Iron (II) Determination in Lipstick Samples using Spectrophotometric and Microfluidic Paper-based Analytical Device (µPADs) Platform via Complexation Reaction with Iron Chelator 1, 10-phenanthroline: A Comparative Study

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Abstract:

This study was undertaken to introduce a fast, accurate, selective, simple and environment-friendly colorimetric method to determine iron (II) concentration in different lipstick brands imported or manufactured locally in Baghdad, Iraq. The samples were collected from 500-Iraqi dinars stores to establish routine tests using the spectrophotometric method and compared with a new microfluidic paper-based analytical device (µPAD) platform as an alternative to cost-effective conventional instrumentation such as Atomic Absorption Spectroscopy (AAS). This method depends on the reaction between iron (II) with iron(II) selective chelator 1, 10-phenanthroline(phen) in the presence of reducing agent hydroxylamine (HOA) and sodium acetate (NaOAc) buffer to yield a reddish/orange colour change proportional to the iron(II) concentration measured at $\lambda_{max} = 510$ nm. Under optimum conditions, the calibration curve was linear in the range between (0.5-150) mg L^{-1} with a limit of detection of 0.09 mg L^{-1}. Compared to a spectrophotometric detection method, µPAD measured colour intensity using captured images using Samsung mobile phone and image J program to give proof of concept that µPAD platform fulfils the purpose of accuracy and at the same time remaining cost-effective and simplistic to be used in both developing and developed countries gave same linear calibration curve with a limit of detection 0.12 mg L^{-1}. ANOVA test was used to compare the proposed method results with conventional method results showing the method was accepted. The antimicrobial activity showed no significant effect from lipstick samples on tested microbes.

Keywords: Colorimetric, Cosmetic, Heavy metals, Iron (II), µPADs platform, Spectrophotometric.

Introduction:

Cosmetic is defined by the European Union cosmetics regulation as substance, preparation or mixture placed in contact with different external human body parts such as hair, nails, epidermis, lips, etc. or applied to the teeth and the mucous membranes of the oral cavity aiming to be used as personal care merchandises to mask body odour, clean, protect, and improve ones' physical appearance to increase confidence and self-esteem[1,2]. The cosmetic industry has increased rapidly due to the huge demand and a sharp rise in product advertisements in electronic media resulting in massive production by the cosmetic industry [3,4]. Various cosmetics marketed globally including perfumes, makeup products, hair products, baby products and skincare products[5] are manufactured from natural environments (soil, water and rocks) which are considered the main ingredients for the cosmetics products industry of raw materials and pigments[6]. As a result, cosmetic products may be contaminated with trace amounts of heavy metals naturally in these ingredients in different proportions due to inadequate purification of the natural raw material or released by the metallic devices used during the manufacturing of cosmetics [7,8].

The prolonged period or daily base use of lipstick has attracted the researchers and clinicians

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health awareness and consumers wellbeing. Women ingest lipstick due to eating and/or drinking, kissing, and licking their lips. Therefore it is said, "women without intention eat about 4 lb of lipsticks in a lifetime"[9]. Oral ingestion of lipstick is considered as the significant route for the bioaccumulation of heavy metals particularly hazardous elements such as iron in the human body as lipstick products are directly applied to the mouth in addition to hand-to-mouth contact and systemically transported to vital internal organs exhibiting their toxicities[10]. The mechanism of toxic metal in the human body is attributed to the absorption and formation of complexes between them and proteins amines ($-NH_2$), carboxylic acids ($-COOH$), and thiols ($-SH$) [11,12], results in malfunctioning, damage or death of the cell and consequently leads to a variety of diseases[13].

Iron is an essential micronutrient utilized in almost every aspect of a living organism particularly crucial for the conservation of energy. Nevertheless, iron concentration in body tissue must be monitor and regulated as iron forms free radicals which in excessive amounts can cause tissue damage[14]. Also, a broad spectrum of clinical manifestations are associated with iron overwhelms in cells that lead to the increase of iron toxicity including neurodegenerative diseases, anaemia, diabetes and cardiovascular disease[15,16]. Extensive screening of literature was carried out on the analysis of iron levels in different lipstick and a variety of analytical techniques have been published including Atomic Absorption Spectrometry (AAS)[17-22], and Inductivity Coupled Plasma-Optical Emission Spectrophotometer(ICP-OES)[9,23]. However, inherited problems related to cost-effective, the need for trained people, and laborious procedures have driven researchers to develop simple, portable, and easy to use detection methods and colorimetric detection is one of the most reliable and promising approaches that can be a good alternative to conventional instrumentation[24-28].

The increasing interest in cosmetics and the lack of a simple and sensitive method for the determination of hazard concentration of iron (II) in cheaply priced lipsticks that are sold in the open market and imported from countries or manufacture locally where there is a lack of regulatory conditions necessitated in this study. In this context, the method emphasizes that iron (II) reaction with phen to form Fe-Phen complex that can be compatible with the colorimetric technique including spectrophotometric and μPADs platform methods considered as a good alternative to conventional techniques such as atomic absorption spectroscopy permitting independent evaluation of toxic metal (iron) in cosmetic (lipstick), these techniques allow fast and accurate method which is in agreement with green chemistry.

Materials and Methods:
Sample Collection and treatment

All different brands of lipsticks were purchased from different 500 ID cosmetic stores in Baghdad the capital of Iraq with unknown origins which sold cheap items suitable for poor and middle-class Iraqi society. The samples were coded as Lip1 (orange), Lip 2 (brown), Lip 3 (coral), Lip 4 (green), Lip 5 (maroon), Lip 6 (golden yellow), Lip 7 (lilac), and Lip 8 (beige). The samples were acid digested for elemental determination in lipstick following the Nnorom et al procedure [18]. A weight of 0.2 g of cosmetic sample (lipstick) was placed into a porcelain crucible and allowed to be heated on a hotplate (Ijlassco, India) near dryness in a fume hood until no fumes were observed(an indication of digestion process end). The content was left to cool to room temperature then 1 mL of concentrated Nitric acid (purity 69.5%, BDH, England) was added. After digestion, the digested sample was filtered through Whatman No. 40 filter paper to remove undigested materials such as glitters, wax, etc. After mineralization, the clear residue was transferred quantitatively into a 10 mL volumetric flask, diluted with deionized water and stored for further experiments.

