VISUAL DETECTION OF SUDAN-IV DYE IN CULINARY SPICES BY PLASMON RESONANCE LIGHT SCATTERING

A Thesis on

Submitted in partial fulfilment of requirement for the award of degree of

MASTER OF SCIENCE
IN
PHYSICS

Submitted by

ALICE GOYAL

Roll No. 301404001

Under the guidance of

Dr. B. N. Chudasama

Associate Professor

SCHOOL OF PHYSICS AND MATERIAL SCIENCE

Thapar University, Patiala

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CERTIFICATE

This is to certify that the thesis entitled, "**Visual Detection of Sudan-IV Dye in Culinary Spices by Plasmon Resonance Light Scattering**"being submitted by **Ms. Alice Goyal (Roll No. 301404001)**of M.Sc. Physics at Thapar University, Patiala was carried out by herunder my supervision.She has not submitted this material for credit towards any other degree at Thapar University, Patiala or any other university.

Date: 18 July 2016

ALICE GOYAL
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This is to certify that above declaration made by the student concerned is correct to the best of our knowledge and belief.

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Alice

ALICE GOYAL

ABSTRACT

Detection of food adulteration is becoming a matter of concern for ensuring proper health of the people. Culinary spices are commonly used in most of the food items, which mostly contain adulterants like Sudan dyes. Sudan dyes has been classified as category III carcinogens which even if present in minute preparation in food than can cause serious health hazards. This work conducts a feasibility study and proposes a novel method enabling the visual detection of Sudan dyes successfully in commercial spices. A method for quantification of Sudan dyes present in culinary spices based on Plasmon Resonance Light Scattering has been devised which can successfully detect presence of Sudan at sub ppm levels. The method proposed in this study excludes the cumbersome separation step as required in other HPLC based methods. In addition, it provides visual evidence of the presence of adulterant in the food samples. The technique can be extended for reliable and rapid detection of adulterants in food products.

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CHAPTER 1

INTRODUCTION

Food is one of the basic necessities among man's everyday needs and to meet the consumer's demand, food sector aims to produce visually appealing food that have good taste. As a result, food adulteration is becoming a matter of concern for both the consumers and industries. Adulterated food products are high commercialized products and are produced in sky-high amounts around the globe. Food safety is becoming a matter of concern for ensuring the proper health of the people. A novel method is required enabling the more subtle detection of adulterations in food articles. Dictionary defines the food adulteration as the mixing of a mediocre and sometimes low quality food to be sold deliberately and hence food becomes contaminated and inadequate for human consumption.

1.1 Food Adulteration

"Adulteration" is a legal term used for food products that do not meet the requirement of state standards [1]. Adulteration also means disagreement with health or welfare standards of the country. Food, Drug and Cosmetics are "adulterated" if they meet any of the following criteria.

1.1.1 Poisonous or Detrimental Substances

Usually if a food contains a poisonous or detrimental substance that can pose serious risk to health, it is adulterated. Firstly, if the adulteration is inherent or naturally occurring and its presence in the food does not customarily provide a health risk, the food will not be considered adulterated [2]. Secondly, if the adulteration is inevitable and is within the prescribed limit, the food will not be considered adulterated.

1.1.2 Filth and Foreign Matter

This includes any obnoxious substances in food such as glass, metal, plastic, wood, stones, cigarette butts (Foreign Matter) and unwanted parts of the raw plant material namely rot, excreta, insects, mold and decomposition [3].

1.1.3 Economic Adulteration

A food is said to be adulterated if it removes a valuable constituent or adds an alternative substance in whole or in parts, for e.g. olive oil diluted with sunflower oil or a fresh fruit with wax on its surface to hide defects. Also, if any substance has been

added to it to increase its weight, minimize its quality and make it appear bigger or of greater value than it is adulterated [4].

1.1.4 Microbiological Contamination and Adulteration of Food

Food that is contaminated with harmful microorganisms such as bacteria, viruses, or protozoa may, or may not, make it adulterated. Usually, for ready-to-eat foods, the presence of microorganisms will make the food adulterated. For e.g., the presence of Campylobacter and Salmonella in raw poultry or in ready-to-eat foods or in vegetables and fresh fruits will make the products adulterated [1].

1.2 Types of Food Adulteration

1.2.1 Milk Adulteration

Milk adulteration involves adding water to milk and removing the beneficial fats from milk. Often soya milk, starch, groundnut milk, and wheat flour are added to milk [5]. This makes the milk less nutritious and it results in milk being useless for the consumer.

1.2.2 Adulteration of Fats and Oils

It is easy to adulterate oils and fats. But it is difficult to detect such adulteration. Common adulterant present in ghee and oil are paraffin wax, hydrocarbons, dyes and Argemone oil. Ghee is often mixed with hydrogenated oils such as groundnut oil, Tallow and Mohua (Mowrah) oil and animal fats [6]. Synthetic colours and flavours are added to other fats to make them appear like ghee.

1.2.3 Food Grain Adulteration

This involves mixing sand or crushed stones in order to increase the weight of food grains. Very often, pulses and cereal grains are mixed with plastic beads that have a similar appearance as grains in color and size [5].

1.2.4 Other Adulterations

Various dyes, brick powder are often added to chili powder while tea leaves are often mixed with used tea leaves. These adulterations are very harmful to the consumer and they should be addressed by consumer organizations and consumers seriously. This category also includes the culinary spices used in daily food products to add taste and color e.g. turmeric, pepper, five-spice powder etc. It is often adulterated with Sudan dyes for colouring [7].

1.3 Detection of Food Adulteration

Adulteration of food deceives the consumers and can render a serious risk to their health. Generally, visual inspection does not fulfil the purpose, especially when adulteration is expected to have high level of sophistication. For example, synthetic colors are added to food to replace the natural colors lost during processing. Conventionally, the colors were added to the food products without knowing their harmful effects. The use of colors for making the foodstuffs attractive and appealing is known for centuries but today colors added to the food products have become important criteria for the choice of food. Coloration of food products is done to maintain the aesthetic quality.

