A Quantitative Study of Carmine Aqueous Solution Based on Drop-Coating Deposition Micro-Raman Spectroscopy (DCDR)

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Abstract. Carmine is a kind of colorant which is widely used in food, beverage, medicine, cosmetics and tobacco industry. However, excessive use of carmine may lead to the risks of carcinogenic, teratogenic and mutagenic, which seriously threaten the health and safety of consumers. In this paper, DCDR technology is utilized to develop a quantitative method for the detection of carmine, which requires only a small volume deposition of analyte solution (several μL) on a suitable hydrophobic substrate. The conventional Raman spectrum of carmine aqueous solution and corresponding Raman spectrum using DCDR method were compared, illustrating a much higher sensitivity for DCDR method. The Raman spectra of carmine aqueous solution with different concentrations of 100, 50, 10, 8, 4 and 2μg/mL are acquired from the spots on the “coffee-ring” with DCDR method. Using DCDR method, a good linear relationship has been observed between the intensities of the two characteristic peaks, 1364cm−1 as well as 1572cm−1, and the concentrations of the solution, with the linear correlation coefficient of R2>0.99. The results illustrate that DCDR method has a good potential in the quantitative analysis of colorant like carmine, providing a promising technique for a rapid detection for food additives.

Keywords. Carmine, DCDR technology, micro-Raman spectroscopy, quantitative detection.

1. Introduction
Carmine is currently one of the most widely used monoazo synthetic colorants in China, which is used for colouring foods such as fruit juice drinks, mixed wines, carbonated beverage, candies, cakes, ice cream and yogurt [1]. Carmine is a compound (CAS Number: 1390-65-4) with a dark red colour in powder, which is 1,3-naphthalenedisulfonic acid, 7-hydroxy-8-[4-sulfo-1-naphthenyl] azo] trisodium salt in aqueous solution and solid state [2]. The molecular formula of Carmine is C20H11N2Na3O10S3, and the chemical structure is shown in figure 1. As a kind of azo compounds, carmine can be metabolized intoβ-naphthylamine and α - amino-1-naphthol in the body which are strong carcinogenic substances. The aromatic amines are further activated by metabolism and turn into electrophilic products which could combine with DNA and RNA to form adducts inducing mutations [3]. Also, carmine can be oxidized to free radicals, and then metabolizes with the substances in the body to produce reactive oxygen species (ROS), which could attack DNA and causing oxidative damage [4]. It has been proved to be certain carcinogenic and mutagenic effects in toxicology experiments [1, 5].
Figure 1. Chemical structure of carmine.

The addition of carmine is prohibited into food in the United States, Canada and Norway. There are strict regulations on the scope of use and maximum usage for carmine as an additive, even though the addition is allowed in food in China, the European Union, Japan and other countries. According to the provisions of the Chinese National Standard of GB/T 5009.35-2016, the detection limit of carmine as an additive is 0.5 mg/kg in foods. And carmine is not allowed to add to the aquatic products or meat in China.

However, food safety incidents of excessive or illegal addition in food happen from time to time, seriously threatening the health and safety of consumers. With more and more attention to the excessive use of colorants in food safety, the study on the detection of carmine is increasingly a need.

Variety of modern analytical methods and instruments are used to detect additives, including thin layer chromatography, spectrophotometry, voltammetry, polarography, capillary electrophoresis, ion chromatography and high performance liquid chromatography (HPLC) etc. [6]. However, these methods either need complicated sample preparation process, or time-consuming, or need expensive analytical instruments, and are not suitable for large-scale rapid field screening requirements [7].

It is of great significance and urgency to develop a simple, fast, portable and method for detecting colorants, especially for azo compounds like carmine.

Drop coating deposition Raman (DCDR) spectroscopy is a technique based on the deposition of a small volume of analyte solution (several μL) on a suitable hydrophobic substrate. Due to the surface hydrophobicity, evaporation of the solvent may lead to preconcentration of studied analyte in ring-shaped drying pattern (known as “coffee-ring”) from which Raman spectra are acquired. DCDR enables the measurement of solutions at very low concentrations and small volumes [8], and has been a widely used technique to study biological molecules and molecular mixtures like lipids, porphyrins, tears, amino acids, peptides, haemoglobin, oligosaccharides, liposomes in cholesterol and other molecules in recent years [9-13].

The aim of this paper is to develop a quantitative method for the detection of carmine based on DCDR technique, excepting to explore a rapid, easy quantitative detection method for azo dye additives.

2. Materials and Methods

2.1. Laboratory Equipment and Materials
Raman spectra of the samples were measured by confocal Raman microscope spectrometer (Invia, Renishaw Inc.), equipped with 1200 l/mm grating. Before the measurement, the wavenumbers were calibrated by using the excited line of 520 cm⁻¹ from silicon. Measurements were carried out with monitoring by a 20x objective of microscope (N. A. =0.4, Leica). The 785 nm laser was used as the excitation source with a power of 50 mw and the final laser power on the sample was ~8 mW.
2.2. Standard Solution Preparation of Carmine
Carmine powder (99%, Sigma-Aldrich Chemicals, St. Louis, MO, USA) was dissolved in deionized water (Millipore-Q) and prepared as a stock solution with a concentration of 1000 μg/mL. The stock solution was subsequently diluted into a series of concentrations of 100, 50, 10, 8, 4, and 2 μg/mL for measurement.

2.3. Measurement of DCDR Spectra for Carmine Solutions
The preparation of samples for DCDR measurements could be divided into two steps as following: (1) Carmine solution with different concentrations were deposited by micropipette as 2μL droplets on an polished aluminium substrate (76×26cm); (2) Then the sample was dried left to dry naturally at ambient temperature of 20℃ for 20min, where the “coffee-rings” are formed.

