cyanidin extracted from red cabbage	Extracted with MeOH and 2 M HCl (85:15 v/v)	1.35 – 2.70 (detection range)	1.35	1300[b]	[36]
Naked-eye and Vis-spectrophotometry	Pectin-rich apple extract-based gold nanoparticles	-	0 – 2.7	0.54	-	[37]
Naked-eye detection	Anthocyanin from black rice immobilized in carboxymethylcellulose/starch film	-	0 - 10	3.0	30 mL	[38]
Smartphone-based	Commercially fermented black carrot juice	-	1 – 50	0.2	2500[b]	[39]
Micro-volume colorimetry	Sappan heartwood extract	Small scale UAE	0.5 – 10.0	0.07	150[b]	This work

[a] sample volume only

[b] total analysis volume (sample+reagent+other solutions)

The effects of interfering species on the Al-natural reagent complex were investigated using the proposed method under optimal conditions. Various concentrations of foreign ions (Na^{+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+}, Mn^{2+}, and Fe^{3+}) were spiked into a standard solution of 3 mg L^{-1} Al. An interfering concentration was considered as the value that caused signal variations higher than ±10%. Results showed that the system tolerated spiked cations up to at least a 1:1 concentration ratio of Al to interfering cations. Higher interference ratios were not studied since these minerals are normally

found in pharmaceutical formulations at lower amounts than Al compounds.

Ultrasound-assisted extraction of Al in pharmaceutical formulations

Aluminium hydroxide gel in suspension and tablet forms was used for optimization. The extraction efficiency or percentage label values (by comparing the amounts of aluminium found in aluminium hydroxide gel samples with their label values) of the results provided by ultrasonic variable parameters were evaluated under the optimal conditions. The mass of the sample, volume of the solvent, and type of solvent followed the standard method [32].

Sonication time

The influence of sonication time (1, 5, 10, 15, and 20 min) on the extraction efficiency of aluminium from aluminium hydroxide gel using hydrochloric acid as the extraction solvent and temperature 80 °C was investigated with results shown in Figure 8. Normally, long extraction time enhances extraction yields [35]. High percentage label of aluminium was obtained within 1 min for aluminium hydroxide gel suspension, while 10 min was required for the aluminium hydroxide gel tablet. Results indicated that sonication time played an important role in the extraction process of aluminium, especially in the form of aluminium hydroxide gel tablet. Therefore, an extraction time of 10 min was selected as suitable.

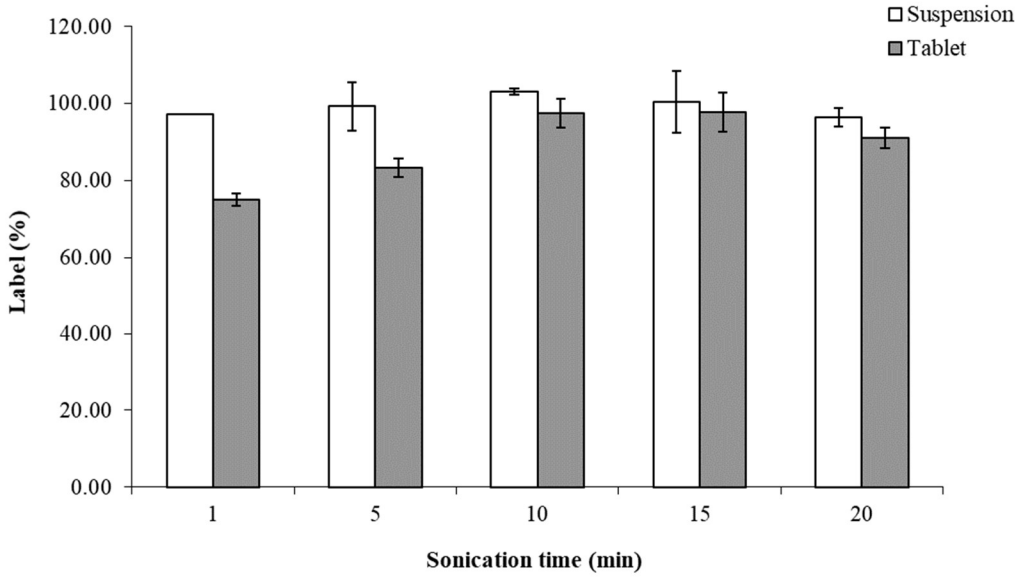

Figure 8. Effect of sonication time on the extraction of aluminium performed with 0.4 mL of $Al(OH)_3$ gel suspension or 11 mg of $Al(OH)_3$ gel tablet powder and 0.3 mL of concentration HCl at 80 °C