Table 1.1. An analysis comparing the sensitivity of different methods to detect adulterant in food articles

Food	Adulterant	Method of Detection	Sensitivity
Milk	Melamine	Hydrogen-bonding recognition-induced colors change of gold nanoparticles	This method allowed a detection concentration as low as 2.5 ppb.
Olive oil	Fatty acids, unrefined hazel nut oil, sunflower oil	FT-Raman Spectroscopy in conjugation with PLSR	Levels greater than 1% were accurately measured.
		Confocal Raman Spectroscopy with Partial least square discriminant analysis (PLS-DA)	Adulteration at concentrations as low as 1% v/v was detected.
Honey	Pyrrolizidine alkaloids, Mycotonins, Sugar Syrups, Proline	MS, NMR, UV-Visible Spectroscopy, IRMS	This method enabled the detection at concentrations 1.6% v/v.
Culinary Spices	Sudan I-IV dyes, Brick Powder, Metanil yellow, Rhodamine B	SERS with Multivariate Chemometrics	The limit of detection (LOD) is 48 µg/kg.
		Voltammetric determination using Gemini surfactant-ionic liquid-multiwalled carbon nanotubes	The detection limit is down to 0.03 µmol l^{-1}.

The possible harmful effects of artificial colors in humans through intake of food are a matter of concern around the world. All ingredients comprising food colors should be listed on the food labels. The color in the food article must be same as that shown on the label as some people are allergic to certain food color. Thus, determination of the artificial food colors is necessary to ensure food safety [8]. Several non-permitted colors such as Sudan dyes, Orange II, Quinoline yellow, Rhodamine B, Malachite Green, Metanil Yellow, Auramine, etc. were detected in food products like culinary

spices [7]. Culinary spices are more prone to adulterants like Sudan which are used for colouring. A number of non-food dyes meant for textiles, paper, polishes and other purposes are used in foods either deliberately or through ignorance due to their low cost and easy accessibility [7]. Majority of non-permitted dyes such as Rhodamine B, Metanil Yellow and Sudan dyes are known to cause various allergic reactions, brain, bladder and kidney tumours, hormone disruption and many serious life threatening diseases. Sudan dyes mostly goes unnoticed in conventional food adulteration tests due to its low amounts (0.1 – 10 ppm). The constant intake of adulterated food even with such low amount of adulterations can pose high risk to health of consumer. Because of this, new method of detection with high sensitivity needs to be developed.

1.4 Surface Plasmon Resonance

Surface plasmon resonance (SPR) is the oscillation of the conduction band electrons in resonance with an incident light of resonant frequency. These resonant electrons are collectively known as plasmons. These plasmons are confined to surface (interface) and interact with light resulting in polaritons. Metal nanoparticles, especially gold and silver are said to have a unique optical property which can be assigned to the SPR of the metal clusters. When small metal nanoparticles are radiated with electromagnetic radiation, the oscillating electric field induces coherent oscillations of conduction electrons (Fig. 1.2). Because of this coherent oscillations electron cloud gets displaced and hence a restoring force arise due to coulomb attraction. This Colombian interaction results into coherent oscillations of conduction electron clouds. The oscillation frequency depends on (i) the electron density, (ii) effective mass of electrons, (iii) shape and (iv) size of the charge distribution.

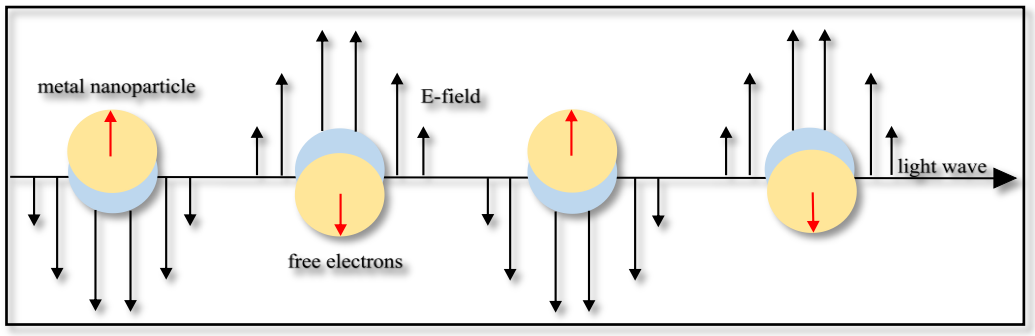

Fig. 1.2 Schematic representation of plasmon oscillation for a sphere, showing the displacement of the conduction electron charge cloud relative to the nuclei as a result of electromagnetic field.

SPR of silver nanoparticles have been largely studied for their better performances due to their SPR in the visible range like gold or copper [12]. Silver nanoparticles are said to have high SPR intensity and this SPR scattering is intense enough to allow even individual nanoparticles to be clearly distinguished. Silver

nanoparticles thus can render better sensitivity for some applications such as biosensing, photonics, electronics and antimicrobial applications. SPR based biosensors are especially alluring in food safety as they may detect analytes in complex matrices with minimum sample treatment. Further, nanomaterial based sensors are useful to detect various adulteration done in food products as these sensors allows the detection at low levels compared to other conventional methods. Nanomaterial-based sensors include binding or reaction of biological components with target species and at the end changing eventually into detectable signals, thereby allowing rapid detection of food adulterants and ensuring food safety. They also provide advantages of fast, sensitive, and adaptable detection, enabling portability for in-field application [13].

Nanotechnology plays a vital role in detection of this kind of adulterants in small amounts and making bio-sensors for the same [9]. In this work, metal nanoparticles (NPs) are explored to detect the presence of adulterants in food products. There are several metal NPs e.g. gold, silver, lead, copper, platinum etc. that have found application in food adulteration detection due to their special optical and electronic properties [10]. The biggest class of adulterants affecting food products are azo dyes. A number of azo dyes such as Acid Red 85, Ponceau, Sudan, and Disperse Yellow 7, etc. [11] are used for colouring food products, as these dyes give bright colors to the food in which they are added. Out of all these azo dyes, Sudan is the main focus of this study as it is widely used in colouring of various food products these days.

