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Review Article

Advances in calorimetric detection; a short review of techniques for interdisciplinary applications

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ABSTRACT

It is evident that the colorimetric techniques, which have been developed and well established in routine analysis for more than 100 years have been fully explored. However, over the last two decades, colorimetric methods have been explored widely due to the advancements and extensive utilization of imaging tools, as well as establishments of portable analytical devices; for example, paper-based sensors which rely on colorimetric experiments. The remarkable changes in the employment of these instruments have led to the development and improvement of many color-detecting models to meet the demands of providing qualitative, semi-quantitative, and complete quantitative analysis of various analytes. Here, we provide a short review of the latest advancements and challenges in colorimetric detection within modern analytical chemistry over the past five years. Additionally, we propose insights and ideas for future directions in this field aimed at enhancing the application of colorimetric detection across various approaches.

1. Introduction

The future of sensing relies on simplicity, affordability, and swift responsiveness. Colorimetric sensors are particularly noteworthy for their ideal characteristics. Earlier sensor designs were bulky and complex, involving multiple functional components such as transducers, processing units, and detection units, which resulted in delayed responses. In contrast, modern colorimetric technology emphasizes miniaturization, cost reduction, and in-situ operation without needing additional instruments. These sensors enable immediate analyte detection, indicated by a visually detectable color change.

Colorimetric sensors are highly attractive because they can change color in response to volatile chemicals emitted by packaged food items. They provide the simplest and most practical method for freshness monitoring that doesn't require instruments, allowing direct observation by the human eye, either on the packaging itself or via an on-package sticker. These sensors detect color changes caused by intermolecular interactions between chromophores and analytes [1-4]. Additionally, colorimetric sensing methods, such as paper-based sensors, are user-friendly and deliver quick results, requiring no specialized training for interpretation.

Developing an effective sensor involves numerous challenges, and an ideal sensor must meet specific criteria such as selectivity, sensitivity, robustness, accuracy, precision, minimal error, reproducibility, and linearity [5]. Selectivity is the sensor's capacity to recognize the target analyte among numerous other interfering substances [6]. Sensitivity is the

sensor's capacity to detect the analyte even at very low concentrations. While cost might not always align with these sensor characteristics, current technology addresses these challenges effectively. Lab-on-chip (LOC) platforms have been highly successful in sensor technology, with microfluidics gaining wide acceptance due to their low footprint and reduced use of analyte-containing reagents. Lab-on-chip technology utilizing paper, known as lab-on-paper (LOP), has become notable for its low cost, rapid detection, and self-sustainability. Whitesides has reported on LOP-based sensor platforms for detecting various biomolecules [7]. LOP is simple, inexpensive, and easily disposable, using cellulose paper to trap molecules at a targeted site, with detection based on colorimetric methods. Microarrays employing LOP have the capability to detect multiple samples simultaneously.

Colorimetric sensors are generally categorized based on the type of molecule interaction, distinguishing between chemical interactions and biomolecular interactions. Accordingly, they are classified as chemical sensors and biosensors.

1.1 Background

Colorimetry is a branch of photometry, which involves detecting light and changes in its intensity. The term "photo" means light. A photometer is an instrument used to measure the energy of electromagnetic waves spanning from infrared to ultraviolet radiation, which includes visible light (Figure 1). It converts light into electric current using a photocell. When the



measured light falls within the visible spectrum of electromagnetic radiation, it is termed as colorimetry. In this technique, a beam of light from a source passes through a

sample holder containing the analyte in solution. The intensity of the transmitted light is lower than that of the light passing through the sample in the cuvette.

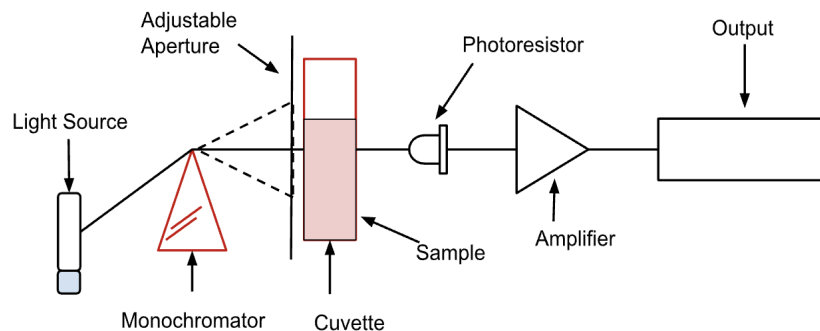


Figure 1: Principle of colorimeter.

