Original Article

Raman spectroscopy in quality control of Chinese herbal medicine

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Abstract

Background: Chinese herbal medicine (CHM) is of noteworthy international interest due to its potential impact on healthcare and manifests numerous opportunities for new drug development. However, solid scientific evidence is still lacking regarding the safety, efficacy, and quality of CHM-derived medicines. Success in the modernization and globalization of CHM is heavily dependent on the achievements in advanced analytical techniques for in-line checks of CHM quality. Raman spectroscopy has become increasingly valued as an analytical technique in the pharmaceutical sector because it can provide a detailed chemical fingerprint. However, earlier research suggests that inadequate attention has been paid to the applications of Raman spectroscopy in CHM.
Methods: Chinese and English literatures were reviewed via PubMed and Medicine databases, and through manual searches using keywords including traditional Chinese medicines, herbs, quality control, and Raman spectroscopy.
Results: Applications of Raman spectroscopy in various aspects of CHM, including the identification and analysis of raw materials, in-line checks of formulation, characterization of adulterants, and detection of counterfeits, were reviewed systematically.
Conclusion: An updated systematic review of the published literature has been conducted to analyze the most important milestones and latest achievements in this topic. Raman spectroscopy is playing an increasingly important role in the quality control of CHM and effectively promotes the modernization of CHM.
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Keywords: analytical technique; Chinese herbal medicine; modernization; Raman spectroscopy

1. Introduction

Chinese herbal medicine (CHM) is attracting global attention due to its potential impact on healthcare and burgeoning opportunities for new drug development.[1,2] Over the past two decades, the CHM field has witnessed great progress in modern research directed at clinical trial evaluation, pharmacological mechanisms/pharmaceutics investigations, and new drug discovery.[2−5] However, we are still facing challenges in shifting experience-based CHM to evidence-based medicine, to promote its modernization and worldwide acceptance. These challenges primarily lie in the multicomponent and multispecies composition features of many CHMs, and the widely reported deficiencies in CHM standardization and quality control. Solid scientific evidence of safety, efficacy, and quality is still lacking, although highly desirable.[1,3,4] The success of these medicines is no doubt strongly dependent on the achievements of the fundamental understanding of pharmacology/pharmaceutics, rigorous

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quality control of herb medicine, and effective development of new drugs. CHM quality control takes priority in the whole chain of research toward its modernization. The herbs should be correctly authenticated to confirm their botanical origin prior to any designed biological and clinical research. Active pharmaceutical ingredients (APIs) should be well identified and their changes during manufacturing process should be well monitored to assure clinical effectiveness and safety. Therefore, development of comprehensive quality control approaches that are suitable for the multiple-component feature of CHM is extremely important.

Analytical methods such as thin-layer chromatography,[6] high-performance liquid chromatography,[7] ultra-high-performance liquid chromatography,[8] and liquid or gas chromatography—mass spectrometry[9,10] have been proposed, developed, and used in the fingerprinting of herbal medicines. These approaches can provide results with high reliability and accuracy, but generally require a cumbersome pretreatment and a time-consuming procedure. In addition, experimental conditions are rigorous and instruments are expensive, which limit their practical application. The use of spectroscopic techniques in terms of infrared (IR) and Raman spectroscopy is advantageous because the analyses are rapid, nondestructive, noninvasive, and simple for purposes of sample preparation.[11—13] These techniques provide fingerprint information (such as molecular conformation, structure, intermolecular interaction, and chemical bonding) by way of probing the associated molecular vibration and rotational energy changes.[14,15] IR spectroscopy is based on absorption, while Raman spectroscopy is based on inelastic light scattering. These vibrations with molecular dipole moment changes are IR active, and those with polarization potential changes are Raman active.[16,17]

