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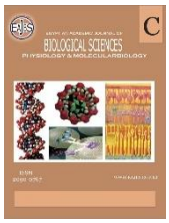

A New Paper-Based Microfluidic Design (µPAD) for Determination of Total Phenols and Biological Applications Using Smartphone Sensor as a Detector

Mahmoud Y. Rashed and Mustafa A. Hussein
Department of Chemistry, Faculty of Science, University of Kufa, Najaf 54001, Iraq.
***E-mail:** mahmoudya116@gmail.com

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ABSTRACT

Phenols are well-known noxious compounds, which are often found in various water sources. In this research, a new method was used to determine the total phenol and measure the biological activity, by using the microfluidic paper-based (µPAD), smartphone Sensor. It is portable, low-cost, and environmentally friendly. The paper was prepared using filter paper by cutting method. The reagent was dried on the paper. The color sensor of the smartphone device was used as the color intensity sensor of the samples through the images captured by the smartphone through software downloaded to the phone. The focus of the samples is measured from the RGB value of the images taken from the (iPhone 14) device, where each image represents a specific focus. The calibration curve for this method was in the range of M (0.00016–0.5), the correlation coefficient (R2) was equal to (0.9753), the limit of detection was in the amount of (0.000032) M, and the relative standard deviation (RSD%) for the concentration is (0.02) M, for which the examination was repeated (10) times and its value was (0 %), and the recovery value (Recovery%) was equal to (100 %).

INTRODUCTION

Phenol is an aromatic chemical compound made comprised of a functional hydroxyl group (-OH). The measurement of phenol and its derivative compounds is crucial for the environment since these species are significant organic pollutants that occur often in ground and surface waters(Ni, Xia, *et al.*, 2011). Phenols are classified as priority pollutants due to their toxicity and persistence in the environment(Colón, Rascón *et al.*, 2023). Even at low concentrations, they produce a distinct, peculiar taste and odor in drinking water. Some of them are also suspected to be mutagenic(Adamski, Nowak, et al. 2010). Many phenolic chemicals are hazardous to animals and plants because they rapidly permeate epidermal and cellular membranes. Because various phenolic compounds act differently and have varied ecological consequences and toxicity, determining specific phenolic compounds is extremely relevant and beneficial. Several methods have been used for the determination of phenolic compounds, such as gas chromatography-mass spectrometry(Colón, Rascón *et al.*, 2023) ,and HPLC(Dini, Graziani, *et al.*, 2020).

The paper has played an important role from the time of its invention right through to modern chemistry. With the creation of the first microfluidic paper-based analytical device (µPAD) in 2007(Sun, Li *et al.*, 2018), it emerged as a promising platform in medical diagnostic and analytical devices. Capillary action, caused by the surface tension of the liquid in a porous medium, pushes fluid within paper without the need for a power source.

µPADs are regarded as an effective POCT(Sinha, Basu *et al.,* 2022), tool since they imply the advantages of paper as a substrate that are environmentally benign and practicable, making them a unique material that is simpler to dispose of safely(Agarwal, Csóka *et al.,* 2019). (µPADs) have several practical benefits including cheaper cost, a simple production method, robust capillary action, and high biological compatibility(Fu and Wang 2018),(Hsu, Liu *et al.,* 2019) with applications in biomedical diagnostics, analytical chemistry, food quality control and environmental sensing. The integration of cell phones with sensors is a potential method for achieving quick, portable, and simple detection(Xu, Huang *et al.,* 2018).

The further integration of µPAD with a smartphone app provides an excellent strategy for smart on-site analysis(Liang, Liu *et al.,* 2019). Making sensors compatible with smartphones is becoming more common in the industry in order to take advantage of its mobility, and connectivity (Daponte, De Vito *et al.,* 2014). Analysis may be done anywhere is convenient thanks to Bluetooth and Internet access. The smartphone has been used in the identification of iron (II) in a pharmaceutical formulation(Ilaybi and Hussien), sensors of environmental(Thio and Park 2022), and detection of enzymatic urea hydrolysis in microfluidic systems(Salman and Hussein 2021). In this study, a new method was included for total phenol determination in prepared samples by basic color analysis (RGB) for samples taken by a smartphone.

MATERIALS AND METHODS

Chemicals:

All solutions of chemicals used in water were prepared with the degree of analytical reagent. All solutions needed in the determination of total phenols by the Folin-Ciocalteu reagent method include phenol solution, A stock solution of phenol was prepared by dissolving 0.9411 g of phenol in a small amount of boiled and cooled distilled water and diluted to a 20 ml. Sodium carbonate, A 75 g L^{-1} was prepared in distilled water.