Reagents, Materials and Standards solutions
1,10-phenanthroline (phen) Solution 0.04 M: prepared by dissolving 0.18 g of 1, 10 phenanthroline (purity, 99.0%, BDH, England) in 25ml deionized water, with simple heating to complete the solubility.

Hydroxylamine Hydrochloride (HOA) Solution 0.2 M: prepared by dissolving 0.35g of hydroxylamine hydrochloride (purity, 99.0%, BDH, England) in 25mL of deionized water.

Acetic acid-sodium acetate buffer (NaOAc) (pH = 5): prepared by mixing 59 mL of 0.1 M glacial acetic acid (prepared by diluting about 0.57 mL of glacial acetic acid (purity, 99.9%, BDH, England)up to 100 mL with deionized water) and 141 mL of 0.1 M sodium acetate (prepared by dissolving 1.65g of sodium acetate (purity, 99.9%, Fluka, Germany) in 200 mL deionized water, the buffer solution was used to control the solution pH.

Stock Solution of Metal ion 400 mg L^{-1}: prepared by accurately weighing 0.19 g of dried iron (III) chloride hexahydrate ($FeCl_3.6H_2O$ (purity, 99.0%, Aldrich, USA)) into a 100 mL beaker, a minimum amount of deionized water was added to dissolve. The content was transferred quantitatively to a 100

mL volumetric flask, diluted to mark with deionized water and mixed thoroughly, this solution was prepared daily.

Robustness (Reagents volume, pH, Order of Addition and Reaction Time)

To investigate the robustness of the developed method, batch experiments were conducted to establish the optimal analytical signal related to iron (II) concentration. The influence of the following variables was studied: phen 0.04 M volume (0.5–2.5) mL, HOA 0.2M volume (0.5 -2.5) mL, pH (3- 8), order of addition, and reaction time (0-35) minutes for spectrophotometric method. Similar variables influence were studied for µPADs method including phen solution 0.04 M volume ranging from (1-9) µL, HOA solution 0.2 M volume ranging from (1-9) µL, and reaction time ranging from (0-35) minutes.

Spectrophotometric Calibration Curve

To construct the calibration graph; 1 mL of HOA(reducing agent) 0.2 M was added to an increasing volume of iron (III) standard solution 400 mg L^{-1} into a series of 10 mL volumetric flask covering the range between (0.5-150) mg L^{-1}. Afterwards, 1 mL of phen (ligand) 0.04 M and 0.2 mL of 0.1 M NaOAc (buffer) were added and completed to the mark with deionized water. The resulted reddish/orange chromogenic product was measurable at 510 nm versus reagent blank (prepared under the same conditions without iron metal addition) after 10 minutes. The calibration curve was constructed from plotting standards concentrations versus absorbance reported as triplicate measurements to ensure precision and accuracy. The accuracy of the procedure was determined by measuring the recovery of metal added to lipstick matrix owing to the unavailability of certified material[29]. Uv-vis spectrophotometer (Shimadzu, Kyoto-Japan) digital double-beam was used equipped with a quartz cell of 1 cm for λmax determination and all absorbance measurements.

Microfluidic paper-based analytical device (µPAD) Calibration Curve

The chromogenic product calibration curve was formed on a µPADs device fabricated according to Peter *et al* method[30]. Initially, by dropping 7 µL of HOA 0.2 M, and 7 µL of iron solution series working solution prepared from standard iron solution (400 mg L^{-1}) into the detection zone using Eppendorf micropipette, allow drying at room temperature (~ 25° C). Afterwards, 7 µL of phen 0.04 M and 3 µL of 0.1 M NaOAc buffer were pipetted into each detection zone (eight separated spots on each chip) producing a coloured product. For additional characterization, images were taken using a Samsung note 9 camera and analysed using Image J freeware (National Institute of Health, USA).

Standard Addition Calibration Graph for Lipsticks Samples

The standard addition method was chosen to determine iron(II) concentration in both spectrophotometric and µPADs methods as in this method the influence of the interference can be eliminated[31]. This method was done by the addition of 1 mL of HOA 0.2M, increment concentration (0, 10, 60, and 100) mg L^{-1} of iron standard solution to 1mL of the solution of prepared samples. Then 1 mL of phen 0.04 M and 0.2 mL of 0.1 M NaOAc buffer were transferred into four volumetric flasks containing the above concentration of iron and sample. The mixture was allowed to react for 10 minutes and measured using spectrophotometric. For µPADs method, a similar method was undertaken using optimum conditions 7 µL of the phen 0.04 M, and 3 µL of 0.1 M NaOAc buffer which was added to detection zone containing 7 µL of HOA 0.2 M, increment concentration (0, 10, 60, 100) mg L^{-1} of iron(III) standard solution and 5 µL of lipstick sample.

In Vitro Antimicrobial Activity Assay

To investigate the antimicrobial of lipsticks from microbial contamination, a range of different gram-positive and gram-negative bacteria was selected in addition to fungi. The isolates included (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *E.coli*, *Klebsiella sp.*, *Pseudomonas aeruginosa* and *Candida albicans*) isolate. The selection of *S. aureus* and *S. epidermidis* bacteria depended on the basis that these bacteria are naturally a skin habitat. Additionally, they also can cause skin infections with *Strep. pyogenes*, and *P. aeruginosa*. The microbial activity of 8 different lipsticks was determined by the agar well diffusion method described by Parekh and Chanda[32]. In this method, a pure isolate of 24hrs growth was cultured in Muller-Hinton Agar plate (HiMedia, Mumbai, India) by using a sterile swab to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter 5.0 mm was used to bore one well in each agar plate. Each well was filled with lipstick suspension (volume 100 µl) from each sample by micropipette into Muller-Hinton Agar plate. The plates were allowed to stand for 1hr or more to allow pre-diffusion to take place and then incubated at 37ºC for 24hrs. The zone of inhibition was recorded in millimetres. Each experiment was performed in a duplicate.