The amount of light absorption increases in direct proportion to the concentration of the analyte. The sample's color is either an inherent characteristic of the solution or can be developed by adding appropriate reagents. The sample's absorption is compared to that of standards, allowing the concentration of the test sample to be calculated [8, 9]. Photometric methods are categorized into visual and physical, with physical photometry being the most practiced. Various analytical techniques are based on photometric principles. In spectrophotometry, atomic absorption, and turbidometry, the absorbed or transmitted light is measured, while in flame emission photometry, the emitted light is measured.

Colorimetry, a branch of photometry, quantifies the concentration of colored substances in sample solutions by measuring the absorbed light within the visible spectrum. This technique uses devices called colorimeters, or absorptimeters, to gauge color intensity in proportion to compound concentration. Since its development in the 19th century, colorimeters like the Duboscq model allowed for visual comparisons of substance concentration but were eventually replaced by more precise spectrophotometers. Modern analytical chemistry relies on various photometric methods, including colorimetry, spectrophotometry, and atomic absorption, to accurately quantify substances based on light interaction.

In the mid-1930s, photoelectric colorimetry rose to prominence when William Henry Summerson introduced a colorimeter with a photocell in 1938. Initially used by the Germans, photocells became more widely adopted after World War II, especially in medical research. Under Dr. Rose's guidance, Arthur Evans developed the first commercial selenium photocell colorimeters, which delivered accurate and reliable results. Evans's company, EEL, was later acquired by Corning, a pioneer in pH-sensitive glass electrodes. Although modern colorimeters no longer use selenium photocells,

innovations such as the Klett Bio Colorimeters have advanced clinical laboratory work by using standard glass color disks for comparisons.

1.2 Principles and parts of colorimetry

When a beam of monochromatic light passes through a colored solution, the coloring substances absorb a portion of the light while the rest is transmitted. The amount of light absorbed is related to the color intensity, which is proportional to the concentration of the chemical (analyte) responsible for the color [7 - 11].

Absorbance (A): Absorbance or optical density (OD) is defined as the logarithmic ratio of the intensity of the incident light to the intensity of the transmitted light when light passes through a medium.

Transmittance (%T): Transmittance is defined as the ratio of the intensity of the transmitted light to the intensity of the incident light when light passes through a medium.

Path Length (l): The path length is the internal length of the cuvette through which light travels, typically 1 cm.

Absorbance Maxima: The wavelength at which a substance shows maximum absorbance is called the absorbance maximum or λ_{max} .

Beer-Lambert Law: A key principle in this field is the Beer-Lambert law, which establishes a direct relationship between a solution's absorbance and its concentration. According to this law, 'A' represents the solution's absorbance, ϵ (epsilon) is the molar absorptivity or molar extinction coefficient of the substance, b is the path length (usually the width of the container holding the solution), and 'c' is the concentration of the substance in the solution. The following equation represents the relationship between the given parameters:

$$A = \epsilon bc$$

Table 1: Parts and uses of colorimetric device.

Components	Functions
Light Source	Colorimeters typically use tungsten filament lamps as their radiation source because they provide stable and continuous radiation output across the visible spectrum (320–700 nm). Although these lamps also emit a considerable amount of energy in the near-infrared region, filters can be used to absorb the excess heat without affecting the radiation energy at other wavelengths. Despite having a relatively short lifespan at higher temperatures, tungsten lamps are still widely used in colorimeters because they offer the necessary stable and adequate radiation intensity. Approximately 15% of the radiant energy from these lamps is within the visible region.
Slit	It permits only a beam of light within the visible spectrum while excluding unwanted light.
Condensing lens	A beam of light passes through the slit and then falls on the condenser lens, which converts it into a parallel beam of light.
Filter	Only the desired wavelength of light passes through the filter or monochromator, while light of other wavelengths is absorbed.
Sample holder/ Cuvette	These specially designed tubes, made from optical glass (borosilicate or quartz), hold the colored sample for accurate measurements in a colorimeter. Available in square, rectangular, or round shapes, cuvettes maintain a fixed optical path length, typically 1 cm. They usually have a capacity of 3–4 ml, or less for micro cuvettes. The colored solution in the cuvette absorbs the complementary color. The material of the cuvette does not absorb light within the wavelength range of interest.
Detector	This device converts light into electrical energy and consists of a metal plate coated with photosensitive elements such as selenium or cadmium (positive electrode). The photosensitive material is covered with a thin, transparent layer of gold or copper (negative electrode). When light hits the selenium layer, electrons are released and pass into the transparent layer, making it electronegative. This creates a potential difference between the transparent layer and the metal plate. The electric current produced is directly proportional to the intensity of the light hitting the photocell.
Readout device	The potential difference or electrical signal generated in the photocell is detected by a readout device. This device, which can be referred to as a galvanometer, ammeter, or digital readout, displays absorbance, transmittance, or both.

