



Exploring smart phone based colorimetric technology for on-site quantitative determination of adulterant (neutralizer) in milk

Vipin Kumar¹  · R. S. Aulakh² · J. P. S. Gill² · A. Sharma²

Revised: 11 January 2022 / Accepted: 30 January 2022 / Published online: 7 March 2022
© Association of Food Scientists & Technologists (India) 2022

Abstract There is a great concern regarding the safety of milk not only for human health but also for its economic consequences. The portable sensing devices, which can collect and analyze data within food supply chains at critical control points are lacking. Smart phones have now emerged as an integral part of each home, lab, farm and factory. It is having a provision of digital camera and computation; with widespread applicability including food analysis. The use of soda as a milk neutralizer is a usual practice but has a detrimental human health impact. This investigation explored an easy, economic, fast, repeatable, and field applicable Smartphone-based sensing technology, which was standardized and in-house validated for the

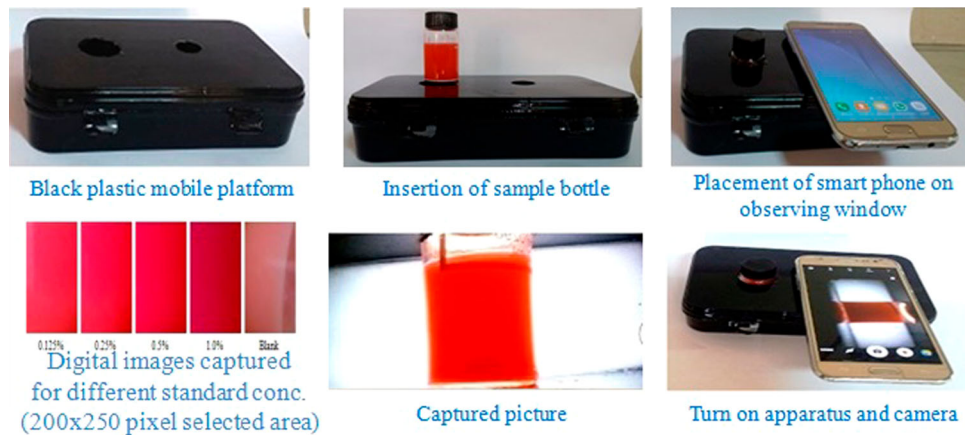
quantitative determination of neutralizer in milk samples. The method had simple steps of spot-test response and digital image evaluation with the Red Green Blue process. The linearity of the method was shown by analytical curves ranging from 0.125% (1250 ppm) to 1% (10,000 ppm) that were characterized by $R^2 > 0.99$. The limit of detection of 0.11% demonstrated the sensitivity of the method which was found better than the existing wet chemical spot test. Comparison with the existing spectroscopic method revealed no statistically significant difference between the observations using paired t-test at a confidence level of 95%.

✉ Vipin Kumar
drvipin80@gmail.com

¹ Department of Veterinary Public Health & Epidemiology,
College of Veterinary Science and A.H., Mhow, Nanaji
Deshmukh Veterinary Science University, Jabalpur,
Madhya Pradesh 453446, India

² School of Public Health and Zoonoses, Guru Angad Dev
Veterinary and Animal Sciences University, Ludhiana,
Punjab 141004, India

Graphical abstract



Keywords Smartphone · Colorimetry · Quantification · Milk · Neutralizer

Introduction

Milk was identified as one of the most frequently defrauded foods in the world (Moore et al. 2012). There is an increasing tendency to use adulterants as neutralizers, preservatives, diluting agents, thickeners, and non-dairy fat emulsifiers; foaming agents in milk (Kumar et al. 2019) to prevent microbial proliferation and spoilage and to increase the bulk and make more profit. The use of soda as a neutralizer was very common. It is added to nullify the developed acidity in the milk (Gupta et al. 2013) consequently prevents the souring and increases the shelf life of the milk. It has immense public health significance so; the investigation of soda in milk plays a crucial role to prevent this counterfeiting and adulterating practice.