Raman spectroscopy is complementary to IR spectroscopy and is of particular interest in the pharmaceutical sector due to the following reasons: (1) inherently high chemical specificity and ability to provide molecular information without requiring staining or labeling,[17,18] and (2) low sensitivity to water, consequently making analysis of aqueous or damp materials, including illegal narcotics, possible.[19] These advantages, coupled with fiber optics and microscopes, have enabled Raman spectroscopy to be an effective quality control tool in the pharmaceutical industry.[18,20] It serves as an ideal instrument for the characterization and authentication of herbs, detection of counterfeits, and facilitation of new drug development. Raman spectroscopy with probes can also be used for continuous quality control in formulation manufacturing. Applications of Raman spectroscopy in the pharmaceutical industry range from use in the laboratory to on dock to production lines until the product is shelved.[21] There are many excellent review articles dealing with the basic principles and applications of Raman spectroscopy.[14,18,21—26] However, among several published reviews, little attention has been paid to the applications of Raman spectroscopy in CHM.

Taking into account the quality and safety requirements of modern CHM, this review aims to summarize and critically discuss the applications of Raman spectroscopy in various aspects of CHM, including the identification and analysis of raw materials, in-line formulation checks, characterization of adulterants, and detection of counterfeits (Fig. 1). A brief introduction to the theory and mechanism of the Raman scatter is also included. Potential areas of future advancements and applications of Raman spectroscopic techniques are also discussed.

2. Methods

This review paper is based on a literature search focusing on PubMed and Medline databases, as well as other resource materials. The key search words used included traditional Chinese medicine, herbs, herbal, quality control, and Raman spectroscopy. In addition, specific searches were carried out using the above keywords with the common and scientific names of the plant products.

3. Results

3.1. Theory of Raman spectroscopy

Irradiation of the electron orbits within the constituent molecules with a monochromatic light always gives rise to two types of light scattering: elastic and inelastic.[27] The elastic scattering that takes place at the same wavelength as that of irradiated light is also known as Rayleigh scattering.[28,29] The inelastic scattering is always accompanied by a shift in photon frequency and a change in wavelength, upon gain or loss of some amount of energy. This phenomenon results in Raman scattering (Fig. 1). In Raman scattering, an incident photon excites a molecule from the ground state to the virtual energy state. Immediate relaxation of the excited molecule to ground electronic or vibrational states would emit photons that undergo a wavelength shift. If the scattered light is of a higher wavelength than the irradiated light, it results in a Stokes—Raman shift; conversely, it is known as an anti-Stokes shift (Fig. 1).[27—29]

The history of Raman spectroscopy began in 1928 when the Indian scientist Sir CV Raman, along with his student Krishnan, discovered the "Raman effect."[30] For decades, it was not possible to obtain successful Raman spectroscopy of pharmaceutical materials due to its intrinsically low efficiency (Raman cross sections are typically in the order of 10^{-28}—10^{-30} cm^2).[16] However, the advancements in new experiments and technical approaches, including Fourier transform (FT) spectrometers and charge coupled device-based experiments, provide a viable strategy to address this limitation.[17,18,21] Various novel types of techniques such as FT-Raman spectroscopy,[31,32] transmission Raman spectroscopy,[22,33,34] and surface-enhanced Raman scattering (SERS)[35—38] have been developed. These technological variations open up a new era for Raman spectroscopy applications in mainstream pharmaceutical analysis.