Results and Discussion:
Method A (Determination of Iron via Conventional Spectrophotometer Technique)

The absorption spectra of the coloured complex formed by complexion reaction between iron metal 80 mg L^{-1} and phen solution 0.04 M in the presence of HOA 0.2 M and 0.2mL of 0.1 M NaOAc buffer were recorded via spectrophotometer, as the complex turned from colourless to reddish/orange complex after 10 minutes with maximum absorbance at 510 nm. The absorbance was proportional to the concentration of iron that exists in the lipstick sample as can be seen in Fig. 1.
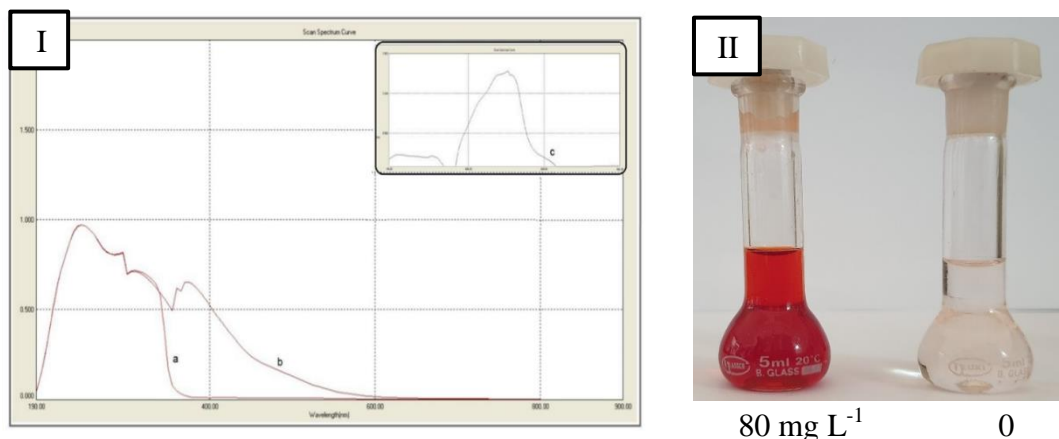

80 mg L^{-1} 0

Figure 1. (I) represent the absorption spectra of (a) phen versus deionized water, (b) iron (III) versus deionized water and (c) 80 mg L^{-1} iron (II) complicated with phen against reagent blank. (II) a photograph of iron chelator phen complex and blank coloured reaction.

Based on literature; the reaction between ferrous ion and phen is highly selective and sensitive to ferrous to form a reddish/orange complex[33]. The mechanism is based on the reduction of ferric to ferrous carried out by a reducing agent such as hydroxylamine, followed by metal complexion with phen which is considered as a selective ligand to iron metal through the implication of three nitrogen groups with iron (II)[34]. The proposed mechanism is shown in scheme 1.
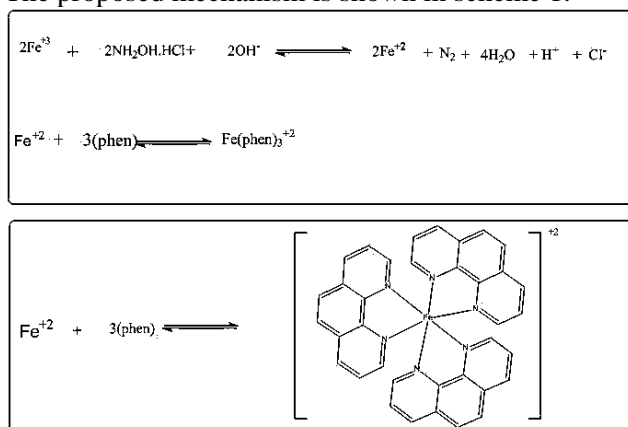
Scheme 1. Illustrate the mechanism of iron (II) metal with 1,10-phenanthroline reaction to form the coloured complex[35].

The present study was undertaken to determine the iron concentration in lipstick samples by conducting batch experiments at the variation that influence the absorption intensity of the coloured product including reagent phen volume, HOA volume, pH, order of addition, and reaction time. All experiments were measured in triplicate and the obtained results were corresponding to the average value. Optimization was carried out by changing one parameter and keeping the other fixed and measured at 510 nm versus reagent blank and so on.

The Effect of 1,10-phenanthroline Volume: The influence of phen volume on the absorption of the reddish/orange product was studied using different volumes ranged from (0.5 -2.5) mL. The results presented in Fig. 2a reveal that the absorbance increased with the increase of phen 0.04M volume up to 1 mL, then there was a decrease in absorbance. Therefore, 1 mL of the phen ligand was used in all consequent experiments.

The Effect of HOA Volume: A series of HOA 0.2M volumes range of (0.5 -2.5) mL was examined. It was observed in Fig. 2b that 1 mL of HOA was enough to obtain a maximum colour intensity absorbance as there was a drop in absorbance with increasing volume. As a result, it was used in all further experiments.

The Effect of pH: The pH solution plays a substantial role in the formation of the metal complex with phen and to avoid error in the analysis, NaOAc buffer solution (pH =5) was added to control the solution pH since the complex formed is very sensitive to the change in pH. Thus, the effect of pH was studied in the range of 3 to 8 using

different pH NaOAc buffer solutions. The result is shown in Fig. 2c reveals that the absorbance increased with pH increasing and reached the maximum at pH 5.0 for iron complexes this can be attributed to low hydrogen-ion activity, which avoids the nitrogen atoms protonation leading to strong bond formation. Afterwards, the absorbance gradually decreased because a higher pH deters iron ions into iron hydroxyl species that alter the complexion between Iron metal and phen [36,37].

The Effect of Order Addition: The development of complex colour is highly affected by the order of reagents addition. Diverse types of addition were carried out (Metal (M), phen (L), HOA (B), NaOAc buffer (AB)) as shown in Fig. 2d. Results show the addition of base, metal, reagent and acetate buffer was recommended as the absorbance reaches 1.00, which can be attributed to the fact that the base

reduces ferric to ferrous and later ferrous reacts with the ligand to form a reddish /orange complex product and thus was followed in the subsequent experiments since it resulted in obtaining maximum absorbance.