1.5 Literature Review

1.5.1 Methods for Detection of Sudan Dyes in Culinary Spices (Chili Powder, Turmeric, Paprika and Curry Powder)

Methods	Conclusion	References
Plasmon Resonance Light Scattering (PRLS) with Silver Nanoparticles	A PRLS signals-based detection of Sudan dyes, without any sample pre-treatments or separation was proposed. The test on real samples of cayenne oil and chili sauce has been performed, which revealed that the method is reliable, sensitive, efficient, and simple. This method can be extended for synthesis of silver nanoparticles. It was found that silver nanoparticles started aggregating as the concentration of Sudan became higher.	L. P. Wu, et. al. "*Anal. Chem.*", 78 **(2006)**, 5570. [10]
SERS with	A gold citrate sol was employed in this study	W. Cheung, et. al.

multivariate chemometrics	as instead of silver sols as they have better homogeneity. The data reported here shows that it is possible to get good sensitivity by employing SERS technique. In this method multivariate chemometrics is essential for better sensitivity. The limit of detection of Sudan-I by this method is found to be 48 µg/kg for a commercial sample of chili powder.	"*J. Phys. Chem.*", 114 **(2010)**, 7285. [14]
Voltammetric determination using Gemini surfactant-ionic liquid-multiwalled carbon nanotubes	Carbon nanotube, ionic liquid and Gemini surfactant were combined to make a novel composite. The composite coated GCE (Glass Carbon Electrode) showed higher sensitivity of Sudan-I detection in comparison with the electrodes have been reported. The limit of detection was 0.03 µmol l^{-1}.	Z. Mo, et. al. "*Food Chemistry*", 121 **(2010)**, 233. [15]
UV-Vis Spectroscopy and Multivariate Classification	This method represents easy and cost-effective detection of Sudan-I in paprika. Partial Least Square-Discriminant Analysis (PLS-DA) gave better results than K-Nearest Neighbour (KNN).	C. V. D. Anibal, et. al. "*Food Anal. Methods*", 7 **(2014)**, 1090. [16]
	Three classification techniques namely, K-nearest neighbour (KNN), soft independent modelling of class analogy (SIMCA) and partial least square-discriminant analysis (PLS-DA) were applied. The three techniques showed that the results were better with PLS-DA. This method enabled the clear detection of Sudan-III in paprika.	C. V. D. Anibal, et. al. "*Talanta*", 79 **(2009)**, 887. [17]
HPLC/APCI-MS	HPLC/APCI-MS method was used to detect Sudan-I in chili powder. This method enabled the detection of Sudan-I in chili powder at very low levels.	F. Tateo And M. Bononi, "*J. Agric. Food Chem.*", 52 **(2004),** 655. [18]
	This method showed a good separation of all analytes under SRM (Single Reaction Monitoring) mode to detect Sudan I to IV dyes. Limit of detection varied from 5-18 µg/l and LOQ from 10-24 µg/l.	M. R. V. S. Murty, et. al. "*Food Chemistry*", 115 **(2009)**, 1556. [19]
LC-UV and LC-MS	A review of the detection methods of Sudan I-IV dyes showed that LC-UV and LC-MS	R. Rebane, et. al. "*J. Chromatography*

	were the dominating methods. Samples of chili powder extracted by using acetonitrile showed 99% recovery for Sudan-IV and Sudan-I.	*A*", 1217 **(2010)**, 2747. [20]
On-line solid phase extraction coupled with HPLC	Results obtained showed that this method is simple and sensitive for the analysis of Sudan dyes, especially Sudan-III. Silica used as an adsorbent was found to be cost effective and has high compatibility for Sudan dyes.	X. Zhixiang, et. al. "*Chromatographia*", 71 **(2010)**, 397. [21]
SFE Coupled with CLC-DAD	This method proved to be a powerful method for the analyses of Sudan dyes in chili powder. SFE method offered reduced sample manipulation, costs and total extraction time. The limit of detection was ranged from 23.2 to 42 ng mL^{-1}.	M. Ávilaa, et. al. "J. Supercritical Fluids", 55 **(2011)**. [22]
SPE coupled with HPLC-DAD	A simple, inexpensive and rapid solid phase extraction (SPE) method coupled with HPLC-DAD for the detection of Sudan dyes in chili powder was developed. The LOD and LOQ were in the range of 4.1-5.8 and 13.2-19.1 µg/kg, respectively.	P. Qi, et. al. "*Food Chemistry*" 125 **(2011)**, 1462. [23]
HNMR combined with chemometric treatment	This was an efficient method for detecting Sudan dyes. The results obtained by PLS-DA were better as compared to other classifications used.	C. V. D. Anibal, et. al. "*Food Chemistry*", 124 **(2011)**, 1139. [24]
UV-Vis, HNMR with PLS-DA	It was concluded that UV-visible and HNMR is a rapid tool for detecting Sudan dyes. Results obtained with UV-Visible were better than NMR techniques. Paprika samples adulterated with Sudan IV showed the highest sensitivity.	C. V. D. Anibal, et. al. "*Talanta*", 84 **(2011)**, 829. [25]

1.6 Gaps in Study

After having a closer look at the literature review, significant literature gaps and the major improvement areas in detection of Sudan dyes in culinary spices are found. Most of the reports were confined to the detection of Sudan family by liquid chromatographic method and related approaches. These methods require large sample preparation which is time taking and make it cumbersome analysis in real time. Limited work has been carried out with UV-Visible Spectroscopy and detection limit are low. Individual detection of Sudan dyes by other methods was a major concern.

Limited work has reported to detect adulterated samples with single or blended dyes via Plasmon Resonance Light Scattering (PRLS). Detection limits of Sudan in culinary spices has been found to be close to 4 ppm. Further improvement in this detection limit is required. In addition, processes needs to be developed that are based on visual detection so that common man can follow the protocols and can check the possibility of adulteration in their spices.