3.2. Applications of Raman spectroscopy in quality control of CHM

Accurate detection, identification, and quantification of raw materials and APIs; in-process checks of CHM quality; and
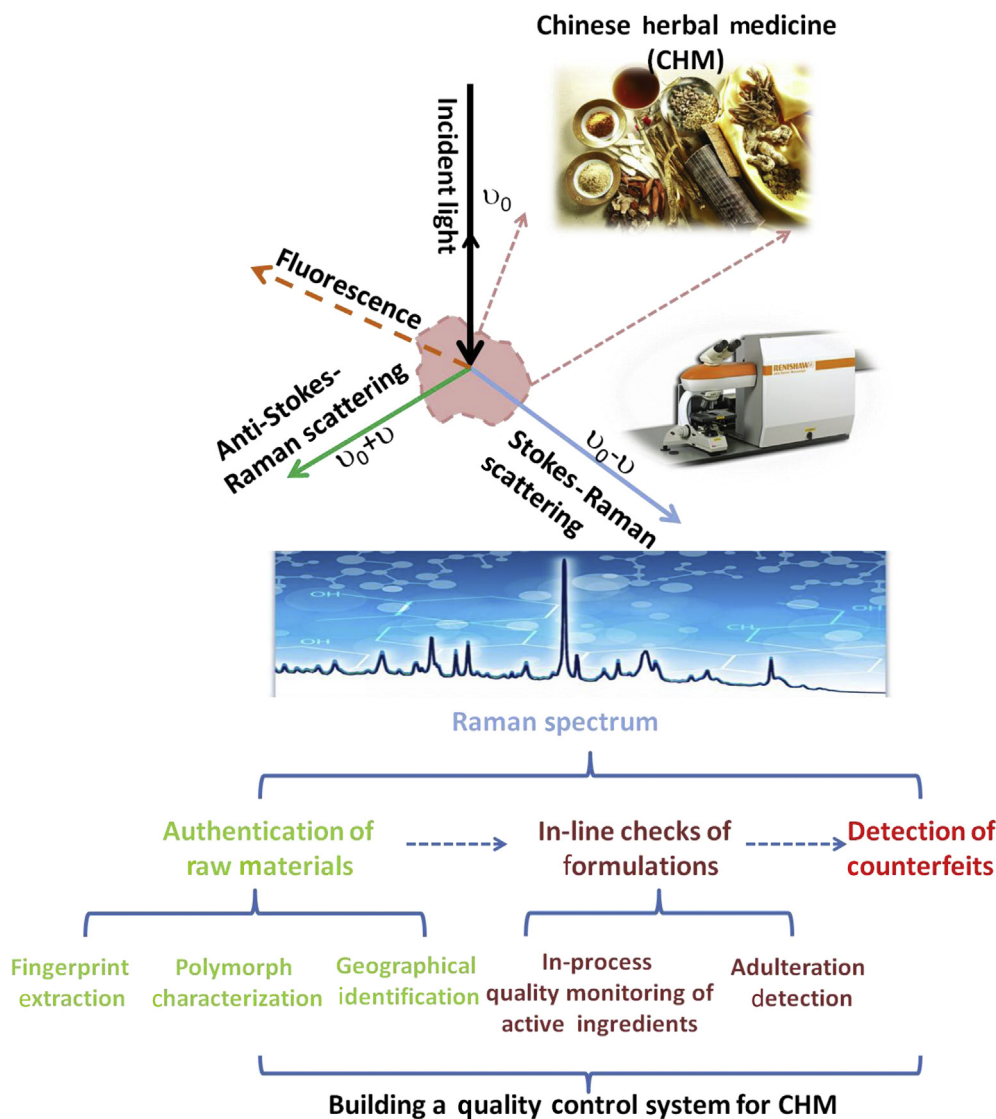
Fig. 1. Schematic illustration of Raman spectroscopy for quality control of CHM. The steps involved include authentication of raw materials (Step I), in-process quality monitoring of active ingredients (Step II), and adulteration detection (Step III). CHM = Chinese herbal medicine.

effective counterfeit detection are fundamental and critical for promoting the modernization and globalization of CHM (Fig. 1).

3.3. Step I: Authentication of raw materials

The authentication of CHM is the first step toward efficient quality control. Traditionally, CHMs are identified qualitatively according to their morphological properties such as color, shape, or smell. This method is simple, although the process was highly subjective. In detail, it is difficult to separate CHMs that are similar in appearance or smell. Moreover, chemical information about the active constituents cannot be collected. However, because Raman spectroscopy has both high chemical specificity and rapid analysis features, it can be coupled with a high-throughput screening method, and used for more accurate and efficacious authentication of CHM.