The Effect of Reaction Time: The optimum reaction time is a fundamental parameter for the iron determination procedure. Therefore, it is essential to study the influence of time over the time interval from (0-35) minutes. Figure 2e shows that there was an initial increase in absorbance with time up to 10 minutes. As a result, in subsequent experiments, 10 minutes was considered more than sufficient for complex colour development at room temperature and the complex was stable at least for one week in solution.
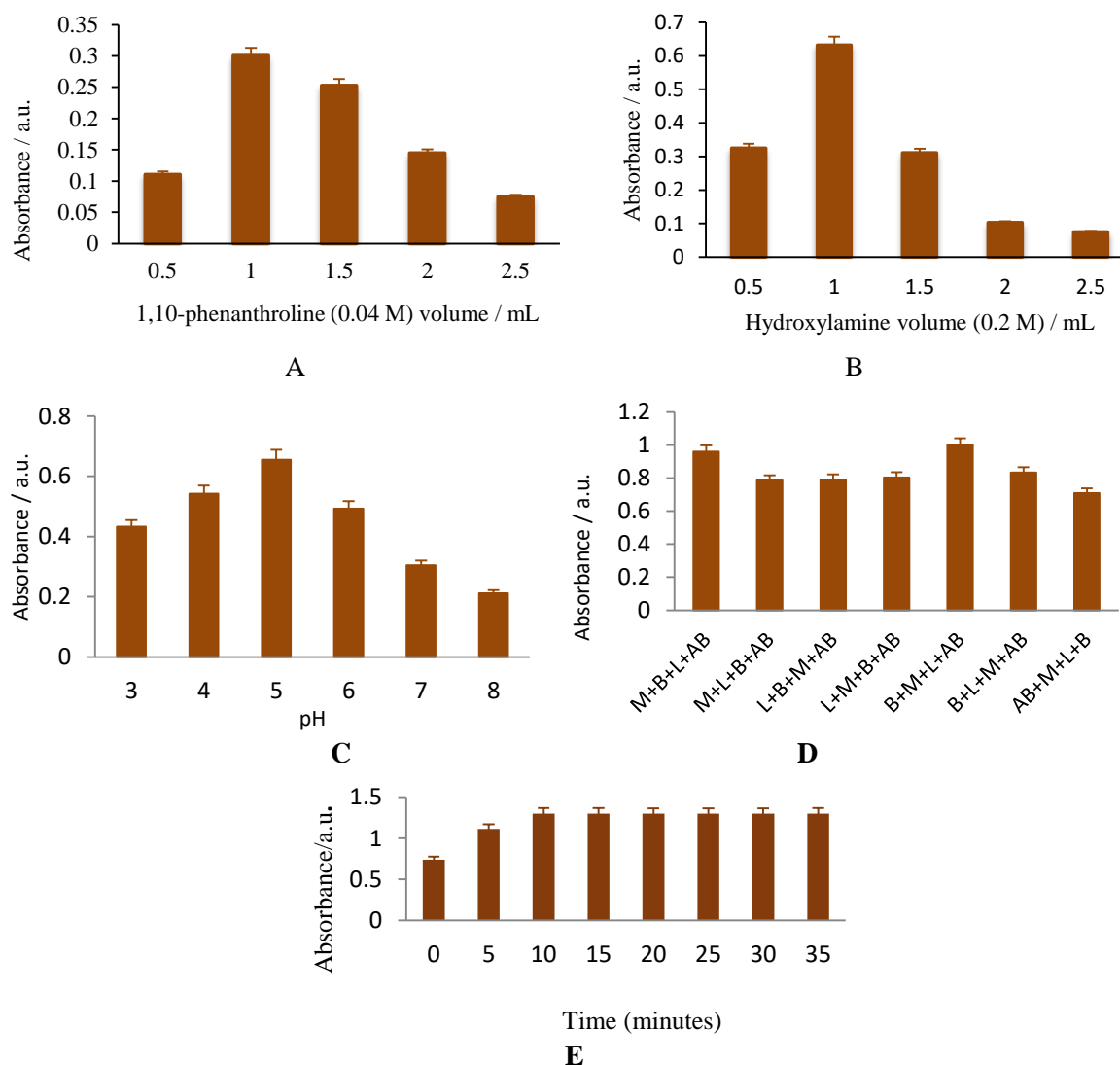

Figure 2. Influence of (a) phen solution 0.04 M volume ranging from (0.5-2.5) mL, (b) HOA solution 0.2 M volume ranging from (0.5-2.5) mL, (c) pH ranging from (3-8), (d) orders of reagents addition were ((M) Metal, (L) phen, (B) HOA, (AB) NaOAc buffer), (e) reaction time ranging from (0-35 minutes) parameters on spectrophotometric batch experiments via the formation of metal complex with phen.

Method B (Determination of iron via µPADs Method)

Investigations were carried out using the µPADs method platform once the spectrophotometric method was established and accurate results were obtained. The initial concept is to prove that the same reproducible results can be achieved using both methods via complex colour change using iron (II) via phen reaction. Preliminary experiments showed that the addition of HOA 0.2 M, standard iron solution, phen solution 0.04 M and 0.1 M NaOAc buffer on paper device lead to colour development as the paper natural matrix would permit the reagents to wick in the detection zone and easily visualize by the human eye (Fig. 3). Sequentially, iron concentrations related to varied complex absorbance were measurable via the image J programme.
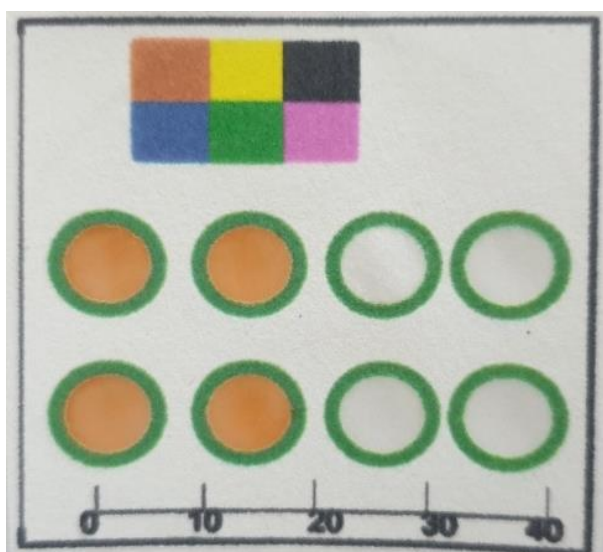

Figure 3. Picture of µPAD platform for iron detection viewing the detection zone containing iron complex with phen in the presence of HOA and NaOAc buffer (first and second vertical spots to the left) and its blank (third and fourth vertical spots).

Parameters that affect the colour development including volume of phen and HOA and reaction time were examined to give the best absorbance for rapid and sensitive measurements. All experiments were done using 80 mg L^{-1} of iron solution.

The Effect of 1,10-phenanthroline Volume: Experiments on the influence of spotted reagent volume were done as the insufficient volume may lead to absorbance reduction at the same time the excess of volume may cause to solution spread outside the detection zone[36]. Phen 0.04 M volume was examined in the range (1-9) µL. As can be seen from Fig. 4a the best volume that gave the higher absorbance was 7 µL, therefore, it was chosen for further experiments.