Modern smartphones are the most recent age telephones, which are very likely a small personal computer (PC) with best in class worked in segments required for a sensing gadget like contact screen, top-notch camera, light sensors, earphones, stockpiling memory, correspondence functionalities, a global positioning system (Younis et al. 2020), computation, etc. Moreover, as per an estimate, around 6.37 billion individuals in the world utilize smart phones, which is practically 80.76% of the total populace (Bankmycell 2021). Along with the progression headway of electronics and information technology areas, the spot of PCs has been replaced by Cell phone gadgets (Sumridetchkajorn et al. 2014). It has now become a necessary aspect of each and everyone's life. Its relevance has been found in the analysis of foods and beverages (Iqbal and Bjorklund 2011), detection of antibiotic residue in milk

(Urapen and Masawat 2015), estimation of preservative (formaldehyde) in milk (Gupta et al. 2019), etc.

Colorimetry is not a wonder; Cell phone-based colorimetric measurement has been proposed and applied by numerous workers (Shen et al. 2012; Sumridetchkajorn et al. 2014; Gupta et al. 2019). Development of digital image-based (DIB) colorimetric methods have been performed and applied in analytical science for exact estimation of analyte (Dos Santos Benedetti et al. 2015) of interest. This technique utilizes the fundamental concept of a primary color pattern i.e. red (R), green (G) and blue (B) as more than 16 million color tones may be possible by different blends of these colors (Gomes et al. 2008). These RGB values changed proportionally with variation in concentration of an analyte of interest during a colorimetric response. Thus the RGB approach can be straightforwardly used to play out a quantitative analysis (Andrade et al. 2013). Indeed, the inconstancy of the light during the application of the DIB technique can be the principal reason for helpless reproducibility. Radiant or overcast climate during the catch of the picture could significantly change the RGB pattern (Martinez et al. 2008) here, we have overcome this drawback by using homogenous illumination and prevention of environmental hindrance during the experimentation. To detect neutralizer in milk, qualitative rosolic acid spot test can be performed using a method described by FSSAI (2016). In this test, aliquot of milk was added with rosolic acid solution. Rosolic acid changes its colour from yellow to red due to raised pH in neutralizer adulterated milk. Development of rose red colour indicates presence of it in the milk. Digital image of the chromogenic reaction was then taken up with the help of a smartphone. Color of the captured pix was then converted into numerical value by using RGB approach and the analytical curve constructed here was useful to estimate concentration of neutralizer in milk.

The food safety agencies regularly performed screening of milk samples by using chromogenic subjective tests. Simply qualitative determination is not corroborative proof of adulteration. It ought to be quantified with a validated analytical approach. There is a paucity of such kind of methods and compact handheld gadgets, which can gather and analyze information inside food chains at focused points. The present investigation was embraced for the development and validation of colorimetric-based technology with a cell phone for the determination of milk neutralizer (soda).

Materials and methods

Design of the device

A 15.5 cm × 9.5 cm × 4.0 cm black plastic platform was designed for a Samsung Galaxy on 7 phone manufactured by Samsung electronics. The manufacturing details includes Android 6.0.1 operating system, 64 bit Qualcomm Snapdragon 410 Quad-core 1.2 GHz Cortex-A53 processor, Adreno 306 GPU, 1.5 GB RAM, 5.5-inch TFT capacitive touch screen, 8 GB of internal storage, a battery of 3000 mAh, 13 MP CCD rear camera and 5 MP front camera. The rear phone camera was positioned over the observing window of the platform. The device was enlightened from a white light LED source with an illumination of 300 lux. A white plastic light diffuser and a mirror reflector were placed between the light source and the sample for a steady enlightening condition (Fig. 1).