1.7 Objectives

The objectives of this thesis are:

- Establish the lowest detection limit of Sudan-IV using UV-Visible Spectroscopy.
- Devise protocols for visual detection of Sudan-IV in Culinary Spices.
- Establishment of experimental protocols for quantification of Sudan-IV using Plasmon Resonance Spectroscopy.

CHAPTER 2

EXPERIMENTAL

2.1 Introduction

Developing a reliable method for the detection of Sudan dyes is important for the quality control in food products. Sudan family includes four types of azo dyes, namely I, II, III and IV. These dyes are carcinogenic in nature and are mostly used in plastics, oil painting, waxes and dyeing since they help in improving the lustre of commercial products [26, 27]. Sudan is not allowed to be used in food products as it is termed as a carcinogen by the country [28]. It is found that among various food products, Sudan dyes are used as an adulterant in culinary spices. However, from the four Sudan dyes, Sudan-IV finds the main focus of interest in this study as to date, detection of Sudan-IV in culinary spices is based on liquid chromatographic methods [29]. In this chapter, experimental protocols have been described which are being developed for detection of Sudan-IV by UV-Visible Spectroscopy.

2.2 UV-Visible Spectroscopy

UV-Visible-NIR (UV-Vis-NIR) spectroscopy is the absorption spectroscopy in the ultraviolet-visible and near infrared (NIR) region. The UV-Vis spectrophotometer measures the absorption at each wavelength [30]. The resulting spectrum is represented as a plot of absorbance (A) vs. wavelength, where the absorbance is related to the path length b and concentration c of the sample and is given by Beer-Lambert law [31]

$$A = \varepsilon bc \qquad (2.1)$$

Where ε is the constant of proportionality known as molar extinction coefficient.

A spectrophotometer can be single beam or double beam. Double beam instruments (Fig. 2.1), improve the accuracy, as they carry a system in which two cells are used; one carries either pure solvent or a blank solution (reference) and the second carries the solution of the sample. [30, 32] The instrument readout is the difference between the absorption in the two cells. Thus, they provide a correction for the absorption of the blank and the changes in the intensity of incident radiation. [32] Samples to be analysed are poured in a transparent rectangular cell, known as cuvette. These cuvettes are made of quartz glass or silica as they are transparent throughout the UV-Vis and NIR region. [30] The spectrometer is capable of recording optical response of the sample in the UV-Vis region (190-900 nm). [33]

Fig. 2.1 Simplified Schematic of a double beam UV-Visible Spectrophotometer [1]

2.3 Detection of Sudan-IV by UV-Visible Spectroscopy

Reagents. Sudan-IV, Silver Nitrate ($AgNO_3$, 99.8%), Triton X-100, Ammonium Hydroxide solution were purchased from Sigma-Aldrich, USA. Dimethylformamide (DMF) was purchased from Loba Chemie PVT LTD. All the reagents were used as received without any purification. Mili-Q ultrapure water was used throughout the synthesis.

Apparatus. The UV-Visible spectra and intensity were measured with a double beam UV-Visible Spectrophotometer, Shimadzu UV-2600, in the wavelength of 350-700 nm. A vortex mixer Remi CM 101 PLUS was used to blend the solution. Centrifugations were done on Mega 17 R centrifuge.

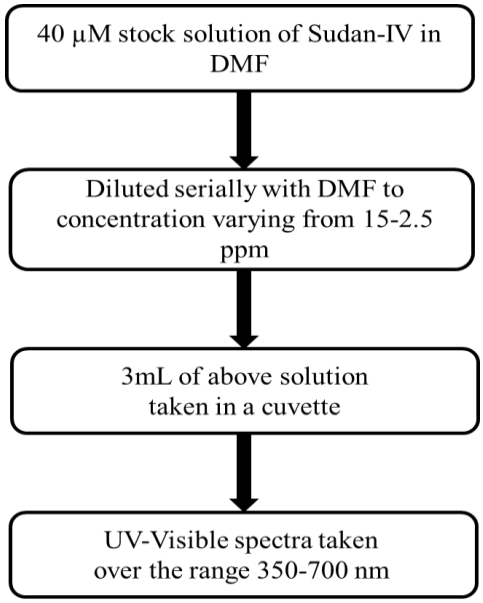

Fig. 2.2 Test protocol for Sudan-IV detection using UV-visible spectroscopy

Procedure: The 40µM stock solution of Sudan-IV was prepared by dissolving appropriate quantity of its commercial product in DMF. The stock solution was then diluted serially with DMF to various concentrations ranging from 15 ppm to 2.5 ppm. UV-Visible spectra were recorded for each of these concentrations of standard Sudan-IV solutions in quartz cuvette at room temperature. From the absorption maximum of UV-Visible spectra for each concentration, a linear correlation was established between absorption maximum and concentration of the Sudan-IV. From linear extrapolation of this data, minimum detection limit of Sudan-IV for UV-visible spectroscopy was established.

2.4 Detection of Sudan-IV using Plasmon Resonance Spectroscopy

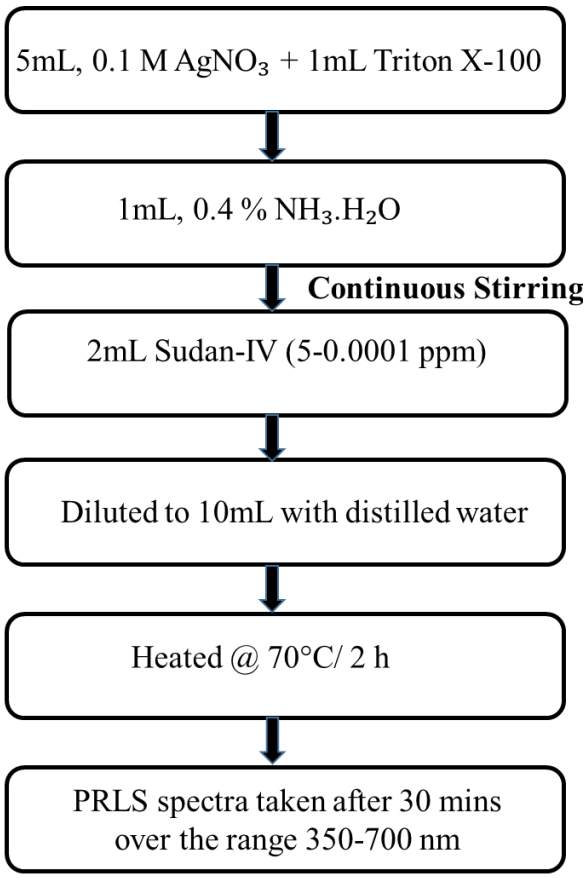

Fig. 2.3 Test protocols for Sudan-IV detection using plasmon resonance light scattering in standard sample