The Effect of Hydroxylamine Hydrochloride Volume: The HOA 0.2 M different volumes of influence were tested to determine the optimum volume that gives the highest intensity. Figure 4b shows there was a slight increase in intensity through the addition of 1, 3, and 7 µL of the base. Then a decrease was observed after 7µL, these results indicate that the optimum base volume that gives the optimum intensity was 7µL.

The Effect of NaOAc Volume: NAOAc solution volume effect on maximum colour intensity was tested and the results showed that 3 µL of NaOAc 0.1 M gave the highest colour intensity, therefore it was selected for further experiments (Fig. 4c).

The Effect of Reaction Time: One of the most fundamental parameters influencing complex formation is reaction time and to ensure maximal colour intensity, reaction time was optimized over time intervals from (0-35) minutes. The colour development initially increased with time up to 10 minutes which was deemed adequate to obtain maximum colour intensity and used for subsequent experiments (Fig. 4d).
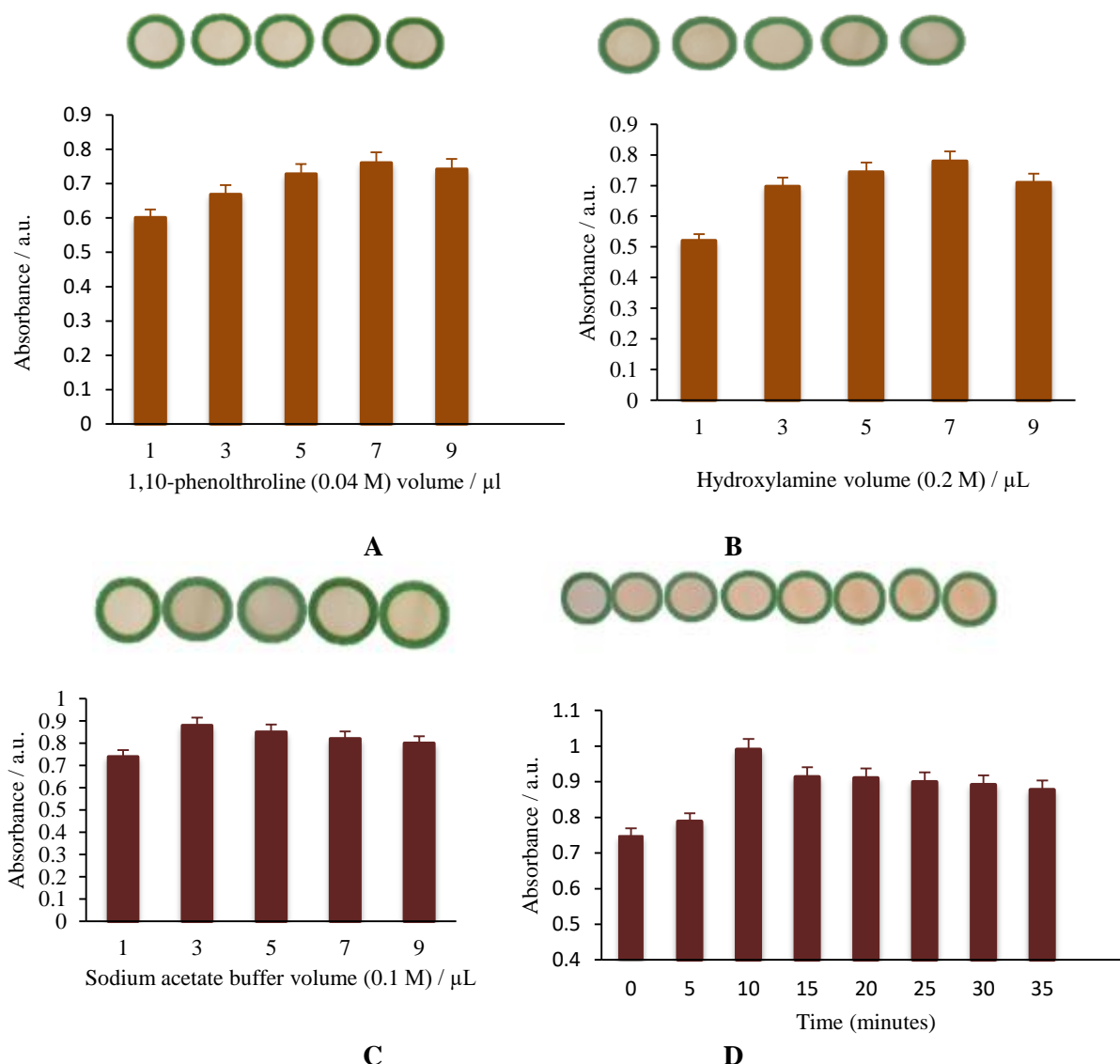
Figure 4. Influence of (a) phen solution 0.04 M volume ranging from (1-9) µL, (b) HOA solution 0.2 M volume ranging from (1-9) µL,(c) NaOAc solution 0.1 M volume ranging from (1-9) µL (d) reaction time ranging from (0-35) minutes parameters on µPAD platform experiments via the formation of the metal complex with phen.

Calibration Graphs and Figure of Merits

The linear calibration graph was constructed for methods A and B using a series of iron concentration solutions via suitable dilution of stock solution. Figure 5a and b show a calibration graph for the spectrophotometric method under optimum experimental variables measured at λmax 510 nm.