Spot test reactions

Rosalic acid spot test for detection of milk neutralizer was performed using the method described by Food safety and standards authority of India (Food safety and standards authority of India 2016) viz. 2 ml aliquot of milk was taken in a test tube; 2 ml of rosalic acid solution was then added. Appearance of rose red colour indicates sodium bi carbonate (neutralizer) was present in milk. Each lot of experimental milk sample was previously assessed for tested neutralizing substance by the approved method and was found free of adulterant. Each batch was then split into 5, 50 ml aliquots, 4 intended for the experimental addition of neutralizer to milk and one for the negative control. After serial dilution, each aliquot has neutralizer in one of the following concentrations: 0.125%, 0.25%, 0.5% and 1% sodium bi carbonate (99.7% NaHCO₃, AR). Calibration measurements were carried out using standard concentrations (0.125%, 0.25%, 0.5% and 1.0%) and with a negative control (0.00%). Each tube of different concentrations was then added by an equal volume of rosalic acid

solution. The pics of the developed rose-red color were taken by Smartphone using black plastic plate-form. Independent repetitions were performed for each of the five standard concentrations.

Colorimetric analysis of digital image data

All digital images captured from Smartphone were processed for colorimetric analysis with the Image J program; a freely available internet download (Andrade et al. 2013). It is a very capable image analysis program. This is designed to import and display an image in the desired pixel area and process it with the RGB approach. It examined the 200 × 250 pixel selected. Captured pics were converted into numerical values by using the program. The user can select the most homogeneous region in the image (usually the middle portion) defining the coordinates for the selected region and to be used for all the images. The software scans all the pixels (column by column) to extract the Red, Green, and Blue (RGB) components for each pixel and calculates a mean value for each RGB component. These values change relatively with developed color in colorimetric spot reaction. The color generated was proportional to concentration of the analyte.

Results were plotted between average intensities of various components (R, G, and B) and standard concentrations of the neutralizer (%). A calibration curve was plotted in concentrations of the neutralizer (%) against analytical response (R) obtained from mean values of the intensities of the red (Red) component, which was found to be most linear.

In order to validate the method for the tested analyte, parameters like sensitivity, linearity, range, limit of detection (LOD) and limit of quantification (LOQ) were evaluated by using a prepared calibration curve for neutralizer estimation in milk. The accuracy, precision was also determined.

New versus existing methods

The comparison of the developed smartphone-based colorimetric method with the spectrophotometric technique was performed. A spectroscopic method (Modi Zeel et al. 2018) was used for the determination of neutralizer in milk as a comparative method. Milk with known standard concentrations of neutralizer i.e. 0.00 mg/5 ml, 6.25 mg/5 ml, 12.50 mg/5 ml, 25.00 mg/5 ml and 50.00 mg/5 ml corresponding to 0.00%, 0.125%, 0.25%, 0.5% and 1% sodium bi carbonate, respectively were admixed with rectified spirit and filtered through Whatman filter paper No.1. Filtrates of various standard concentrations were then collected and the rosalic acid spot tests for chromogenic reaction were performed. The analytical measurements