Procedure: A total mixture of 5 mL, 0.1 M $AgNO_3$, 1 mL Triton X-100 and 1 mL of 0.4% $NH_3.H_2O$ working solution was added in a 30 mL test tube. The mixture was stirred well. 2 mL of Sudan-IV solution was added at various concentrations (5-0.0001 ppm). The mixture was then diluted to 10 mL with distilled water and stirred thoroughly. It was then heated to 70°C for approximately two hours. PRLS spectra of the solution were recorded from 350-700 nm after thirty minutes and the absorbance was measured against the blank solution (Distilled water). The absorbance maximum

was measured for each concentration and again linear correlation was established between absorbance and concentration of Sudan-IV. The minimum detection limit of standard Sudan-IV solution by plasmon resonance spectroscopy is thus established.

2.5 Detection of Sudan-IV in Turmeric Samples

Pre-treatment of turmeric samples: Three turmeric powders of different commercial trade were purchased directly from the market and were treated as real samples. 1.0 g of the turmeric powder to be analysed were placed in a 30 mL test tube and then dissolved with 10 mL DMF. The mixture then vortexed thoroughly and was centrifuged at 10,000 rpm for 800 seconds. 10 mins later, the top translucent layer of liquid will undergo the sample preparation steps as followed for standard samples.

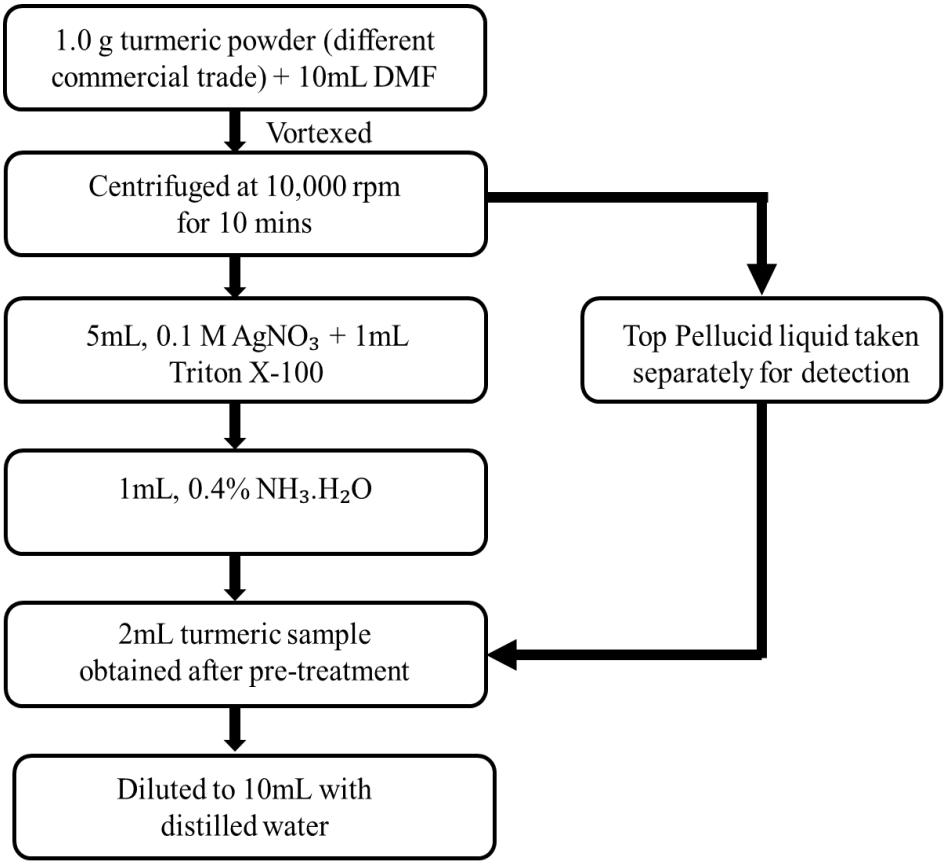

Fig. 2.4 Test protocols for Sudan-IV detection using plasmon resonance light scattering in turmeric samples

Sample Preparation for PRLS Spectroscopy. The top pellucid liquid obtained from the pre-treatment of turmeric powders were added to the mixture of a total of 5mL, 0.1 M $AgNO_3$, 1mL Triton X-100 , 0.4% $NH_3.H_2O$ diluted to 10mL with distilled water according to the general procedure. 3mL from the above mixture is then placed in a cuvette and PRLS spectra was scanned from 350-700 nm.

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1 Detection of Sudan-IV by UV-Visible Spectroscopy

The UV-Visible spectra for Sudan-IV in alkaline state were measured at different concentrations (15-2.5 ppm). It is shown in Fig. 3.1 for various Sudan-IV concentrations. Sudan-IV contains a phenol group (-OH) as shown in Fig. 3.2 with a napthol part attached to it in a suitable position which is responsible for azohydrazone tautomerism. The isomers present in Sudan-IV shows equilibrium in DMF solution. This leads to the absorption band of the hydrazine form and as a result shows the characteristic peak of Sudan-IV in DMF at 519 nm [34].