Figure 5c and d show the calibration graph for a µPADs method using optimum conditions. Table 1 summarizes the regression equation, correlation coefficient, molar absorptivity, Sandell's sensitivity, the limit of the quantification (LOQ) and limit of detection (LOD) for both iron methods.
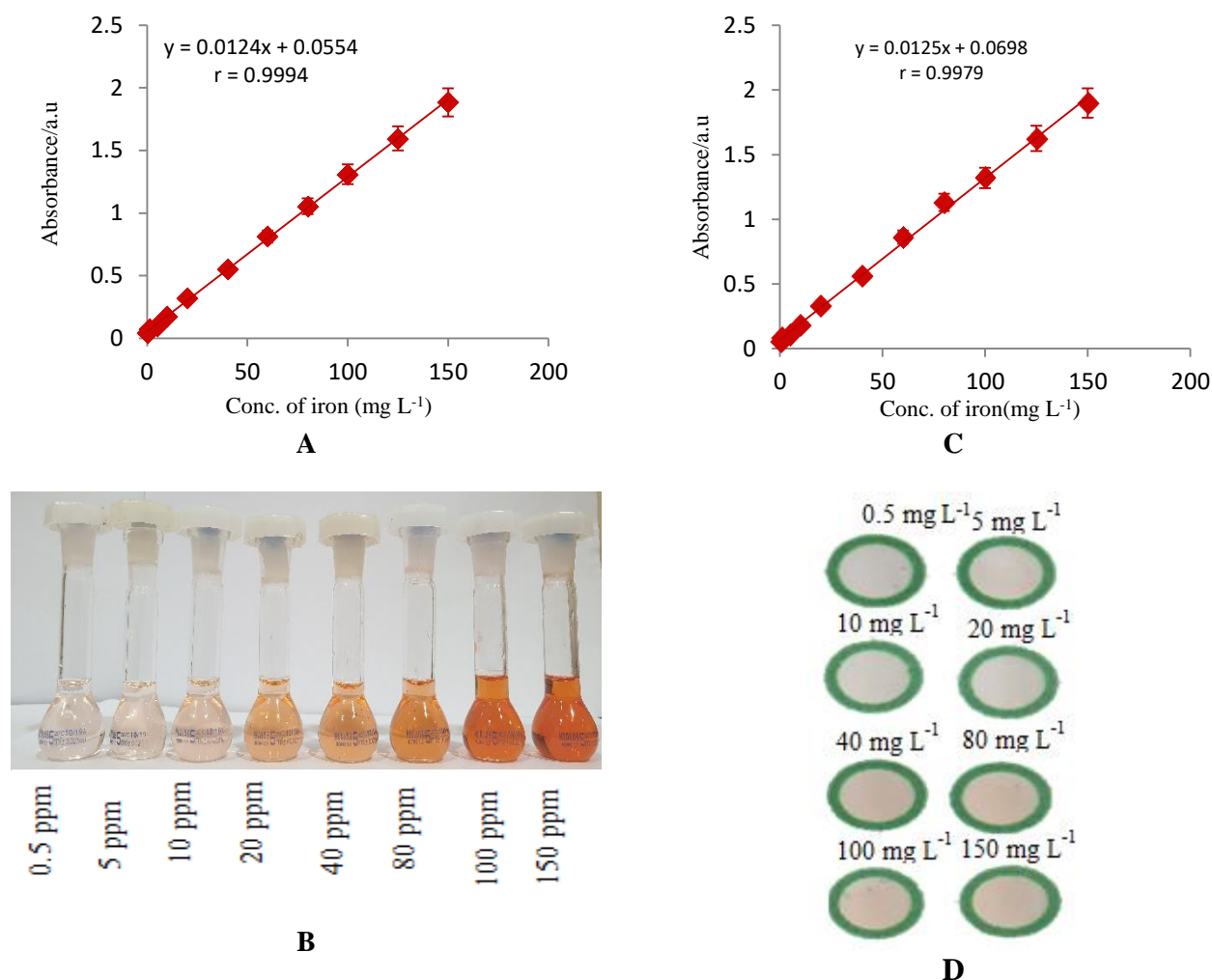
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Figure 5. (a) Linear calibration graph for spectrophotometric determination of iron (II) concentration in the range between (0.5-150) mg L^{-1}, (b) an image of serial concentration of iron in a volumetric flask, (c) Calibration graph for µPADs determination of iron (II) concentration (0.5-150) mg L^{-1}, (d) an image of the paper microfluidic device with serial standard concentrations of iron spotted inside the sensing zone.

Table 1. Analytical values of statistical treatments for iron calibration graphs using spectrophotometric and µPADs methods, respectively.

Parameter	Values	
	Spectrophotometric method	**µPADs method**
Linear coefficient, r	0.9994	0.9979
Linearity percentage	99.88	99.58
Regression equation	y=0.0124x + 0.0554	y=0.0125x + 0.0698
Slope, b	1.24×10^{-2}	1.25×10^{-2}
Intercept, a	5.54×10^{-2}	6.98×10^{-2}
Conf. limit for slope b±t$_{sb}$	$0.0124 \pm 2.915 \times 10^{-4}$	$0.0125 \pm 4.27 \times 10^{-4}$
The conf. limit for intercept a±t$_{sa}$	$0.0554 \pm 2.147 \times 10^{-2}$	$0.0698 \pm 3.16 \times 10^{-2}$
Standard deviation of the residuals, S$_{y/x}$	2.17×10^{-2}	3.2×10^{-2}
Standard deviation of the slop, S$_b$	1.29×10^{-4}	1.89×10^{-4}
Standard deviation of the intercept, S$_a$	9.5×10^{-3}	1.4×10^{-2}
Linearity range (mg L^{-1})	0.5 - 150	0.5-150
Molar absorptivity (L.mol^{-1}. cm^{-1})	692.478	698.06
Sandell's sensitivity (mg L^{-1})	8.1×10^{-2}	8×10^{-2}
Limit of detection, LOD (mg L^{-1})	0.09	0.12
Limit of quantification, LOQ (mg L^{-1})	0.31	0.47

The precision and accuracy for iron (II) proposed methods were studied by calculating the values of relative standard deviation (RSD %) and percentage of relative error (Er %). The results in Table 2 showed that RSD values were low between (0.39 – 1.85), relative error less than 3%, and acceptable recoveries values between (98.18 – 101.33) were obtained indicating to good precision and accuracy of the proposed methods.

Table 2. Statistical data of iron procedure accuracy and precision using spectrophotometric and µPADs detection methods, respectively.

Method	Taken (mg L^{-1})	Found (mg L^{-1})	Rec.%	RSD%	Erel%
Spectrophotometry	10	10.12	101.2	0.74	1.2
	60	60.21	100.35	0.39	0.35
	100	99.59	99.59	0.61	- 0.41
µPADs	10	9.818	98.18	1.851	-1.813
	60	60.66	101.10	0.76	1.10
	100	101.33	101.33	0.988	1.33