Instrumental:

Electric balance, Bp301S, Sartorius, Germany. pH meter, Romania .smart phone device (iPhone 14), U.S.A.. Filter paper, China. Paper punch.

A new method that relies on the use of a (µPAD) with color sensor on the smartphone device (RGB):

It is a new method that relies on the use of a (µPAD) with the color sensor on the smartphone device (iPhone 14) via an application downloaded on the smartphone device, which analyzes the color intensity of the major colors (RGB) of the captured images, as shown in Figure 1.

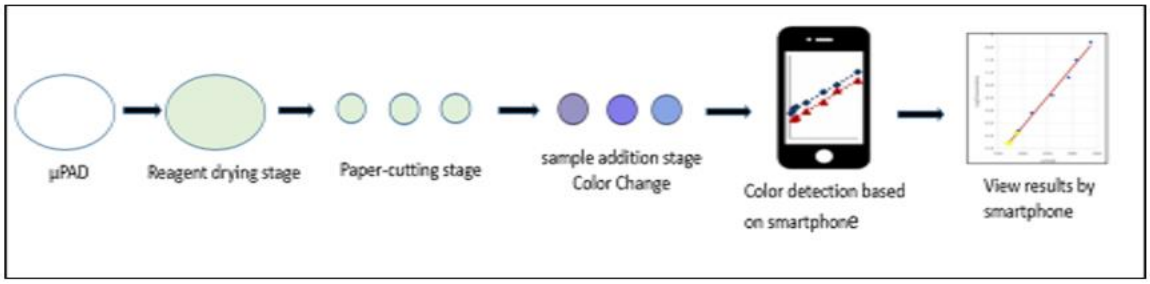

Fig1: Show using a smartphone with µPAD.

Biological activity, Bacterial Isolates:

The following microbial pathogenic isolates of multidrug resistance (MDR): Staphylococcus aureus and Enterococcus faecalis, six Gram +ve bacteria, were identified, together with Proteus mirabilis and Enterobacter cloacae, two Gram +ve bacteria. Clinical examples include a wound, burns, and diabetic ulcers. The isolates were discovered by phenotypic and biochemical

studies, and they were recently validated with the portable Vitek-2 equipment. Both bacterial isolates were procured using an automated bacterial detection tool for GP and GN cards d in glycerol-added BHI broth at (-20°C) (15 percent). Prior to use, the isolates were sterilized at 37°C for 24 hours and subcultured on BHIA(Samie, Tambani et al. 2010).

RESULTS AND DISCUSSION

Design of (µPAD) and RGB Measurement Method Using A Smartphone:

Designed a new form of microfluidic paper-based analytical device (µPAD) that is fast, easy to use, portable to the job site, and inexpensive for the measurement of phenol samples by smartphone using an image color (RGB) color analysis system, as shown in (Fig 2), the design was carried out according to the following steps:

1- Filter paper saturated with Folin-Ciocalteu reagent was prepared after drying it.

2- Filter paper was cut into circles of equal diameter (5 mm) using a paper punch.

3- A series of standard concentrations prepared from phenol were added to each paper at a specific concentration.

4- Sodium carbonate solution was added to each paper to show the blue color as a phenol detector.

5- A smartphone camera was used to take a picture of each colored concentration.

The volume added was fixed for both the sample and the pH(10). After taking pictures, they are processed using color image analysis software (Color M1) using the (RGB) system installed on the smartphone in order to extract the standard calibration curve of the phenol solution at the same time, and then studied for optimal conditions such as detection limit, matching, and analysis of the prepared samples, and the results analyzed statistically.