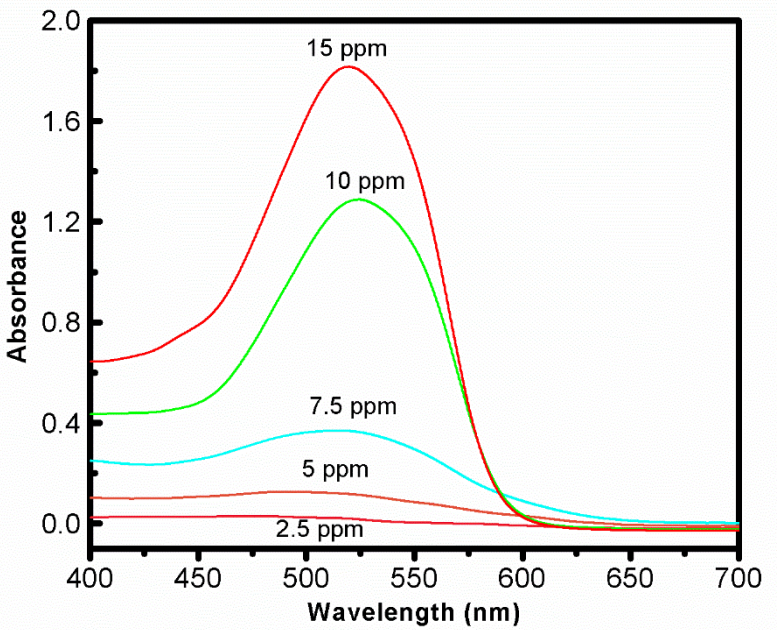

Fig. 3.1 UV-Visible spectra of Sudan-IV in alkaline condition

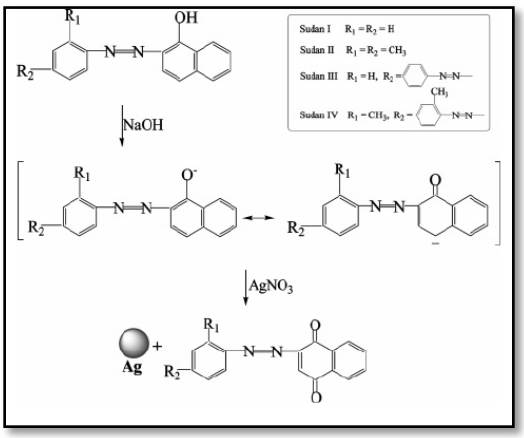

Fig. 3.2 Molecular structure of Sudan and the chart of their reactions with $AgNO_3$

This peak maximum was utilized as signature peak in UV-Visible spectra to verify the presence of Sudan-IV in a given solution. It was observed that the characteristic peak due to Sudan-IV in the UV-Visible spectra vanishing below 5 ppm (Fig. 3.1). No peak corresponding to Sudan-IV was observed for concentrations ≤ 2.5 ppm. Hence, 5 ppm is the limit of detection (LOD) for Sudan-IV.

Calibration curve for Sudan-IV at different concentrations was constructed as shown in Fig. 3.3. A linear correlation between absorption maximum in UV-visible spectra and concentration of standard Sudan-IV was observed. This linear correlation can be further explored to determine unknown concentrations of Sudan-IV.

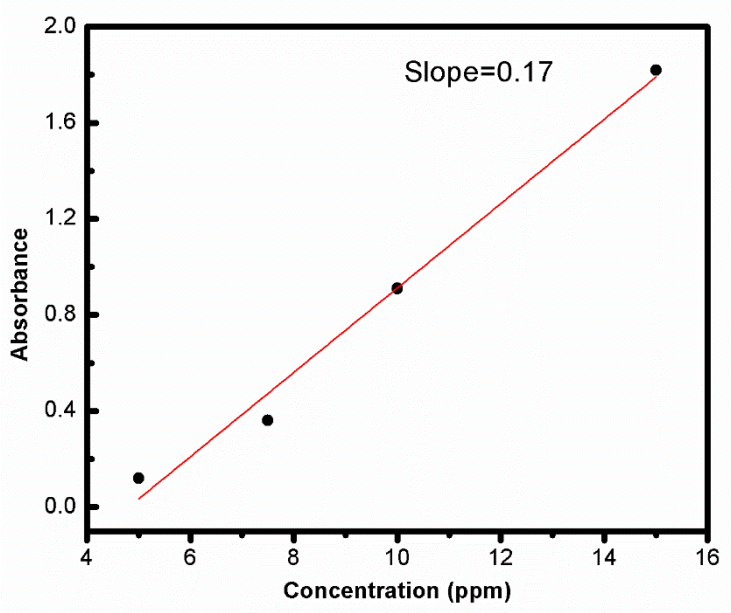

Fig. 3.3 Calibration curve for Sudan-IV over the corresponding range of 15-2.5 ppm. The limit of determination is 5 ppm

3.2 Detection of Sudan-IV by Plasmon Resonance light Spectroscopy

To perform plasmon resonance light scattering on standard sample of Sudan-IV, the sample for measurement was prepared as per the protocols described in previous chapter. The PRLS spectra thus recorded is shown 5 ppm of Sudan-IV. The absorption spectra as observed in Fig. 3.1 greatly changes when PRLS spectra were recorded in the presence of AgNO3.This is because of the reduction of $AgNO_3$ by the Sudan-IV. Such reduction reaction results into the formation of fine spherical silver nanoparticles, which gives strong surface plasmon resonance. Fig. 3.4 shows the plasmon resonance spectra of 5 ppm standard Sudan-IV. A strong absorption band centred at 425 nm is observed, which can be attributed to the formation of silver nanoparticles. Formation of silver nanoparticle can also is observed visually by the colour change in the solution. Fig. 3.5 shows the change in color from pink (due to

Sudan-IV) to the brown (due to formation of silver nanoparticles). This colour change can be utilized as visual detection probe for Sudan-IV. Sudan dyes have two chemical groups which are a phenol group and a nitrogen-nitrogen double bond. The phenol group turns into a phenate ion at a high enough pH and gets reduced (Fig. 3.2) and hence, could be oxidized by $AgNO_3$. $AgNO_3$ is deoxidized and forms silver nanoparticles [35].