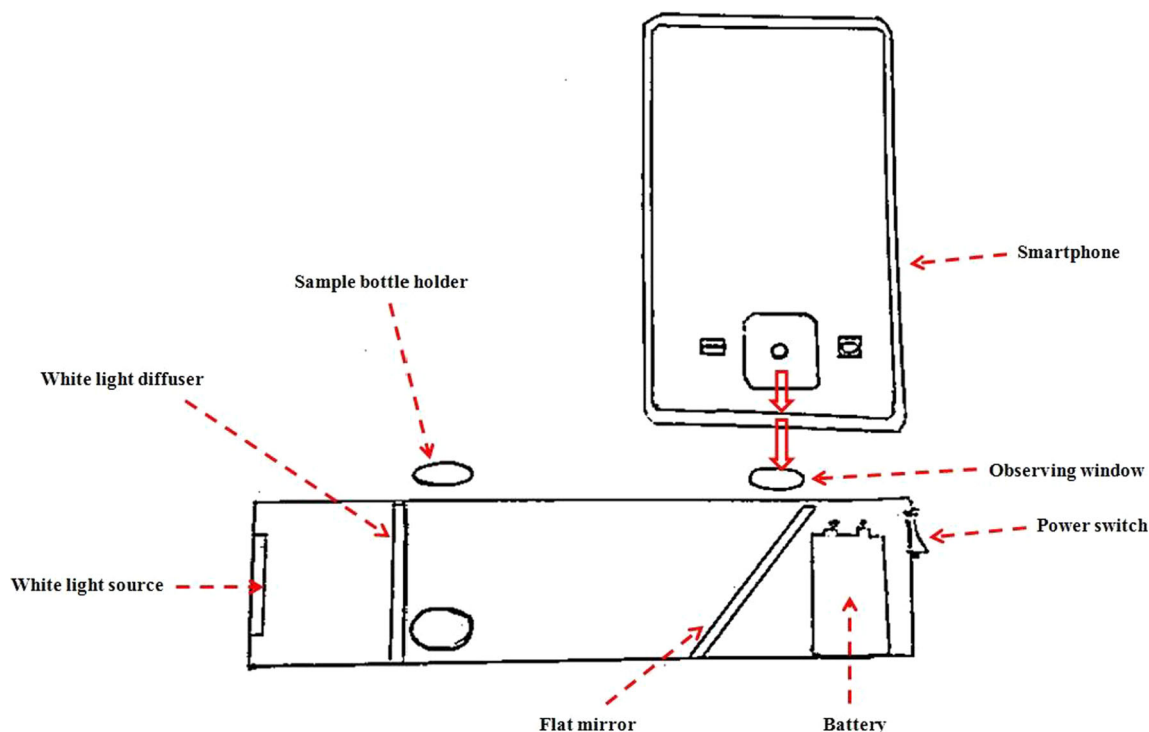


Fig. 1 Schematic diagram of the Smartphone based colorimetric device. Dimension of the black plastic platform is 15.5 cm × 9.5 cm × 4.0 cm. Components include white light source, diffuser, mirror/reflector, sample bottle holder, observing window, and power assembly

(absorbance) were taken at a wavelength of 554 nm in the visible region. Results of both the techniques were compared using a paired t-test (CL-95%).

Comparison of analytical sensitivity of developed smartphone-based colorimetric method and routine wet biochemical method was also carried out. The limit of detection (LOD) of the developed method was compared with the existing wet biochemical method for the detection of neutralizer in milk. For this purpose, LOD reported by the Food safety and standards authority of India (Food safety and standards authority of India 2016) was taken from literature.

Results and discussion

Color analysis

Rosalic acid spot test is intended to detect neutralizer in milk. Test reagent changed its color from yellowish/brownish to rose-red. The color response pixs captured in smartphone were converted into numerical values by using the Image J. program. Digital images for standard solutions with different concentrations were depicted in Fig. 2. All images represent a selected area equal to 200 × 250 pixels. Visual examination revealed that images present a

difference in intensity for the red color, relative to the neutralizer concentration in each calibration solution.

The intensity of the red “R” component decreases with increasing concentrations of the standard solution, the intensities associated with the green “G” and the blue “B” components remain practically almost constant (Fig. 3).

Preparation of analytical curve

The analytical curve for the neutralizer was constructed using the analytical responses (R), which were calculated according to the following equation (Kohl et al. 2006; Gomes et al. 2008). The curve was prepared by using standard neutralizer solutions of various concentrations from 0.125 to 1.0% (w/v).

$$R = \log \left(\frac{I_0^{RGB}}{I^{RGB}} \right)$$

where,

I_0^{RGB} = Mean values of the intensities for the R, G, or B components in the selected area associated with the blank solution,

I^{RGB} = Mean values of the intensities for the R, G, or B components in the selected area associated with standard solutions.