3.3.1. Chemical "fingerprint" extraction of raw materials/APIs

Yam is a frequently used and important ingredient in many CHMs, in addition to serving as a health food in diet therapy. Using FT-Raman spectroscopy, Liao et al[32] successfully investigated the protein structure of various yam species including *Dioscorea alata* L, *Dioscorea alata* L var. *purpurea*, and *Dioscorea japonica*. The Raman intensity ratios of $I_{643/621}$, $I_{853/828}$, and $I_{878/759}$ are found to be distinct and comparable, revealing their intrinsically compositional differences and distinct spatial arrangements. Secondary structures of protein for *D. alata* L and *D. alata* L. var. *purpurea* were mainly in an α-helix and an antiparallel β-sheet, respectively. The protein structure of *D. japonica* is in a mixed form of α-helix and antiparallel β-sheet. It is apparent that the differences in the Raman profiles of various yam proteins provide an efficient method to identify various yam cultivars.

Radix puerariae, another commonly used CHM, is the dried roots of *Pueraria lobata* (Wild) *ohwi* (puerariae lobatae radix, PLR) and *Pueraria thomsonii* Benth. (puerariae thomsonii radix, PTR). It is effective in the treatment of fever, diabetes, diarrhea, and cardiovascular and cerebrovascular diseases. Studies on its pharmacology and use in clinical practice have shown that the active constituents in radix puerariae are isoflavones; puerarin has been found to be the main constituent of isoflavones. Qi et al[39] qualitatively identified puerarin using Raman spectroscopy. Several characteristic Raman bands such as 725 cm^{-1} (bending of C–H), 894/801 cm^{-1} (stretching vibrations of C–C), 1449/1571 cm^{-1} (bending of O–H), and 1628 cm^{-1} (C–C$_{ring}$ and C=O stretching vibrations) have been detected. The strongest Raman peak at 1628 cm^{-1} was regarded as a marker of puerarin because it is not easily interfered with other peaks. Moreover, by building a quantitative model based on the intensity ratio between the characteristic peaks of puerarin and ethanol, they successfully quantified the concentration of puerarin in ethanol. Subsequently, Wong et al[12] quantified the content of starch and polyphenols in PLR and PTR, using Raman spectroscopy coupled with partial least squares analysis. They found that the contents of starch and polyphenols can be used as references to differentiate PLR from PTR, and to predict the total phenolic content and antioxidant capacities. Wang et al[40] also demonstrated that Raman spectroscopy can be used both qualitatively and quantitatively for the analysis of forsythin, which is the main AIP in fructus forsythia. The peak intensity and peak area ratios were chosen as the reference parameters for determining the content of forsythin. Accurate results were obtained by way of well-defined mathematical processing. CHMs including radix aconiti kusnezoffii,[41] *Gynostemma pentaphyllum*,[42] cinnabar,[43] and radix sanguisorbae[44] were also well characterized using Raman spectroscopy, with the establishment of corresponding Raman spectrum database.

SERS is another ultrasensitive vibrational spectroscopic technique used for the analysis of CHM. Zhao et al[35] utilized SERS for analyzing *Coptis chinensis* and *Phellodendron amurense*, and their main active constituent berberine. The most intense peak located at 727 cm^{-1} was identified in the experimental spectra, which was consistent with density functional theory calculation results. Strong SERS peak signals were detected at 727 cm^{-1}, 752 cm^{-1}, 770 cm^{-1}, 1142 cm^{-1}, 1274 cm^{-1}, 1394 cm^{-1}, 1421 cm^{-1}, and 1566 cm^{-1}, corresponding to characteristic Raman peaks of berberine. Furthermore, liquid chromatography–mass spectra were adopted to quantify the berberine concentration. Their results demonstrated the capacity of SERS to identify CHM and its active constituents. Quick detection of another CHM, namely, atractylodis macrocephalae rhizoma, was also achieved by surface-enhanced Raman spectroscopy.[37] This occurred when intense SERS bands were observed due to the strong interaction between atractylodis macrocephalae rhizoma and silver colloid. Similarly, Wei et al[45] rapidly indentified and analyzed the APIs in *Panax notoginseng* using SERS. All these factors together suggested that the SERS technique shows great potential for a quick, effective,

accurate, and nondestructive analysis of CHMs without sample extraction and separation.