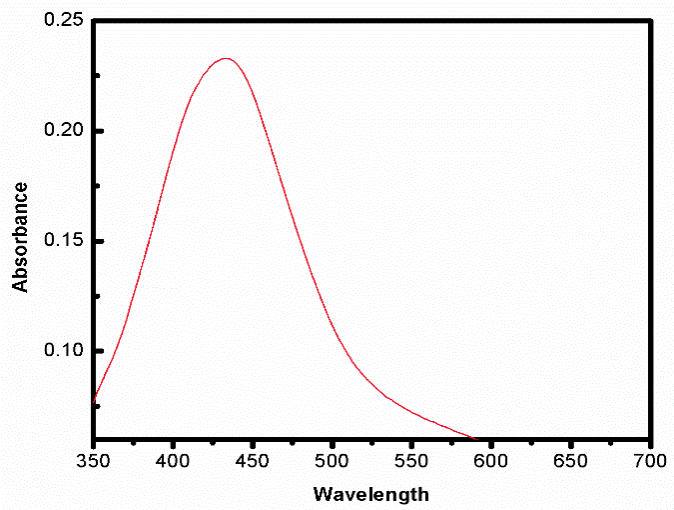

Fig. 3.4 Plasmon Resonance Absorption spectra of the reaction by-product (silver nanoparticles) formed due reaction between $AgNO_3$ and Sudan-IV formed silver NPs

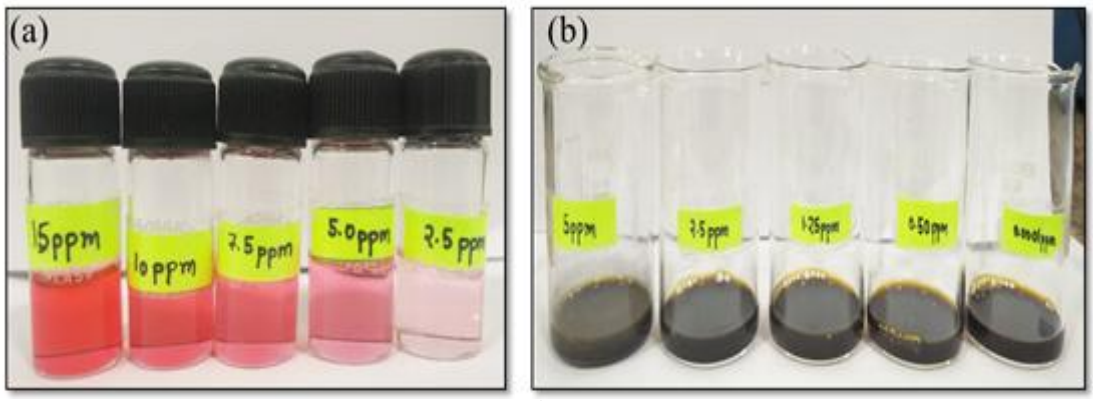

Fig. 3.5 Visual detection of Sudan-IV showing the color change with varying concentration (a) Before reaction with $AgNO_3$ and (b) After reaction with $AgNO_3$. The brown color is due to the formation of silver NPs. The Surface Plasmon Resonance signal will be used for quantification of Sudan-IV

The number of silver NPs formed by the reaction of sudan-IV with $AgNO_3$ was found to be more at high concentration of sudan-IV as compared to that at a low concentration, as seen from the calibration curve of Sudan-IV constructed according to general procedure (Fig. 3.3). Thus, the concentration of silver NPs was increased with increase in concentration of Sudan-IV. Thus, the UV-Vis spectra of the emerged

silver NPs in the range 350-525 nm should be due to the plasmon absorption of silver NPs [35-37]. A liner correlation between the plasmon resonance absorption band due to the formation of silver nanoparticles and concentration of Sudan-IV used was again established (Fig. 3.6). From this minimum limit of detection is established for Sudan-IV, which is found out to be 0.1 ppb. Thus a significant enhancement (x 10^4) in limit of detection for Sudan-IV has been observed when UV-visible spectroscopy is replaced with plasmon resonance light scattering.

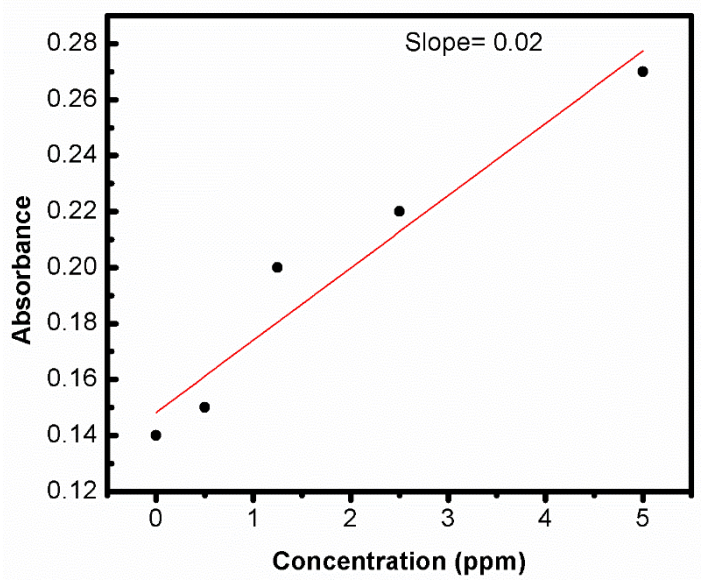

Fig. 3.6 Plot of Absorption vs. Concentration of standard Sudan-IV solution

3.3 Detection of Sudan-IV in turmeric by Plasmon Resonance light Spectroscopy

Fig. 3.7 shows the visual evidence of detection of Sudan-IV in turmeric samples. It can be seen that the color of the real samples changes after reaction with $AgNO_3$. The brown color of the mixture indicated the formation of silver NPs, which in turn showed the presence of Sudan-IV.