2. Advances in calorimetric detection over the years

One of the earliest sensing techniques humans learn is to distinguish between various colors and intensities. Color is highly significant in the field of research, and it has not been explored much. Earlier, scientists discovered that color changes in a solution suggest the occurrence of a chemical reaction, and the intensity of the hue can be correlated with the concentration of the analytes involved in the reaction. Selective colorimetric techniques became an effective method for qualitative analysis. One such report of colorimetric detection was reported in 1838,

where the levels of nickel and iron in cobalt ore is quantified by analyzing the filtrate color with standard solutions by Lampadius et. Al [10]. The development of these colorimetric techniques and procedures occurred with the proposal of color vision theory by Herman von Helmholtz and Clerk Maxwell. The development of different techniques, theories and devices by the 19th century resulted in employing modern technologies for color measurement. Figure 2 includes the advantages and disadvantages of colorimetric techniques.

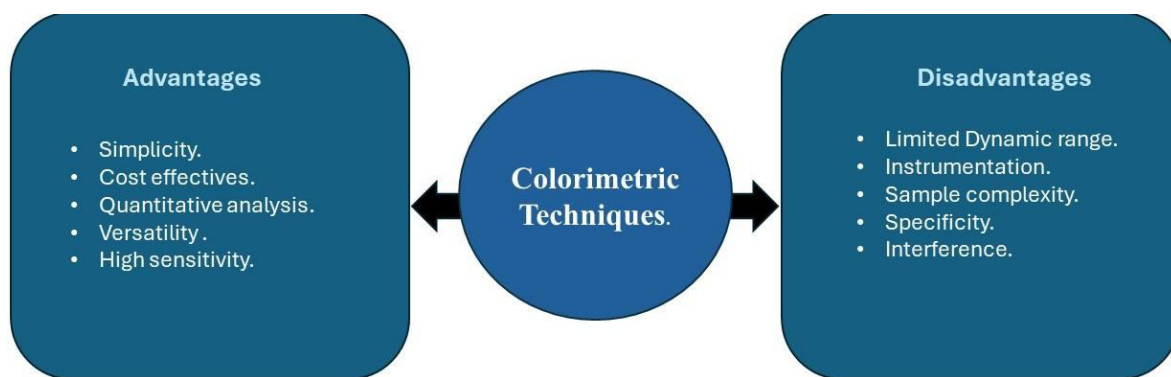


Figure 2: Schematic representation of pros and cons of colorimetric techniques.

Although these colorimetric techniques are straightforward compared to the modern advanced techniques like mass spectrometry, they are widely employed in various applications and have been evolved over time with different approaches. This detection approach has seen significant advances across various fields, namely in material

science, nanotechnology and biotechnology. The notable advances in these fields include:

- 1. Nanoparticle based sensor:** Silver, gold or quantum dot nanoparticles can alter color with respect to the analytes due to surface plasmon resonance or quantum confinement effect. Modifying these nanoparticles with receptors such as

antibodies or aptamers enables selective identification of analytes [11-14].

2. **Plasmonic sensors:** Plasmonic colorimetric nano sensor enables detection of analytes at a very low concentration with localized surface plasmon resonance [15-17].
3. **Paper based sensor:** Paper strips or devices integrated with microfluidic devices equips in onsite detection of analytes at low cost. These devices use dyes as an indicator that changes color upon interaction with analytes. The analyte detection is quantifiable and visible to the naked eye [18-21].
4. **Smartphone integrated sensor:** Integration of smartphone application and camera with colorimetric device helps in quantification of color changes allowing in point of care testing without specialized equipment [22-25].
5. **Digital color analysis:** Advances in image processing and computer algorithms allow for the precise measurement of analytes, improving the accuracy and reliability of colorimetric sensors [26, 27].