3.3.2. Polymorph characterization of APIs

Polymorphism is of critical interest in the development of pharmaceutical formulations. Variations in the crystalline forms of APIs may affect their physiochemical properties, which in turn impact the therapeutic effect, biocompatibility, and manufacturing processing.[46] Therefore, it is critically important to develop advanced analytic tools to understand the crystalline behavior of APIs at an early stage. Raman spectroscopy offers several particularly important advantages for polymorph screening: (1) highly sensitive to crystalline geometry and (2) requiring little or no sample preparation, thus preserving crystals in their original form.[47]

Qu et al[48] described an application of Raman spectroscopy for the chemical profiling of ginsenoside Rg3, which is the main API in ginseng. Their results revealed that two isomers of ginsenoside Rg3, 20-(R)-Rg3 and 20-(S)-Rg3, exhibit obvious differences in peak intensity for Raman bands at 640 cm^{-1}, 772 cm^{-1}, and 1674 cm^{-1}. Based on these chemical profiles, the authors were able to differentiate between the isomers. These findings pave the way to establish clear relations between physicochemical properties and crystalline forms.

Realgar, an arsenic sulfide mineral, has been used in Chinese medicine for more than 2400 years. Recently, As$_4$S$_4$, the main component of realgar, has attracted increasing attention due to its antitumor activities in several cancers, especially acute promyelocytic leukemia, *in vitro* and *in vivo*.[49] However, four crystal types (α-As$_4$S$_4$, β-As$_4$S$_4$, χ-As$_4$S$_4$, and pararealgar) have been reported for realgar, and there is no conclusive report on the type of crystal structure of medicinal realgar produced from China. Zhang el al[50] addressed this challenge using Raman spectroscopy in combination with X-ray diffraction. By correlating Raman spectra and X-ray diffraction pattern with chemical profiles of realgar, they were able to confirm α-As$_4$S$_4$ as the crystal structure of Chinese realgar.

3.3.3. Geographical origin identification of raw materials

Apart from the above applications, Raman spectroscopy can also be used for analytical discrimination between the same CHM with different geographical origins. Minor differences in concentrations of different nutrients within the samples can be identified by Raman spectroscopy. Ginseng is widely used in traditional herbal remedies to promote stamina and increase antifatigue capacity. They are mainly grown in China, Korea, and America. American ginseng is classified as an endangered species, and so its export and import is illegal in certain countries. In addition, the different forms of ginseng from variable sources are different in their potential beneficial effects. Therefore, it is highly desirable to distinguish between different types of ginseng. Edwards et al[51] investigated various forms of ginseng using Raman spectroscopy. By comparing the sets of spectra, they successfully identified the geographical origins of different types of ginseng. The discrimination is

based on the fact that the ginsenoside content differs in both conformation and concentration. This in turn translates into the presence or absence of certain Raman spectral features. For example, the Chinese ginseng exhibits a characteristic Raman peak at 980 cm^{-1}, which is absent in both the Korean and the American ginseng spectra. The peak at 1003 cm^{-1} is detected in both American and Chinese ginseng, while a peak at 1600 cm^{-1} appears in both American and Korean ginseng. Taking the above three facts into consideration, it is possible to deduce that it will be American ginseng if the peak is at 1003 cm^{-1} and peak at 1600 cm^{-1} are both detected. Similarly, Huang et al[52] demonstrated the difference in Raman spectra of radix astragali produced in different areas in China and proposed a valid classification model for geographical origin identification. Another example of this is the geographical origin identification of *Lycium barbarum* using confocal microprobe Raman spectroscopy, as described by Shi et al.[53]

3.4. Step II: In-process checks of CHM formulation and their characterization

Quality by design in drug manufacturing begins with verifying the raw materials and ends with the quality evaluation of the product. The latter requires assurance that the product, such as a gel cap, tablet, etc., contains the correct amount of active materials with appropriate polymorphs, excipients, and other additives (e.g., dyes). These require robust analytical tools that can effectively monitor the quality of the incoming raw materials, intermediates, and final products in real time. Raman spectroscopy serves as such an ideal candidate for process analysis.[54]