Linear regression was found between concentrations of the neutralizer (%) and analytical responses (R) obtained from mean values of the intensities for the R (Red)

Fig. 2 Digital images captured for different standard concentrations (200 × 250 pixel selected area)

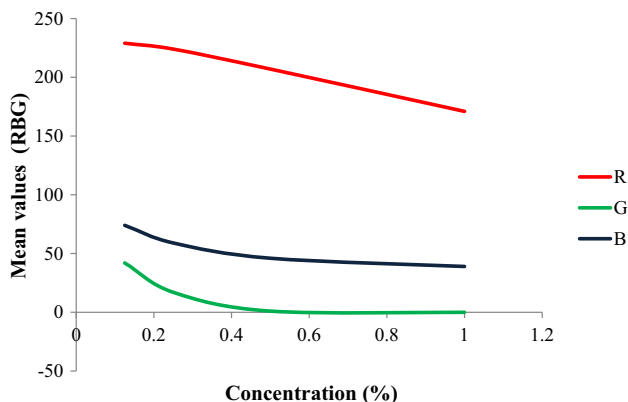
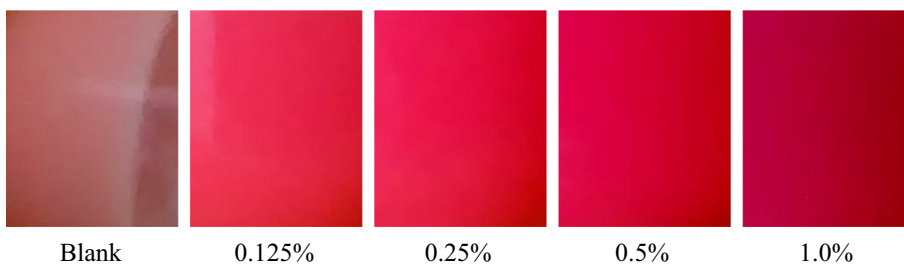


Fig. 3 Neutralizer concentration versus intensity for RGB color scheme. Mean values were plotted against the different concentrations (0.125–1.0% w/v)

component (Fig. 4). Other linear relationships between concentrations of neutralizer and the responses of those concerned with mean values of the intensities for the G or B components were also tried to maximize precision. But, in every case, the results were poor compared to those using “R” (Red) for neutralizer.

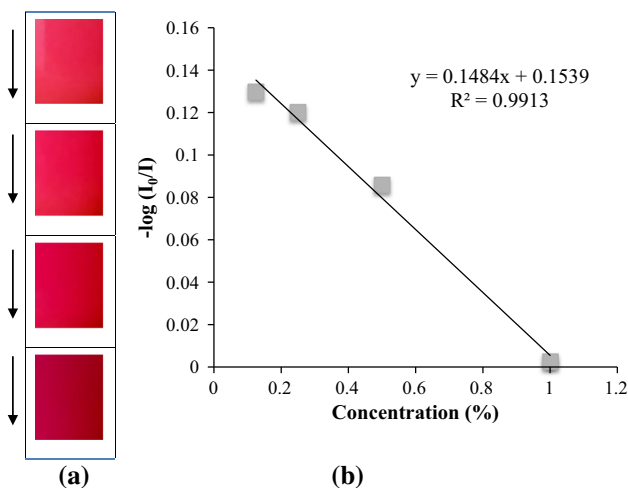


Fig. 4 a Digital images containing spot test reaction mixtures with neutralizer contents from 0.25 to 1.0% (w/v). The arrows indicate the direction of the increase of neutralizer content b Analytical curves obtained for neutralizer contents ranging from 0.25 to 1.0% (w/v) by using the results from the R channel

Application

The current technique acquired a good scientific bend for the estimation of neutralizer in milk with the regression equation $y = 0.1484 x + 0.1539$; where.

$y = R$ is the analytical response

$x =$ the analyte concentration in % (w/v) of neutralizer

The coefficient of determination, R^2 was 0.9913 in the reach between 1250 ppm (mg/liter) and 10,000 ppm (mg/liter). The technique indicated a linear regression at an examined concentration up to 1% (10,000 ppm). For assessment of the concentration of neutralizer up to that level above portrayed direct condition can be utilized; nonetheless, if the obscure aliquot contains a higher grouping of neutralizer in milk, dilution of such samples were needed. The LOD of the technique for neutralizer determination was found as 0.11%. The mean % recovery of analyte was found to be 94.7 and mean % RSD to be 2.05. No other investigation was observed in the literature review for comparison of neutralizer determination in milk through Smartphone-based colorimetry. Although, the explored method is comparable with earlier report by (Gupta et al. 2019), who applied smartphone for colorimetric determination of formaldehyde residues in milk. All the method validation parameters are presented in Table 1.