Temperature

The choice of extraction temperature is also important. The temperature strongly impacts the solvent's properties [33]. High temperature enhances solvent diffusion rates, while, low temperature enhances cavitation [35]. In this study, different temperatures (40, 50, 60, 70, and 80 °C) were investigated for extraction efficiency. All the other conditions were set according to the optimal parameters, with results displayed in Figure 9. High temperature during sonication did not influence the extraction of aluminium from aluminium hydroxide gel suspension and tablet. It might be due to aluminium ions were easily to dissolve from aluminium hydroxide gel under the acidic condition. Therefore, an extraction temperature of 40 °C was selected.

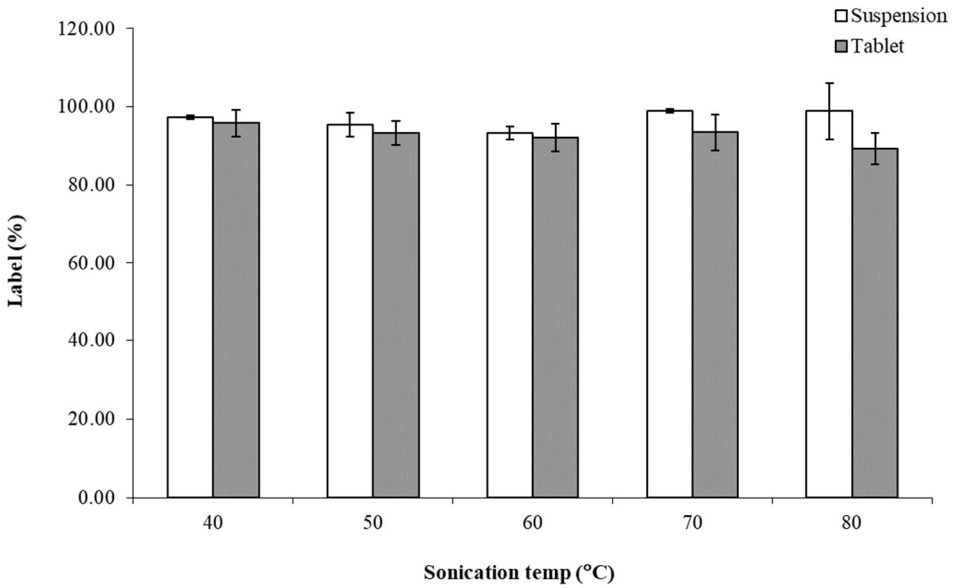

Figure 9. Effect of sonication temperature on the extraction of aluminium performed with 0.4 mL of $Al(OH)_3$ gel suspension or 11 mg of $Al(OH)_3$ gel tablet powder and 0.3 mL of conc. HCl for 10 min

Extraction efficiency

Small-scale ultrasound-assisted extraction efficiency was evaluated by comparing the results obtained from the official method for pharmaceutical formulations, as presented in Table 2. Satisfactory recoveries for both procedures were obtained at 103.3 and 96.8% for suspension and tablet forms, respectively. Results indicated that the extraction efficiency of the proposed small-scale ultrasound-assisted extraction procedure was comparable to the official procedure. Relative standard deviations of the aluminium concentration obtained after extraction by the proposed procedure were lower than for the official procedure.

Table 2. Extraction efficiency of the proposed ultrasound-assisted extraction procedure under optimal conditions

Pharmaceutical Form	Amount of Aluminium Hydroxide (mg) in 15 mL of Suspension or 1 Tablet		% Recovery[a]
	Reference Method (n = 10)	Proposed Method (n = 10)	
Suspension (960 mg Al / 15 mL)	960.5 ± 36.9 (3.8% RSD)	992.4 ± 12.9 (1.3% RSD)	103.3
Tablet (188.8 mg Al / tablet)	186.0 ± 9.4 (5.1% RSD)	180.0 ± 6.4 (3.6% RSD)	96.8

[a]Recovery (%) = [proposed method / reference method] x 100

Application to pharmaceutical samples

The proposed assay was used to determine aluminium content in pharmaceutical preparations, and the amounts of aluminium found in aluminium hydroxide gel samples were compared with their label values. Results are presented in Table 3. The aluminium contents in aluminium hydroxide gel suspension and tablet samples were acceptable at 90.0 to 110.0 % of the labeled amount of aluminium hydroxide [32]. Satisfactory recoveries ranging 91 to 107% for pharmaceutical samples were obtained.