3.4.1. In-line quality monitoring of active ingredients

Zhang et al[50] used Raman spectroscopy to investigate the quality variations of medicinal realgar processed with different methods, such as grind to powder, refine with water, and wash with water/acid/alkali/vinegar. They compared the sets of Raman spectra and found that they are similar in both peak shapes and intensity. This result indicates that no or negligible-quality degradation is taking place during processing procedures, and corroborates the good stability of APIs in medicinal realgar. Another application was described by Edwards et al[51] using Raman spectroscopy for monitoring quality degradation in Korea ginseng. Significant differences in peak definitions and intensities were observed for the sliced Korean ginseng root before and after the powdering process, suggesting that the processing of ginseng may affect its molecular configuration.

3.4.2. Adulteration detection

Adulteration with synthetic drugs is a common problem with herbal medicine, which may result in adverse effects. It is, therefore, critically important to detect and identify adulterants in herbal medicine to ensure patient safety.[55,56]

Honey is a natural product with significant nutritional and medical value, depending on its purity.[57,58] However, honey can easily be adulterated with various cheaper sweeteners, resulting in a reduced therapeutic effect and considerably higher commercial profit. Li et al[59] investigated adulteration of honey using Raman spectroscopy in combination with the partial least squares-linear discriminant analysis. By correlating Raman spectra with chemical profiles of adulterated honey, they could discriminate genuine from adulterated honey, and moreover, detect different adulterants (e.g., high-fructose corn syrup and maltose syrup). Employing silver-nanoparticle-based SERS wiper, Li et al[60] detected dye adulteration of medicinal herbs. The detection limits were demonstrated to be 10^{-6} g/mL for malachite green, 10^{-7} g/mL for rhodamine 6G, and 5×10^{-8} g/mL for methylene blue.

Illegal chemicals, which could cause unpredictable side effects, may also be added into CHM for a rapid healing effect. Zhang et al[38] described a SERS analysis method to detect illegally added drugs. This method is able to analyze drug mixtures in Chinese traditional patent medicine without any separation process. Five kinds of illegally added drugs, including rosiglitazone maleate, phenformin hydrochloride, metformin hydrochloride, pioglitazone hydrochloride, and sibutramine hydrochloride, have been detected. Similarly, Cao et al[61] applied confocal Raman microscopy coupled with spatial mapping, and successfully identified the illegally added chemicals in hypoglycemic agent via multivariate analysis of Raman maps. Another example was given by Zhu et al,[62] who used thin-layer chromatography in combination with SERS to investigate the illegally added chemicals in antihypertensive CHM. Four kinds of illegally added chemicals such as nicardipine hydrochloride, doxazosin mesylate, propranolol hydrochloride, and hydrochlorothiazide were detected.

3.5. Step III: Counterfeit detection

Medicine counterfeiting is a growing and imposing threat to the pharmaceutical industries and society as a whole. Counterfeit medicines are drugs without any active ingredients, without sufficient active ingredients, or with fake packaging.[63,64] Raman spectroscopy coupled with multivariable analysis has provided a viable approach for fast and no-destructive medicine counterfeit detection.[65−69] This discrimination is based on the presence or absence of different excipients that could be identified with Raman scattering.[65,66,70]

Radix glehniae, *Anthriscus sylvestris*, and *Platycodon grandiflorus* are three kinds of medicinal herbs that share a common appearance with ginseng. They are generally used to counterfeit ginseng in commercial market for increased profit. Wan et al[71] suggested using Raman spectroscopy for high-throughput screening of various ginseng counterfeits. Additional "fingerprint" Raman peaks were detected in the counterfeits, in addition to the peaks shared by both genuine ginseng and counterfeits. In detail, radix glehniae exhibits an additional peak at 2206 cm^{-1}, while two additional peaks at 1050 cm^{-1} and 1869 cm^{-1} were identified in Raman spectrum of *A. sylvestris*. *P. grandiflorus* displays characteristic peaks at 600 cm^{-1}, 691 cm^{-1}, and 1227 cm^{-1}. These results indicated that Raman spectroscopy, in combination with second

derivative transformation, is able to efficiently discriminate between genuine and counterfeit ginseng.