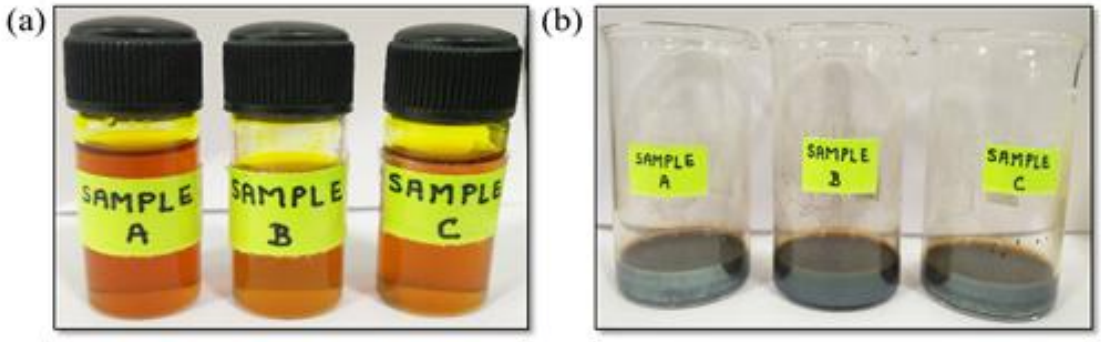

Fig. 3.7 (a) Turmeric samples from three different commercial trades after extraction. (b) Real samples of turmeric when treated with $AgNO_3$. Intense bright brown color due to Surface Plasmon Resonance confirms the formation of silver nanoparticles

The PRLS spectra of commercial samples of turmeric have been recorded as per the protocols described in previous chapter. The PRLS spectra are shown in figure 3.8. A strong plasmon resonance band again centred at 419 nm was observed confirming the presence of Sudan-IV in tested samples. The origin of this band is same as explained in the previous section. The slope of the linear correlation obtained for standard sudan-IV was used for quantitative estimation of Sudan-IV. Results of this estimate are also shown in Table 3.1. Amongst the three tested commercial samples, significant quantity of Sudan-IV was detected, with highest concentration in sample C at 3.28 ppm.

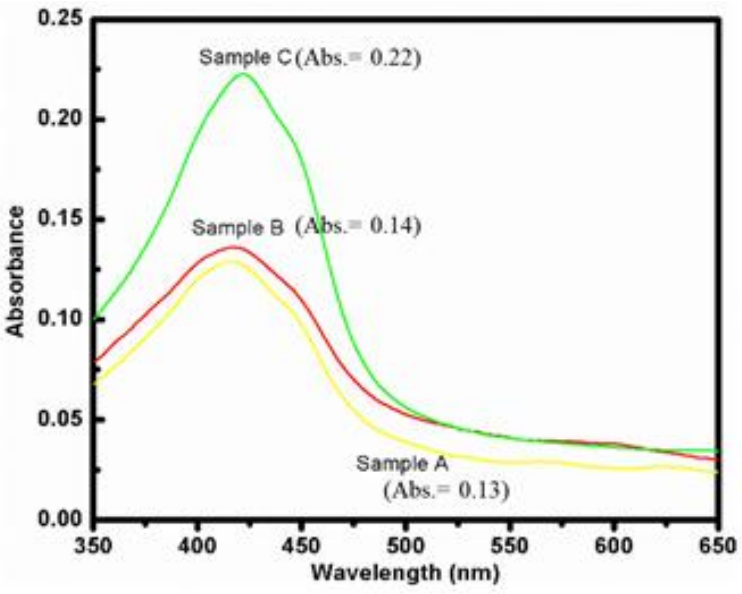

Fig. 3.8 PRLS spectra for Sudan-IV detection in real samples of turmeric purchased from different commercial trades.

Table 3.1. Estimation of Sudan in Turmeric Samples

Sample	PRLS Signal	Quantity (ppm)
A	0.13	1.94
B	0.14	2.09
C	0.22	3.28

CHAPTER 4

CONCLUSIONS AND FUTURE DIRECTIONS

4.1 Conclusions

With the objective of the detection of adulterants in food products, such as culinary spices adulterated with Sudan family I-IV, various methods and different approaches were studied. In such a context, it was found that the chromatographic techniques used in detection of Sudan family and related approaches, for instance, HPLC/APCI-MS, HPLC-UV and HPLC-DAD etc. require separation with HPLC first, which requires a higher detection time and include large sample preparation steps.

In this study, we have developed a much more reliable and easy method of visual detection based on the PRLS signals of the formed silver NPs for the qualitative and quantitative determination of Sudan in culinary spices. Silver NPs were employed in this study as Plasmon Resonance Probes. After developing a standard test outline, the presence of Sudan Dyes in three different turmeric samples purchased from different commercial trades were detected. The standard detection of Sudan-IV dye with $AgNO_3$ showed a proficient linear relationship in the range of 5-0.0001 ppm. The limit of detection was 0.1 ppb. This is currently higher that HPLC-based approaches and other spectroscopic techniques.

In conclusion, this method proved to be a fast, efficient, reliable and economical for the accurate detection of Sudan dyes in culinary spices without any large sample preparation steps. The determination for real samples of turmeric displays that this method is effective, sensitive and have potential to be used into practice.

4.2 Future Directions

In this thesis a simple, rapid, cost-effective process of determination of Sudan-IV in turmeric powder has been demonstrated. This study can be extended to other culinary spices like chili powder and paprika in which Sudan is normally found as adulterant. The present study is focused at Sudan-IV detection. With appropriate modification in measurement entire family of Sudan (I, II, III and IV) can be detected. In the present study in-situ formation of silver nanoparticles and its Plasmon Resonance was used as detector probe. The Plasmon Resonance and its characteristic absorption spectra depend on type of Plasmonic nanoparticles, their shape and surface spices present. Thus, this study can be extended to cover this aspects in protocol development for detection of adulterant in a particular food product.

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