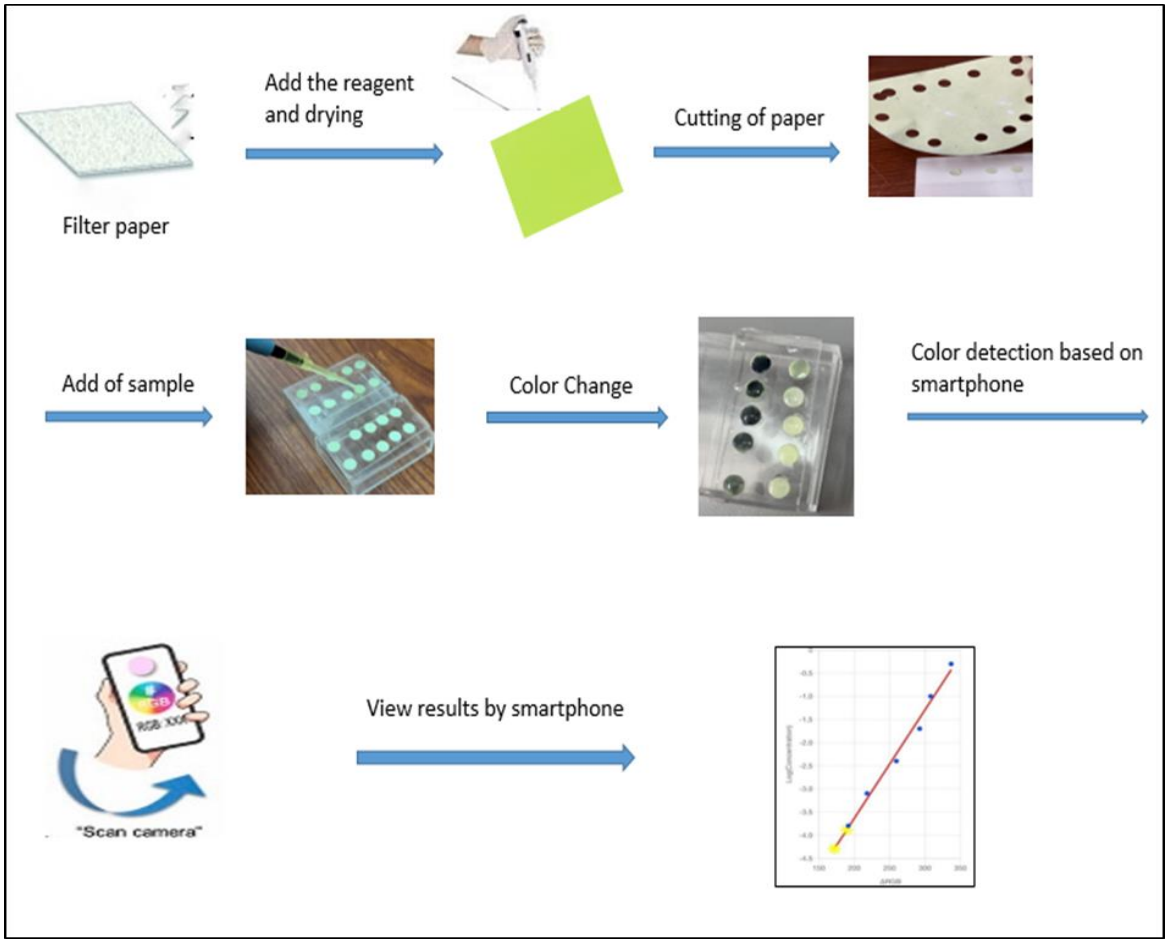

Fig 2: Shows the design stages of a microfluidic paper-based analytical device (µPAD).

Congruence Study:

To ensure the conformity of the results obtained by color density (RGB) by the smartphone, we studied the congruence of the results of tests for (10) duplicate images taken at a concentration of (0.02 M), and the (RSD) value of the match was (0%) and the recovery value (Recovery) equals (100%), as shown in Table 1 and (Figs. 3 and 4**)** below.

Table 1: Shows the congruence results of the RGB method.

Value B		Concentrations measured by chromatic intensity method (M)	RSD %	Recovery %
1	147	0.02	0 %	100 %
2	146	0.02		
3	147	0.02		
4	147	0.02		
5	147	0.02		
6	147	0.02		
7	146	0.02		
8	147	0.02		
9	147	0.02		
10	146	0.02		