Artesunate is derived from artemisinin and is a key drug in the treatment of multidrug-resistant *Plasmodium falciparum* malaria in Southeast Asia. de Veij et al[72] showed that Raman spectroscopy allows fast screening of the tablets and then separated counterfeit artesunate from genuine ones by careful spectroscopic interpretation coupled with an automatic approach. Starch, calcite ($CaCO_3$), and paracetamol (4-acet-amidophenol) were identified in counterfeits without detectable levels of artesunate. The counterfeit with a mixture of rutile and artesunate was also detected. Furthermore, their results indicated that the "chemical fingerprint" of different types of counterfeit artesunate could also be collected in

Table 1
Examples of various CHM resources analyzed by Raman spectroscopy.

Category	Example	Type of Raman spectroscopy used	Refs
Raw materials & APIs			
Ginseng	Fingerprint of 980 cm^{-1} is detected only for Chinese ginseng, & 1600 cm^{-1} lies in Korea & American ginseng.	Dispersive Raman spectroscopy	[51]
Yam	Protein structures of various yam species were analyzed.	FT-Raman spectroscopy	[32]
Radix puerariae	The API puerarin was identified & characterized qualitatively & quantitatively. The content of starch & polyphenols in PLR & PTR was detected.	Dispersive Raman spectroscopy in combination with partial least squares analysis	[12] [39]
Fructus forsythia	The API forsythin was identified & characterized qualitatively & quantitatively.	Dispersive Raman spectroscopy	[40]
Coptis chinensis & *Phellodendron amurense*	The API berberine was identified & characterized qualitatively & quantitatively.	Surface-enhanced Raman spectroscopy coupled with liquid chromatography−mass spectra	[35]
Atractylodis macrocephalae rhizoma & *Panax notoginseng*	Chemical "fingerprint" information was collected.	Surface-enhanced Raman spectroscopy	[37] [45]
Gynostemma pentaphyllum	Chemical "fingerprint" information was collected.	FT-Raman spectroscopy	[42]
Aconiti kusnezoffii & cinnabar	Chemical "fingerprint" information was collected.	Dispersive Raman spectroscopy	[41] [43]
Radix sanguisorbae	Chemical "fingerprint" information was collected.	Confocal Raman spectroscopy	[44]
Ginsenoside	Two isomers of ginsenoside Rg3, 20-(R)-Rg3 & 20-(S)-Rg3, were identified (polymorphs).	Dispersive Raman spectroscopy	[48]
Realgar	Crystal structure of the main component in Chinese realgar was determined as α-As_4S_4 (polymorphs).	Dispersive Raman spectroscopy	[50]
Radix astragali	Geographical origins of various sources were discriminated.	Dispersive Raman spectroscopy	[52]
Lycium barbarum	Geographical origins of various sources were discriminated.	Confocal Raman spectroscopy	[53]
Adulterants			
Sweeteners	High-fructose corn syrup & maltose syrup were identified & discriminated.	Dispersive Raman Spectroscopy in combination with partial least squares-linear discriminant analysis	[59]
Dyes	Dyes including rhodamine 6G & methylene blue were identified & discriminated.	Surface-enhanced Raman spectroscopy	[60]
Illegal chemicals	Five kinds of illegally added drugs have been detected	Dispersive Raman spectroscopy	[38]
	Synthetic hypoglycemic drugs adulterated in CHM were identified.	Confocal Raman spectroscopy coupled with spatial mapping	[61]
	Four antihypertensive chemicals adulterated in traditional Chinese medicine were identified.	Surface-enhanced Raman spectroscopy in combination with thin-layer chromatography	[62]
Counterfeits			
Ginseng versus radix glehniae et al	Additional "fingerprint" Raman peaks were detected in counterfeits in addition to the peaks shared by both genuine ginseng & counterfeits.	Dispersive Raman spectroscopy	[71]
Artesunate	Starch, calcite ($CaCO_3$), & paracetamol (4-acetamidophenol) were identified in counterfeits without detectable levels of artesunate. The counterfeit was a mixture of rutile & artesunate.	Dispersive Raman spectroscopy coupled with an automatic approach (PCA/HCA)	[72]
Fritillaria cirrhosa versus bulbus Fritillariae ussuriensis et al	Additional "fingerprint" Raman peaks were detected in counterfeits in addition to the peaks shared by both genuine ginseng & counterfeits.	Dispersive Raman spectroscopy	[73]
Chinese star anise & cortex cinnamomi	Additional "fingerprint" Raman peaks were detected in counterfeits in addition to the peaks shared by both genuine ginseng & counterfeits.	FT-Raman spectroscopy	[74] [75]