The prepared dried deposits on the substrate were placed on the stage of microscope Raman measurement. “Coffee-rings” of the deposits could be observed that most of the molecules of samples have aggregated in ring-shape to the edge of the deposits with the monitor of 20× objective. As shown in figure 2, the Raman spectra of samples from 400 to 2000 cm\(^{-1}\) were obtained with 3 spectral scans, and each with an accumulation time of 20s.

![Figure 2. Schematic diagram of DCDR method for the detection of carmine: (a) Layout of the measuring spots on the coffee-ring; (b) White light image of carmine deposit under 20× objective.](image)

For DCDR measurements, a minimum of 6 spectra from different spots with uniform distribution on the coffee-ring of the dried deposits were acquired in order to minimized the non-uniform distribution on the coffee-rings (shown in figure 2(a)). The white image of the measured spot on the “coffee-rings” is shown in figure 2(b) under 20× objective.

The 6 spectra from a coffee-ring were averaged as the DCDR spectrum of the dried deposit. All the samples were kept at ambient temperature of 25℃ during the measurements.

3. Results and Discussion
The original spectra acquired were only subtracted by the baseline, without any other treatments, so as to reflecting the intrinsic characteristics of spectra from different sample such as intensities and wavenumbers of the peaks.

In order to investigate the advantages of DCDR technique in the detection of carmine solution samples, the conventional Raman measurement using droplet method and the DCDR method were carried out on the same sample droplet. As shown in figure 3, the DCDR method has a higher sensitivity than that of conventional Raman measurement.
The Raman peaks of carmine can be clearly observed in figure 3, including that of 1240 cm\(^{-1}\), 1300 cm\(^{-1}\), 1364 cm\(^{-1}\), 1439 cm\(^{-1}\), 1514 cm\(^{-1}\), 1572 cm\(^{-1}\). Tentative assignments of peaks from the DCDR spectra of carmine were shown in table 1 [2]. Specially, the peak at 1364 cm\(^{-1}\) assigned to stretching of CC (\(\nu_{CC}\)), as well as the peak at 1572 cm\(^{-1}\) assigned to the bending of NH(\(\delta_{NH}\)), deformation of CH(\(\beta_{CH}\)) and stretching of CC (\(\nu_{CC}\)), were characteristic peaks for the identity of carmine as an azo colorant.

Table 1. Characteristic Raman shifts and tentative assignments of peaks from the DCDR spectra of carmine [2].

| Raman Shifts(cm\(^{-1}\)) | Assignments                          |
|---------------------------|--------------------------------------|
| 1572                      | \(\delta_{NH}, \beta_{CH}, \nu_{CC}\) |
| 1514                      | \(\delta_{NH}, \nu_{C=O}, \nu_{C=N}, \beta_{CH}\) |
| 1439                      | \(\beta_{CH}\)                        |
| 1364                      | \(\nu_{CC}\)                         |
| 1300                      | \(\delta_{NH}, \nu_{N-N}, \beta_{CH}, \nu_{CC}\) |
| 1240                      | \(\delta_{NH}, \nu_{N-N}, \beta_{CH}, \nu_{CC}\) |

\(^{a}\)Bending.  
\(^{b}\)Deformation  
\(^{c}\)Stretching.

The averaged DCDR spectra were obtained from deposits of carmine solution with different concentrations of 100, 50, 10, 8, 4 and 2 \(\mu\)g/mL, which are shown in figure 4. It is illustrated clearly that the intensities of DCDR spectra showed a trend of decline with the decreasing concentration of the carmine solutions. That is, the intensities of DCDR are positively correlated with concentrations.
The two characteristic peaks of 1362 cm\(^{-1}\) and 1572 cm\(^{-1}\) can be also clearly observed when the concentration is as low as 2 \(\mu\)g/mL, indicating a promising sensitivity for the detection of carmine solution with a detection limit less than 2 \(\mu\)g/mL.

In order to verify the potential in the quantitative detection of carmine solution, the linearity of DCDR method using the intensities of the two characteristic peaks of 1362 cm\(^{-1}\) and 1572 cm\(^{-1}\) was further investigated.

It can be seen from figure 5 and figure 6, that there is a good linear relationship between the intensities of the two characteristic peaks of DCDR spectra and concentrations of carmine solutions in the range of 2-50 \(\mu\)g/mL, with a linear correlation coefficient \(R^2\) greater than 0.99.

**Figure 4.** DCDR spectra of carmine from a dried drop in deposited concentration of 100, 50, 10, 8, 4 and 2 \(\mu\)g/mL.

**Figure 5.** Fitting result of carmine DCDR spectra for intensities of 1365 cm\(^{-1}\) within concentrations of 2-50 \(\mu\)g/mL.

**Figure 6.** Fitting result of carmine DCDR spectra for intensities of 1572 cm\(^{-1}\) within concentrations of 2-50 \(\mu\)g/mL.

**4. Conclusion**

In this paper, DCDR technique was utilized as a quantitative study of the detection of azo colorant carmine. DCDR spectra from dried deposits of different carmine solution concentrations were obtained for a quantitative investigation.
Intensities of the two characteristic peaks of 1364 cm$^{-1}$ and 1572 cm$^{-1}$ showed a good linear relationship within concentrations of 2-50 μg/mL, with a correlation coefficient of $R^2 > 0.99$. The results show that DCDR technique has a good potential in the quantitative detection of colorant like carmine, providing a promising technique for a rapid detection for food additives.

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