*Average of three replicate

Analytical Application and Comparison Method

The ability of the proposed method to determine iron (II) in commercial lipstick collected from 500 I.D. stores in Baghdad were investigated using the standard addition method to eliminate interferences influence by comparing the proposed method results with standard analytical method results that are usually used for iron analysis; therefore atomic absorption spectrometry (AAS) was adopted. The results presented in Fig. 6 showed that the concentration of iron (II) from Spectrophotometric and µPAD were not significantly different from those of conventional AAS. Table 3 presents the analysis of variance including eight samples indicating F_{tab} = 3.4668 is much higher than F_{Stat} = 0.2526 Therefore, it can be concluded that there was an insignificant difference between the three methods. Also, comparing the results from spectrophotometric, paper-based device and atomic absorption spectrophotometric methods showed a good correlation between results via the three methods i.e. (r = 0.9987), (r = 0.9969) and (r = 0.9981), respectively.
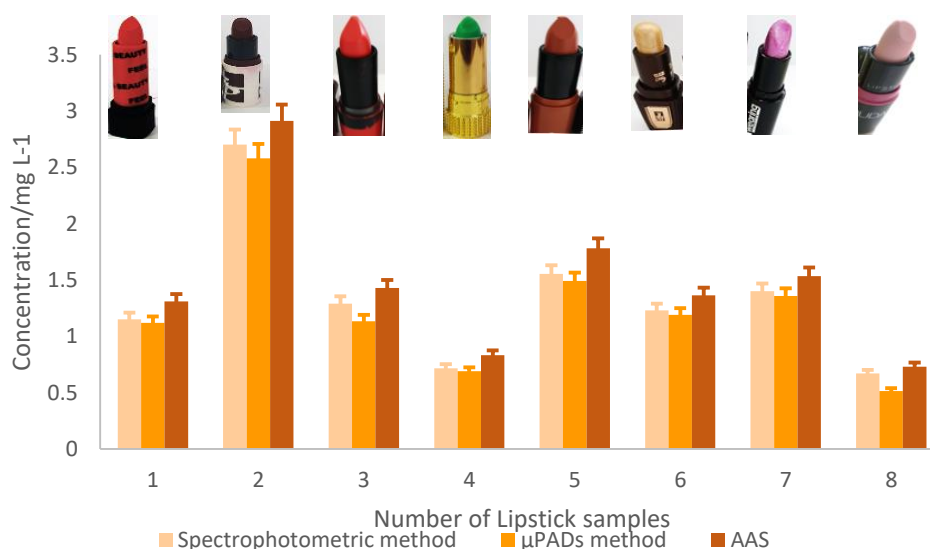

Figure 6. Iron (II) concentrations in different lipstick samples were collected from 500 I.D. stores, Baghdad, Iraq using spectrophotometric and µPADs and AAS methods via the reaction of iron chelator 1, 10-phenanthroline with iron (II) in the presence of reducing agent(hydroxylamine) and sodium acetate buffer.

Source of Variation	SS	df	MS	F		F crit
Between Groups	0.208847	2	0.104423	0.252645		3.4668
Within Groups	8.67972	21	0.41332			
Total	8.888567	23				

Table 3. Statistical analysis using ANOVA program and correlation between methods

Method	AAS	Spector.	µPADs
AAS	1		
Spect.	0.9987	1	
µPADs	0.9969	0.9981	1

Study of Biological Activity

The antimicrobial activity of local lipsticks against clinical and normal flora microorganisms was examined using agar well diffusion method. The antimicrobial efficacy testing revealed that all the lipsticks samples showed no effect on *Staphylococcus aureus* while *Staphylococcus epidermidis* and *Streptococcus pyogenes* isolates were sensitive to all lipsticks samples and samples 7 and 8 showed the most antimicrobial effect on *Staphylococcus epidermidis* and *Streptococcus pyogenes* isolate Fig. 7a. For gram-negative bacterial isolates, all isolates were sensitive to all lipsticks samples and sample 7 showed the most antimicrobial effects on *Pseudomonas aeruginosa* isolate Fig. 7b. While the lipsticks samples showed no antimicrobial activity against *Candida albicans* isolate Fig. 7c.
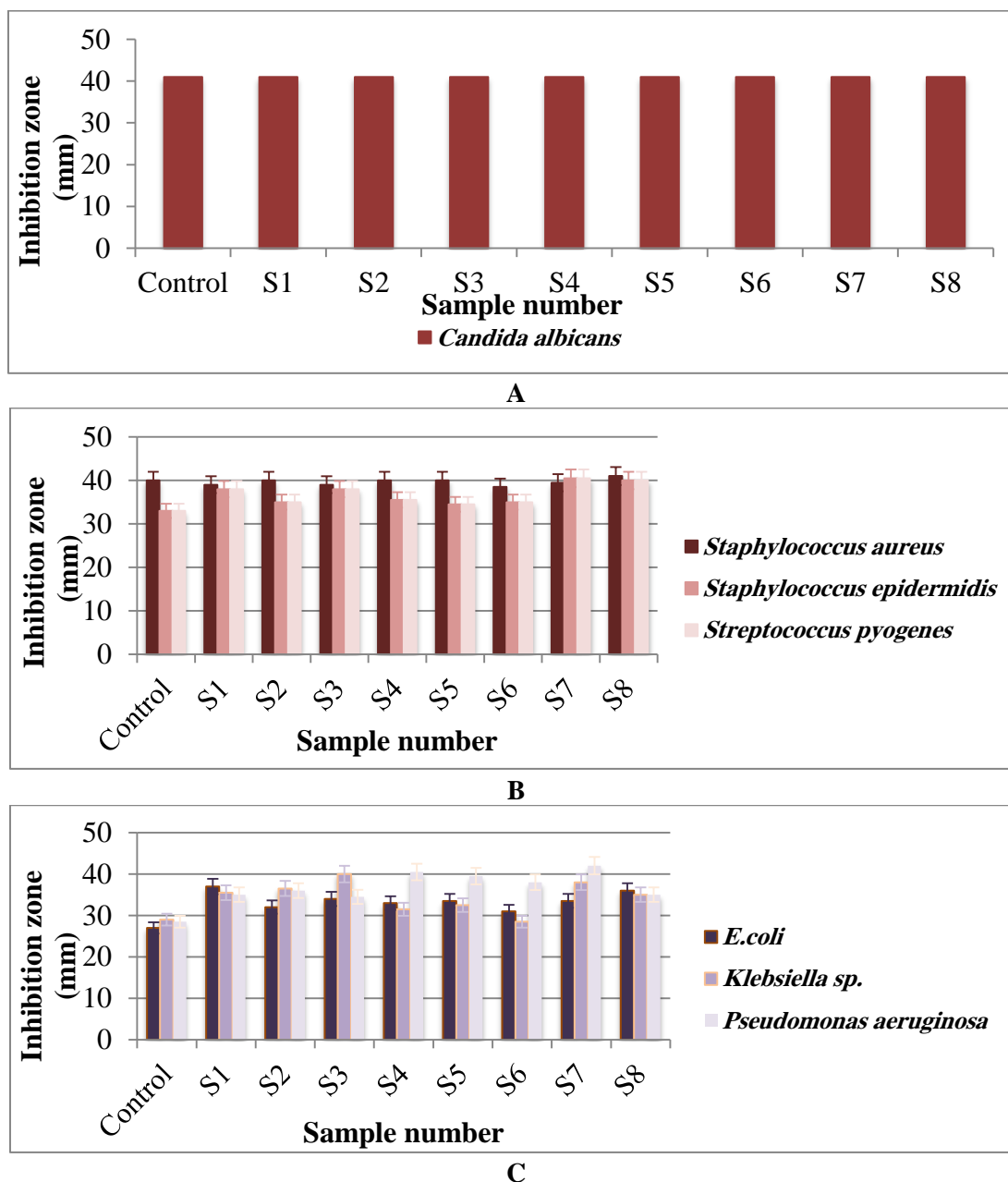