API = active pharmaceutical ingredient; CHM = Chinese herbal medicine; FT = Fourier transform; PLR = puerariae lobatae radix; PTR = puerariae thomsonii radix; HCA = Hierarchical cluster analysis; PCA = Principal components analysis.

combination with chemometry. This provides an important Raman spectrum database for counterfeits.

Another example was illustrated by Wang et al[73] involving *Fritillaria cirrhosa*. They found that the major spectral features observed in both spectra originated from alkaloids. However, upon further inspection of the spectral data, additional peaks are observed in the Raman spectrum of the suspect cases (e.g., bulbus Fritillariae ussuriensis, *Bolbostemma paniculatum*, *Tulipa edulis*, and lphigenia indica knuth et benth). These features were not identified in *F. cirrhosa*, which help distinguish *F. cirrhosa* from its allotropes. Similarly, CHMs such as Chinese star anise[74] and cortex cinnamomi[75] were also discriminated from their counterfeits using FT-Raman spectroscopy. Raman spectroscopy can also be used in portable devices for detecting counterfeit medicines. One example of this was its use for artesunate tables as described by Ricci et al.[76]

4. Discussion

It is clear from a review of the current literature that Raman spectroscopy has become increasingly important in the field of CHM over the past two decades. The technique can identify and analyze raw materials in terms of a chemical "fingerprint," polymorph, and geographical origin. During formulation manufacturing, Raman spectroscopy can be used for in-line checks and analysis such as monitoring quality variability in active ingredients, characterization of adulterants, and detection of counterfeits, among other benefits (Fig. 1 and Table 1). These advantages, coupled with the possibility of online monitoring and availability of accessible spectrometers, ensure that Raman spectroscopy will contribute more to the modernization and globalization of CHM in the future.

In spite of the significant progress mentioned above, a wide scope of intriguing possibilities regarding the application of Raman spectroscopy in CHM requires further exploration. (1) Efforts in the direction of establishing a comprehensive Raman spectrum database of CHM are required. CHM resource investigation demonstrated that over 11,000 plant species representing 2309 genera and 383 families were used in the CHM practice.[1] The huge amount of CHM resources present great challenges for documenting and organizing information. Hence, more efforts toward establishment, organization, and development of these databases in the CHM field should be made to make full use of Raman spectroscopy. (2) Exploration in the area of new drug discovery is needed. The huge amount of CHM resources provides rich resources for new drug discovery. Over the past decades, this field has witnessed success in the development of a number of new drugs, such as anti-Alzheimer drug huperzine A, antimalarial drug artemisinin, antihepatitis drug bicyclol, etc., from traditional CHM. However, exploration of Raman spectroscopy in the area of drug discovery remains limited. Opportunities for advanced and wide applications of Raman spectroscopy in new drug discovery can be found in state-of-the-art drug identification applications. (3) Applications in compatibility studies between APIs and excipients, and stability studies of

APIs in CHM are lacking. Raman spectroscopy exhibits promising potential in these areas.

In addition, most of the literature reports on the use of Raman spectroscopy for herbal medicine analysis, especially in the field of CHM, are found in Chinese-language publications. This existing language limitation hampers effective communication and dissemination of the latest research results at the international level. In order to speed up the modernization and globalization of CHM, inclusion of their benefits reported in English-language publications is highly desirable.

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