New versus existing methods

The proposed method can successfully be applied for the quantitative determination of neutralizer in milk. The results obtained for the samples analyzed by newly

Table 1 Method validation parameters for neutralizer determination in milk

Parameter	Neutralizer
Linear regression equation	$y = 0.1484 x + 0.1539$
R^2	0.9913
Range (%)	0.125–1
LOD (%)	0.11
LOQ (%)	0.38
Accuracy (% recovery)	94.7
Precision (% RSD)	2.05

Table 2 Results for the neutralizer determination in milk samples by the proposed and comparative methods

Samples	Smartphone based colorimetric technique (mg/5 ml)	Spectro-photometric method (mg/5 ml)	Relative error (%)
1	0 ± 0	0 ± 0	Undefined
2	6.25 ± 0.0050	5.61 ± 0.0020	− 11.4
3	11.93 ± 0.0065	11.77 ± 0.0019	− 1.4
4	23.28 ± 0.0030	25.32 ± 0.0072	+ 8.1
5	50.43 ± 0.0058	50.29 ± 0.0072	− 0.3

developed v/s comparative spectroscopic methods (Table 2) were statistically compared using the paired t-test at a confidence level of 95%. The t exp value (0.47) was found lower than the t critical value (2.776), so that, it is concluded that there is no statistically significant difference between the results obtained using the two analytical techniques.

Comparison of analytical sensitivity of developed smartphone-based colorimetric method and routine wet chemical method The limit of detection of the developed method was also compared with the existing wet chemical method for detection of neutralizer in milk. For comparison purposes, LOD reported by Food safety and standards authority of India (Food safety and standards authority of India 2016) was taken from literature. The LOD of the DIB method (0.11%) was found better than the wet chemical test (0.2%) i.e. analytical sensitivity of the spot test has been improved by some extent with the use of digital image-based sensitive technique.

Conclusion

A simple, cost-efficient, smartphone-based sensing technology for colorimetric determination of neutralizer in milk based on a chromogenic spot check and digital image analysis is proposed. This study blends the talent of spot test (rapid, less volume and screening evaluation) and digital pix processing (economic gadget with color quantification, portability, and field applicability). Moreover, technology has the potential to provide a cheaper analytical alternative for a different range of analytes particularly in low-income nations with limited resources. Thus, we devised a technology that uses an omnipresent tool with the facility of on-site quantification of neutralizer residue in the milk supply chain. This technique is not only beneficial for the end-users but also helps in achieving extension goals by shifting out of the lab to the land.

Acknowledgements The authors are grateful to Director, School of Public Health and Zoonoses, GADVASU, Ludhiana for the facility provided. We also acknowledged the enormous help acquired from the research scholars whose articles are mentioned and covered in this manuscript.

Authors contributions Dr. VK—Method validation and laboratory analysis; Dr. RSA—help in design of study and research methodology; Dr. JPSG—Experimental design and set up; Dr. AS—help in lab investigation and data analysis.

Funding No funding received to conduct this work.

Data availability All data generated or analyzed during this study are included in the manuscript and availability reported in the reference section of the article.

Declarations

Conflicts of interest The authors declare that they have no conflict of interest.