6. **Multi analyte sensor:** Innovations in colorimetric design and signal processing enable simultaneous detection of multiple analytes using a single device expanding the utilization of colorimetry in multi analyte matrices [28-30].

All these wide range of advances in the field helps in cost effective, rapid and highly sensitive diagnostic tools for numerous applications covering a wide range of fields from healthcare to environmental monitoring to various other fields.

3 Colorimetric Assays – Importance and applications

Colorimetric assays are analytical tools used in science fields to identify the concentration and quantity of an analyte in a sample by estimating the intensity of color produced because of a chemical reaction. Colorimetric assays are widely employed in various fields such as environmental monitoring, research laboratories, clinical diagnostics and food quality and testing. It is popularized due to its simplicity, cost effectiveness and rapidity. The key points of colorimetric assays are shown in Figure 3.

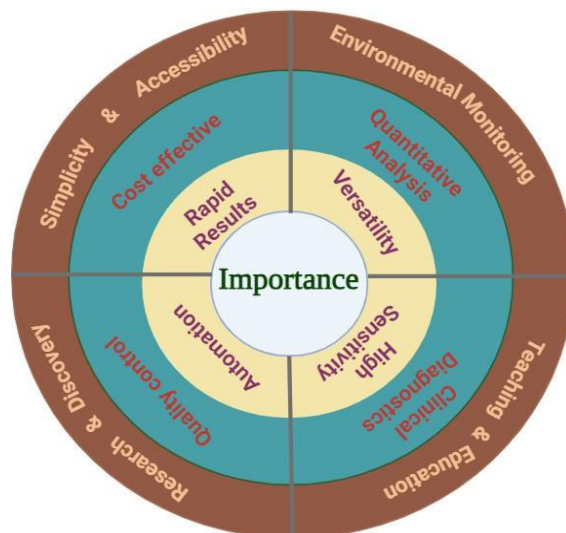


Figure 3: Key aspects and importance of colorimetric Assays

3.1 Health monitoring

Wide varieties of colorimetric assays are available in the markets. It is widely used in different areas according to its applications. Most used colorimetric assays are for clinical diagnostics. They are branched into various assays, namely enzyme linked immunosorbent assay (ELISA) and blood glucose monitoring. The former is employed to detect antigens, antibodies, hormones and other biomarkers in samples for disease diagnostics and the latter is utilized in detecting glucose levels of diabetics' patients from blood. Clinical lab tests have permitted the recognition of various substances, the requirement of mobile health applications and simple assays has driven the development of devices for monitoring health at the point of patient care [31, 32]. Clinical diagnostics across different diseases rely on test systems identifying specific biomarkers in patient blood primarily employing antibodies for molecular identification. ELISA has many strengths such as high sensitivity, selectivity, broad availability of antibodies and commercial kits. It also possesses many weaknesses such as storage conditions of antibodies and its precision during prolonged research [33]. Currently, different commercially

obtainable diagnostics kits employing aptamers are available for identifying specific biomarkers in biological samples [34, 35]. Mizaj et al., fabricated colorimetric detection for detecting H_2O_2 as a biomarker for bronchiectasis in humans. The UV-vis absorption analysis of the assay indicated that the dye system was capable of detecting levels of H_2O_2 as low as approximately 0.011 parts per million [36]. Different VOCs such as NO [37] and ketone [38] were also detected from breath via colorimetry to detect oxidative stress and diabetic ketoacidosis in patients. These detection techniques provide the users with a low cost and rapid identification of NO and ketone. Figure 4 includes various colorimetric sensors employed for monitoring different diseases.

Diabetes is a family of metabolic conditions characterized by significant insulin deficiency, typically resulting in either sudden disruption of pancreatic beta cells leading to hyperglycemia or gradual onset due to insulin resistance [39]. Numerous reviews have extensively investigated the potential of computer assisted methods for screening diabetes, yet less tangible applications have unfolded as commercially available products. There are few detection methods for diabetes that can

do the same as the alternative ways including the ones for early diagnosis and image management such as electrochemical or colorimetric techniques. These techniques typically involve

detecting biomarkers, potentially indicating the presence of the target disease at an earlier stage than image-based methods, which rely on observing already-damaged tissues.