Table 3. Determination of aluminium in pharmaceutical formulations (n = 3)

Sample[a]	Amount of aluminium hydroxide (mg) in 15 mL of suspension or 1 tablet		% Relative error[b]	%Recovery
	Label value	Found		
S1	960	986.3 ± 13.5	2.7	97.2 ± 3.4
S2	918	938.1 ± 6.9	2.2	101.2 ± 2.7
S3	900	910.7 ± 8.8	1.2	100.6 ± 3.5
S4	900	930.9 ± 7.4	3.4	104.0 ± 4.7
S5	600	603.4 ± 13.1	0.6	103.8 ± 3.6
S6	459	465.5 ± 6.6	1.4	106.6 ± 1.2
T1	188.8	183.8 ± 3.0	-2.7	90.9 ± 2.6
T2	191.3	188.7 ± 2.7	-1.4	91.2 ± 1.2
T3	248.6	235.2 ± 6.5	-5.4	95.2 ± 9.1
T4	191.3	179.8 ± 1.1	-6.0	101.0 ± 4.8
T5	188.8	184.6 ± 15.8	-2.2	104.4 ± 6.2
T6	153	147.6 ± 4.3	-3.5	94.6 ± 10.1

[a]S1-S6 : aluminium hydroxide gel suspension, T1-T6 : dried aluminium hydroxide gel tablet
[b]relative error = [(found - label value) / label value] x 100

Greenness assessment

The greenness of the proposed method was compared with the official method for Al in pharmaceutical sample [32] according to Analytical GREEnness (AGREE) metrics approach as seen in Figure 10. The calculation of the AGREE metrics software carried out by the application depends on 12 parameters that correspond to the 12 green analytical chemistry (GAC) principles [40]. Every principle or factor has a score range. According to the AGREE method, the proposed method scored 0.84 and stood out with portable miniaturized system, less amount of samples/reagent, less amount of waste, low energy consumption for sample pretreatment, high sample throughput and the use of bio-based reagents, while the reference method scored 0.42.

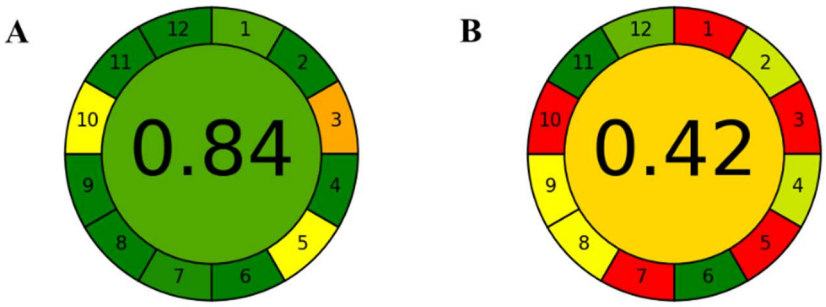

Figure 10. Results of AGREE analysis for the proposed micro-volume colorimetric assay (a) and the official method (b) for Al in pharmaceutical sample

Conclusions

A simple, reliable, cost-effective, and green analytical approach for the colorimetric determination of aluminium in pharmaceutical samples was presented. High efficiency sample preparation procedures based on ultrasound-assisted extraction were successfully applied

for small-scale extraction of natural reagent from Sappan heartwood, and also for aluminium from aluminium hydroxide gel samples. High throughput of more than 12 samples treated simultaneously was achieved using only a simple ultrasonic bath with a built-in temperature control. Our proposed micro-volume colorimetric assay significantly increased performance for the determination of aluminium in terms of cost-effectiveness, field-portability, and low chemical consumption. Satisfactory recoveries and high sample measurement frequency suggested that our proposed system showed high potential as a good alternative assay for the quality assurance of antacid suspensions and tablet products in the pharmaceutical industry. The "naked-eye" detection of this green approach can be used as a screening method for high aluminium content in wastewater samples from factories to improve environmental safety for the local residents.

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