References

- Andrade SIE, Lima MB, Barreto IS, Lyra WS, Almeida LF, Araújo MCU, Silva EC (2013) A digital image-based flow-batch analyzer for determining Al(III) and Cr(VI) in water. *Microchem J* 109:106–111. <https://doi.org/10.1016/j.microc.2012.03.029>
- Bankmycell (2021) How many smart phones are in the world. <https://www.bankmycell.com/blog/how-many-phones-are-in-the-world#1579705085743-b3697bdb-9a8f>. Accessed 26 October 2021
- Dos Santos Benedetti LP, Dos Santos VB, Silva TA, Filho EB, Martins VL, Fatibello-Filho O (2015) A digital image-based method employing a spot-test for quantification of ethanol in drinks. *Anal Methods* 7:4138–4144. <https://doi.org/10.1039/c5ay00529a>
- Food safety and standards authority of India (2016) Manual of methods of analysis of foods (Milk and milk products) Food safety and standards authority of India, Ministry of health and family welfare, Government of India, New Delhi
- Gomes MS, Trevizan LC, Nóbrega JA, Kamogawa MY (2008) Molecular absorption spectrophotometry using a scanner: Proposal of a didactic experiment for ascorbic acid determination. *Quim Nova* 31:1577–1581. <https://doi.org/10.1590/S0100-40422008000600050>
- Gupta VK, Shukla S, Singh RV, Gupta P (2013) Assessment of milk food safety status: common milk adulterants in rural and urban areas of Indian Malwa Plateau and their public health significance. *Vet Pract* 14:536–539
- Gupta VK, Aulakh RS, Tomar SS (2019) Novel method for the determination of preservative (formaldehyde) in bovine milk through smart phone-based colorimetric technology. *Indian J Vet Sci Biotechnol* 15:30–33. <https://doi.org/10.21887/ijvsbt.15.2.8>
- Iqbal Z, Bjorklund RB (2011) Assessment of a mobile phone for use as a spectroscopic analytical tool for foods and beverages. *Int J Food Sci Technol* 46:2428–2436. <https://doi.org/10.1111/j.1365-2621.2011.02766.x>

- Kohl SK, Landmark JD, Stickle DF (2006) Demonstration of absorbance using digital color image analysis and colored solutions. *J Chem Educ* 83:644–646. <https://doi.org/10.1021/ed083p644>
- Kumar V, Aulakh RS, Gill JPS, Bedi JS (2019) Analyzing efficacy of milk adulteration testing kits and conventional biochemical methods prevailing in India. *Vet Pract* 20:256–259
- Martinez AW, Phillips ST, Carrilho E, Thomas SW, Sindi H, Whitesides G (2008) Simple telemedicine for developing regions: camera phones and paper-based microfluidic devices for real-time, off-site diagnosis. *Anal Chem* 80:3699–3707. <https://doi.org/10.1021/ac800112r>
- Modi Zeel S, Desai Bhoomikaben S, Parmar SC, Jain AK, Shaikh AI, Aparnathi KD (2018) Development of spectroscopic method for quantification of sodium hydroxide in milk. *Int J Chem Stud* 6:44–48
- Moore JC, Spink J, Lipp M (2012) Development and application of a database of food ingredient fraud and economically motivated adulteration from 1980 to 2010. *J Food Sci* 77:118–126. <https://doi.org/10.1111/j.1750-3841.2012.02657.x>
- Shen L, Hagen JA, Papautsky I (2012) Point-of-care colorimetric detection with a smartphone. *Lab Chip* 12:4240–4243. <https://doi.org/10.1039/c2lc40741h>
- Sumriddetchkajorn S, Chaitavon K, Intaravanne Y (2014) Mobile-platform based colorimeter for monitoring chlorine concentration in water. *Sens Actuators B Chem* 191:561–566. <https://doi.org/10.1016/j.snb.2013.10.024>
- Urapen R, Masawat P (2015) Novel method for the determination of tetracycline antibiotics in bovine milk based on digital-image-based colorimetry. *Int Dairy J* 44:1–5. <https://doi.org/10.1016/j.idairyj.2014.12.002>
- Younis MR, Wang C, Younis MA, Xia X (2020) Smartphone based biosensors. In: Wu A, Khan WS (eds) *Nanobiosensors*. Wiley-VCH, Weinheim, Germany, pp 357–387. <https://doi.org/10.1002/9783527345137.ch15>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.