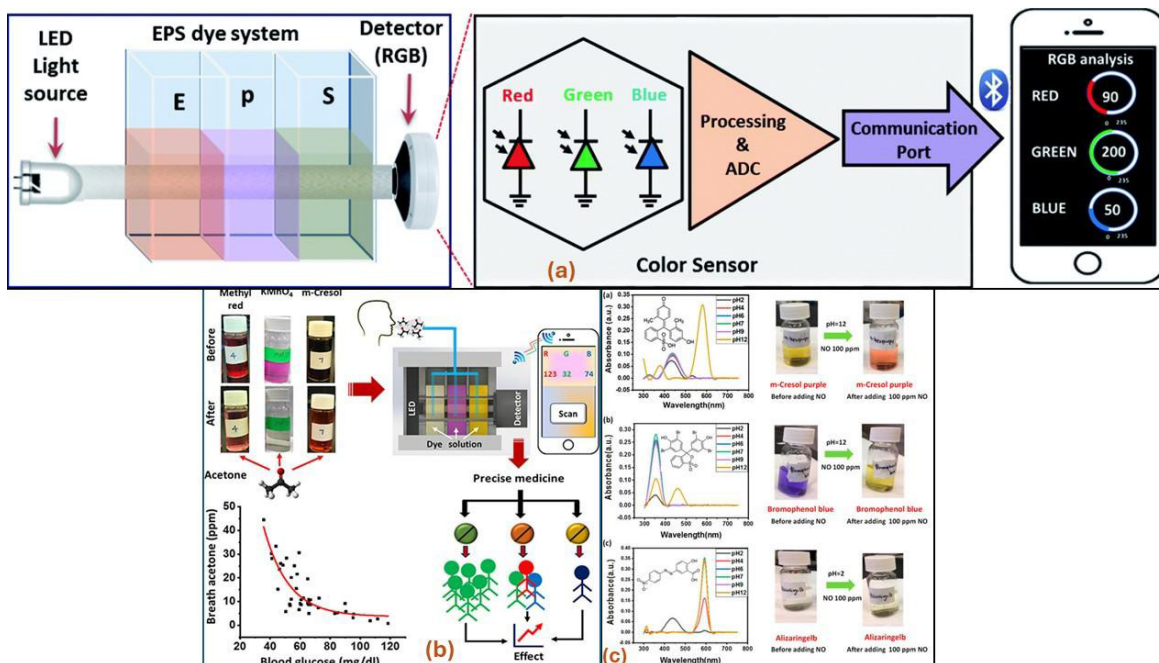


Figure 4: The colorimetric detection of (a) H_2O_2 [36], (b) acetone [38] and (c) Nitric oxide (NO) [37].

3.2 Environmental monitoring

The recent focus on environmental impacts arises from the reflection that human activities are interfering with the natural habitats, resulting in a threat to quality of life. For instance, the rapid increase in population and high utilization of energy resources causes serious threats to the environment. Environmental pollution involves release of toxic chemicals into the environment [40]. Various pollutants, including organic pollutants, dyes, pesticides, pharmaceuticals, heavy metal ions can adversely affect air, soil and water [41]. Moreover, a very small amount of these toxic materials can induce ecological damage and potentially harm living beings [42, 43]. The

concentration of fluorine in groundwater was quantified using colorimetry by Sritama et al., as higher levels of fluoride concentration adversely affect human health. The fabricated sensor is highly selective towards fluoride ions and it could effectively detect fluoride concentration in a range of 0.1–5 ppm [44]. Various heavy metal ions have been detected via colorimetry. Zhuo et al., provides a comprehensive review on different chromogenic materials which are employed to detect heavy metal ions via colorimetry, which comprises organic, inorganic, and diverse material types. Figure 5 contains schematic representation of colorimetric sensing of various heavy metal ions [45].