Figure 7. Antimicrobial activity of lipsticks samples. (a) Inhibition of different gram-positive bacteria isolates by lipsticks samples, (b) Inhibition of different gram-negative bacteria isolates by lipsticks samples and (c) Inhibition of Fungal isolate by lipsticks samples. Each bar graph represents the inhibition zone in millimetres.

The lack of expiry date and an ingredients label on lipsticks with unknown brands pose a potential high risk to consumer health. The possible contamination of lipsticks by microorganisms before and after use by the consumer is a major problem for the cosmetic industry. *Staphylococcus aureus, E. coli*, and *Pseudomonas aeruginosa* and *Candida albicans* are the most common tests strains that are recommended to test antimicrobial efficacy in addition to normal flora such as Staphylococcus epidermidis[38]. Using pyrosequencing analysis, a study detected 105 bacterial genera in 20 lipstick samples and Streptococcus were one of four genera predominant in 92% of the 19,863 total sequence reads while Staphylococcus, Pseudomonas, Escherichia a potentially pathogenic bacteria represented 27.6% of the total 105 genera. According to a recent study by Siya 2019, *S. epidermidis* represented 40% while *S. aureus* represented 32% from identified microorganisms[39]. *S. epidermidis* is responsible for biofilm formation in the prosthetic devices and implants[40] and *S. aureus* is involved in causing bacteremia in patients suffering from endocarditis and metastatic infections[41].

Conclusion:

Two new, simple, reliable and economical colorimetric assays including spectrophotometric and paper-based analytical microfluidic devices (µPADs) were used for the development of in situ system for measuring the iron (II) concentration in cheap lipsticks samples collected from the local market of Baghdad, Iraq as this metal could cause toxic metal poisoning to lipstick consumers due to daily use. This paper used the employment of 1,10-phenanthroline as a mostly used ligand in coordination chemistry for iron (II) detection to form a measurable complex and its resulted absorption due to colour change is proportional to the concentration of iron(II) that exist in lipstick. This gives easily interpretable results without sophisticated equipment such as atomic absorption spectroscopy, additional advantage relies upon a short time frame to complete the colourimetric assay. The important findings of this study, iron (II) levels were found to be lower than the permissible limit in lipstick samples, antimicrobial activity testing revealed that the tested microbes showed varied sensitivity all lipsticks samples with exception of *Staphylococcus aureus* and *Candida albicans* that were not affected by lipsticks samples and sample 7 showed the highest effect, and optimization of experimental results showed that these two methods give similar accuracy.

Authors' declaration:
- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in Mustansiriyah University.

Authors' contributions statement:

EA Abdulkareem has done the experimental work. JO Abdulsattar has designed the experiments, analysed the data, wrote, edited, revised, and proofreading the manuscript. BO Abdulsattar has been consulted for the biological work with the data analysis.

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تقدير الحديد الثنائي في عينات احمر الشفاه باستخدام الطريقة الطيفية وتقنية السوائل المايكروية الورقية عبر تفاعل التعقيد مع مخلب الحديد 1,10- الفينانثرولين. دراسة مقارنة

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الخلاصة :

اجريت هذه الدراسة لتقديم طريقة لونية سريعة ، دقيقة ، انتقائية، بسيطة و صديقة للبيئة لتقدير تركيز الحديد الثنائي في مختلف العلامات التجارية لاحمر الشفاه المستورد او المصنع محليا في العراق والمجمع من متاجر 500 دينارعراقي واعتماده كاختبار روتيني باستخدام الطريقة الطيفية ومقارنتها مع تقنية السوائل المايكروية الورقية الجديدة كبديل للاجهزة التقليدية المكلفة مثل جهاز الامتصاص الذري الطيفي . وتعتمد هذه الطريقة على تفاعل بين الحديد الثنائي مع مخلب الحديد الثنائي الانتقائي1,10- الفينانثرولين بوجود العامل المختزل هيدروكسي امين و المحلول المنظم اسيتات الصوديوم لتكوين معقد ملون ذي لون برتقالي محمر والذي يتناسب طرديا مع تركيز الحديد التنائي المقاس عند الطول الموجي الاعظم 510 نانوميتر. تحت الظروف الفضلى منحني المعايرة كان خطيا ضمن المدى (150-0.5) ملغرام لتر[-1] مع حد كشف 0.09 ملغرام لتر[-1] للطريقة الطيفية. ومقارنة مع طريقة التقدير الطيفية فأن طريقة السوائل المايكروية الورقية تم قياس شدة اللون الناتج بأستخدام صور الملتقطة بأستخدام جهاز سامسونج وبرنامج Image J. لاعطاء دليل على ان منصة السوائل المايكريوية الورقية تحقق الغرض من كونها طريقة ذات دقة وفي الوقت نفسه ذات كلفة قليلة وبسيطة لاستخدامها في كل من البلدان النامية والمتقدمة حيث اعطت نفس منحني المعايرة الخطي مع حد كشف 0.12 ملغرام لتر[-1] تمت المقارنة مع الطريقة التقليدية بواسطة برنامج ANOVAمعطيا نتائج مقاربة .لم يظهر النشاط المضاد للميكروبات اي تأثير ملحوظ من عينات احمر الشفاه على الميكروبات المختبرة.

الكلمات المفتاحية: الطرق اللونية، مستحضرات التجميل، العناصرالثقيلة ، الحديد الثنائي، تقنية السوائل المايكروية الورقية، المطيافية.