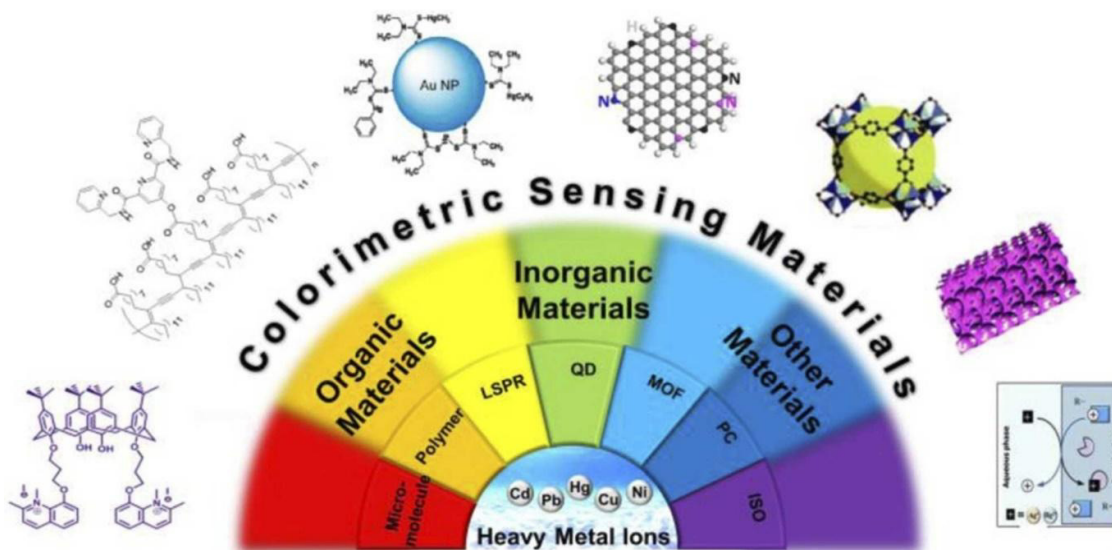


Figure 5: Colorimetric detection of various heavy metals [45].

Detection of VOCs using colorimetry has gained significant interest due to its simplicity and rapidness and visual identification. Moreover, it has demonstrated its capacity in identifying various VOCs in air samples [5, 46-48], enabling promising technology for monitoring indoor VOC pollution. Colorimetric sensor works by detecting the analyte vapors by changing the color of the pigment, relying on the chemical interaction between the analyte and sensor [49]. This technique has the advantage of distinguishing different analytes and solely depends on non-specific interactions like physical adsorption or rely on the analyte or sensor surface physical properties [50, 51]. Emar et al., fabricated a sensor array incorporating N, N-dimethyl-4,4'-azodianiline, pH indicators and parosaniline as sensing materials for detecting VOCs in indoor air in households. Effective sensing of VOC's could improve the air quality [52]. Similarly, CO₂ causes

global warming as a key greenhouse gas. Hence, converting CO₂ into useful products reduces its concentration in the atmosphere, thereby helping to control climate change. Mizaj et al., proposed an innovative method of detection of these converted products namely ethanol, methanol and formic acid from CO₂ using dye solutions such as Phenyl red, Eosin blue and KMnO₄ and the limit of detection of the proposed technique is as low as 0.03-0.06 ppm [53]. There are several types of sensing mechanisms associated with colorimetric technology, for instance nanoparticle aggregation, nanoparticle decomposition, nanozymes, fluorescence modulation, ligand-receptor interactions, and photonic structures. The advanced colorimetric sensors and instrument based colorimetric sensors exhibit high performance in environmental monitoring [54]. The colorimetric analysis of various environmental analytes are briefly shown in Figure 6.

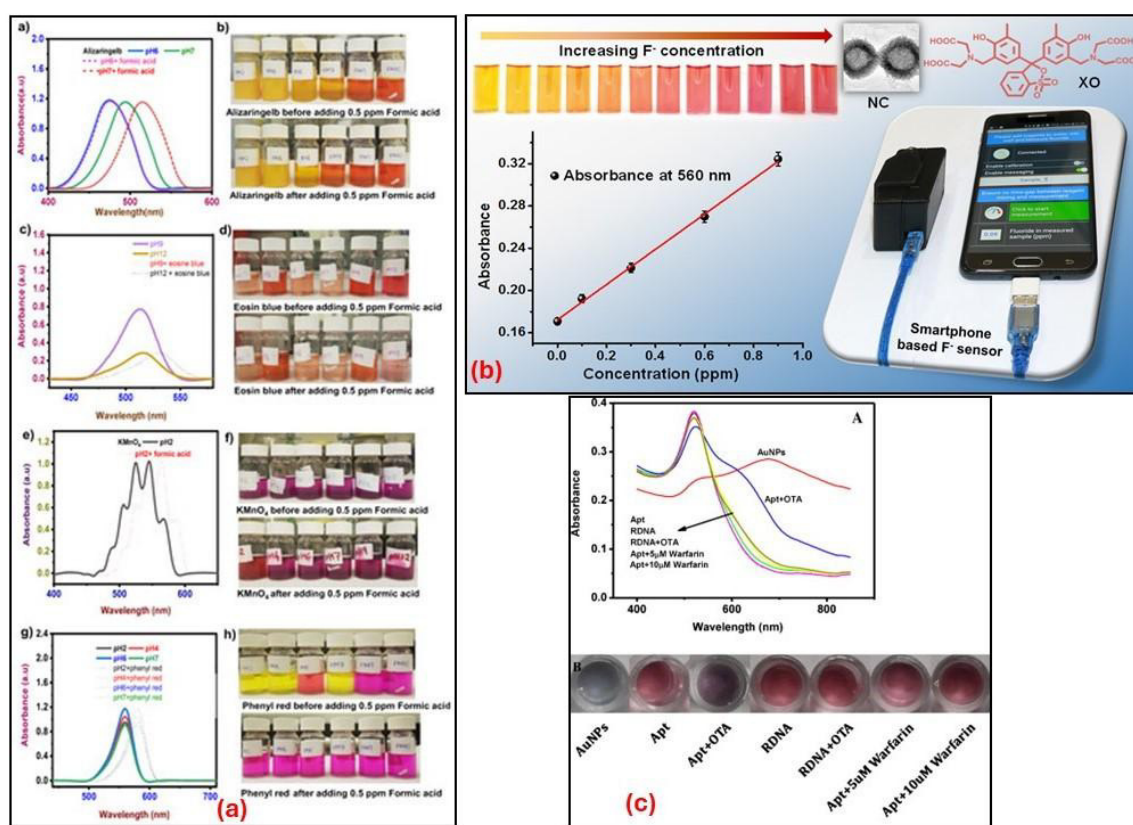


Figure 6: Colorimetric environmental monitoring of various analytes such as (a) CO₂ converted compounds [53], (b) Fluoride detection in ground water [44] and (c) UV absorption spectra of detection of Ochratoxin A using gold nanoparticles [51].

3.3 Food monitoring

The boom and expansion of the food industry, along with increasing consumer demand for improved shelf life and extended storage of food products, have created a need for techniques to monitor and maintain food quality and safety. These techniques are crucial throughout the food's life cycle—from production and storage to shipment and consumption.

The food quality is monitored using several different colorimetric approaches. For instance, changes in gas emissions or accumulation of microorganisms can provide insights on the food's condition, including its freshness and deterioration [55]. Sensors capable of detecting these changes

can provide a thorough evaluation of food quality. One such example is the utilization of pH indicators on food packaging which shows color change as the food spoils, reflecting the pH change caused by the emission of volatile amines when fish or meats spoils [56, 57]. Mithra et al., proposed a versatile colorimetric paper sensor comprising 3 dye systems for detecting trimethyl amine for monitoring the freshness of fish. Using this unique paper-based sensor, the researchers could effectively detect the trimethyl amine with a low concentration of 4.5 ppm with a low detection time of 2 seconds [58]. An IoT based sensor was developed by Surya et al., for detecting sodium sulfite in beverages. A visual based paper sensor with a

low detection limit of 0.05M which shows great selectivity at room temperature. Additionally, paper based sensors replace sophisticated instruments due to its rapidness and low cost [59]. Various colorimetric sensors are available in the market for detecting the quality and freshness of food products [60-65] generally and specifically, Milk [66-69], Meat [70, 71], Shrimp

[72-75] and sea foods [76, 77] etc. Colorimetric studies in this area plays a pivotal role in the food sector by providing quality, safety and significant information to the consumer. Four different food monitoring studies using colorimetry are shown in Figure 7.

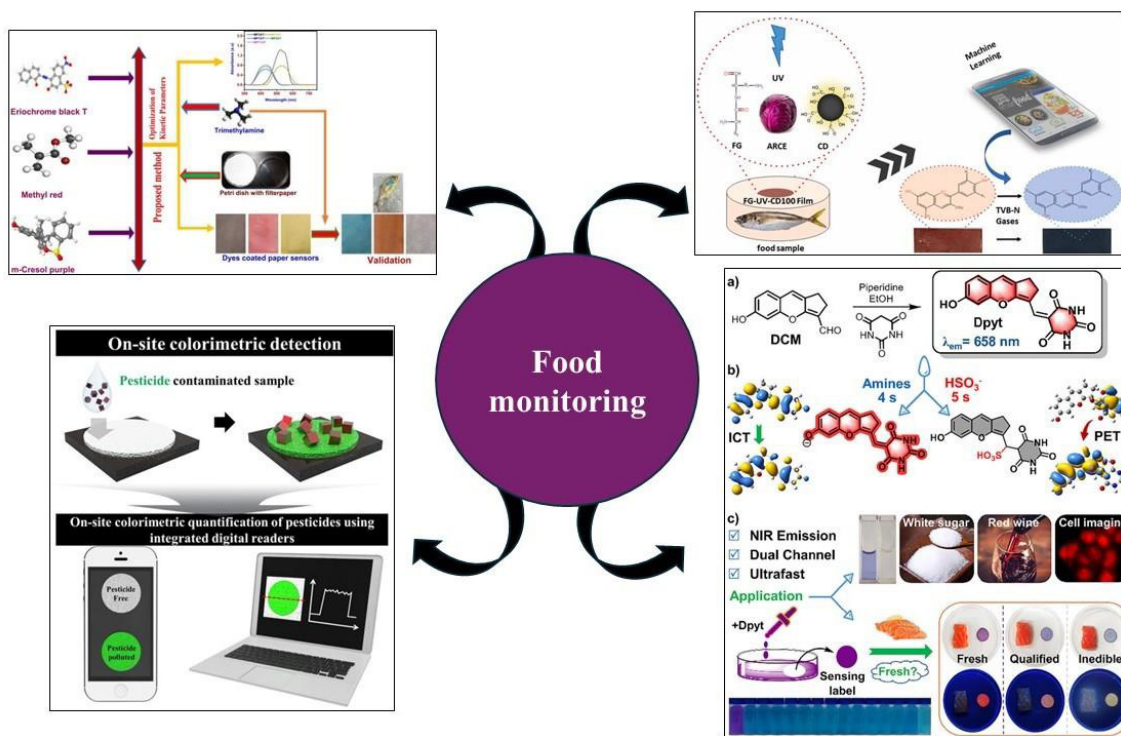


Figure 7: Schematic representation of various colorimetric studies for food quality monitoring [58, 78-80]

Colorimetric analyses are widely employed in other various fields such as Pharmaceuticals (drug assay and screening), Biotechnology and molecular biology, Animal health monitoring, Agriculture industry, petroleum and oil industries etc. The integration of colorimetry with smartphones provides a wider range of applications as its principle is simple and user friendly [25]. The accessibility of smartphones and rapid developments in mobile applications is having a great impact on our lifestyle. Smartphones embedded with color devices which can produce Red, Green and Blue (RGB) color codes enable simple examination of analytes, as this strategy is straightforward and accessible.

4. Challenges and future perspectives

When the new sensors are designed, an analytical method which measures the optical properties such as reflectance and absorbance from the colored species can be constructive. The crucial parameters to consider when creating a new colorimetric device, such as a paper-based sensor, smartphone-based sensor, or spot test, are how these methods affect the optical behavior of colored materials. Paper based and spot test analysis utilizes dyes that can be easily analyzed using smartphone applications or software. On the other hand, due to the transient nature of absorbance and reflectance, microfluidic devices require real time analysis. Furthermore, data processing is crucial for methods based on RGB values that require meticulous calibration to establish linear relation with

the analyte concentration. This calibration involves logarithmic conversions which are complex.

Additionally, the analyzing platforms are usually defined in relation to a particular reference. This results in the development of various RGB software such as Adobe RGB, Apple RGB etc, which have developed over time due to technological advancements. Therefore, it is crucial to evaluate the applicability of RGB values across various devices as there is a possibility for obtaining identical RGB values for different colors in different devices. This challenge can be addressed by developing calibration curves specific to individual devices or by utilizing single device throughout the studies. Furthermore, there is a need for further advancements in multi analyte colorimetric detection, emphasizing it as a critical area demanding extensive research.

5. Conclusions

In conclusion, the recent developments in colorimetric detection techniques have revolutionized various fields by providing rapid, cost-effective, and user-friendly solutions for detecting a wide range of analytes. Advances such as nanoparticle-based sensors, smartphone integration, and smart materials have significantly enhanced specificity, sensitivity and applicability. These colorimetric techniques continue to evolve, promising even greater impact in fields such as healthcare diagnostics, environmental monitoring, and food safety. With ongoing studies and development, colorimetric

detection continues to play a vital role in addressing global challenges and improving quality of life.

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