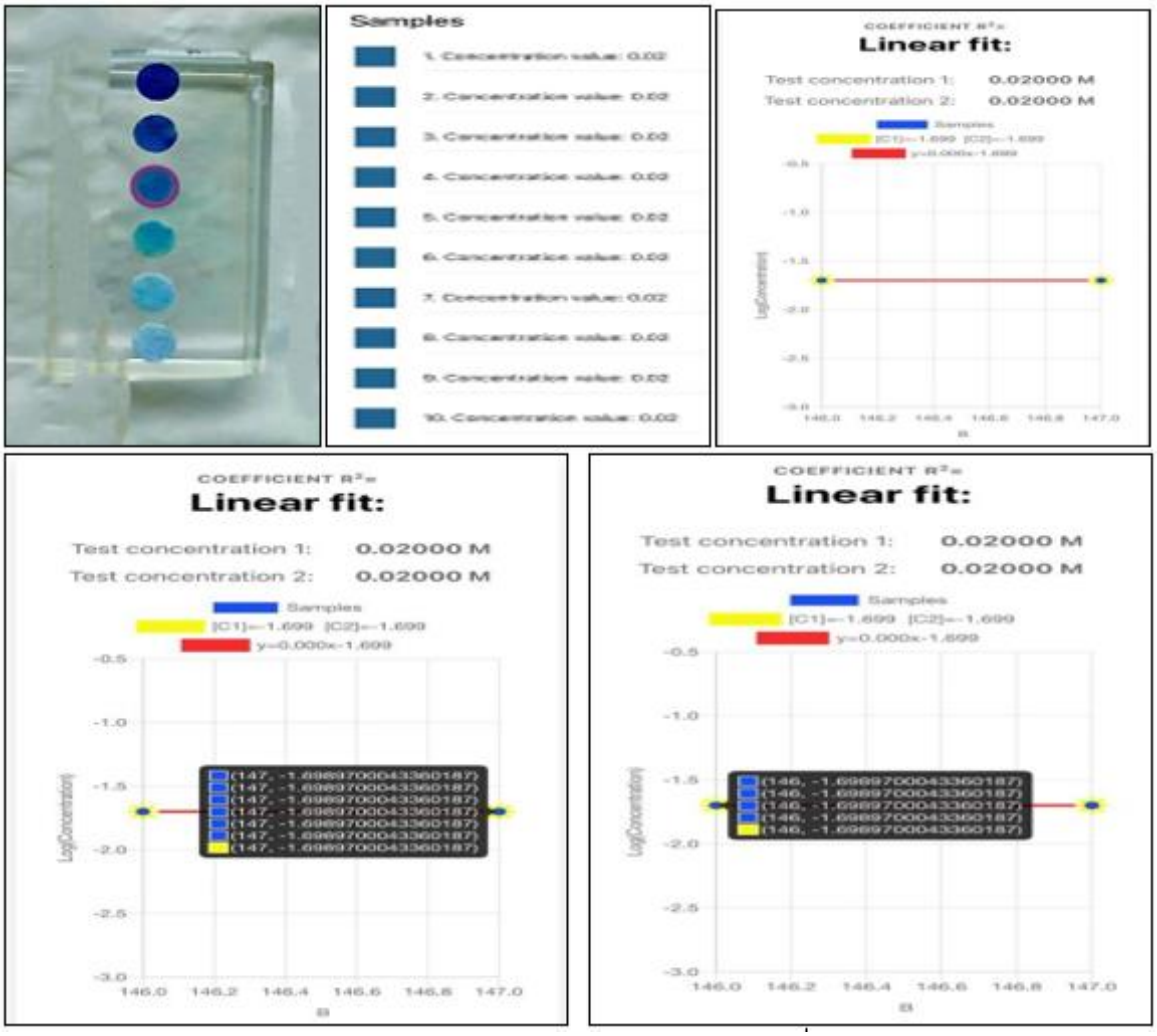

Fig 3: Pictures of the reproducibility study of concentration (0.02).

	A	B	C	D	E	F	G	H	I	J	K	L
1	Name	R	G	B	ΔRGB	RGB	H	S	V	Concentration		
2	Calibration s	32	43	118	336.81152	-2.551425	0.6444444	0.7288	0.4627	0.5		
3	Calibration s	37	73	136	307.9107	-1.660411	0.6055556	0.7279	0.5333	0.1		
4	Calibration s	31	102	146	292.34569	-1.147728	0.5638889	0.7877	0.5725	0.02		
5	Calibration s	46	141	152	259.39545	-0.314221	0.5166667	0.6974	0.5961	0.004		
6	Calibration s	78	155	176	218.10548	0.9360234	0.5361111	0.5568	0.6902	0.0008		
7	Calibration s	103	162	185	191.44973	1.6149112	0.5472222	0.4432	0.7255	0.00016		
8	Test sample	125	180	171								
9	Test sample	111	170	166								
10												
11												
12												
13												
14		Linear adjustment y=a*x+b					Cuadratic adjustment y=a*x^2+b*x+c					
15	Name	Result for te	Result for te	R2	a	b	Result for te	Result for te	R2	a	b	c
16	LOG(C) vs Δ	5.113E-05	0.000131	0.9861861	0.0234421	-8.323193	9.236E-05	0.0001754	0.992224	4.558E-05	-0.000501	-5.296758
17	LOG(C) vs R	0.0001142	0.0002678	0.9882274	-0.82079	-2.475656	0.0001494	0.0002941	0.9919368	0.0427639	-0.785585	-2.558304
18	LOG(C) vs V	0.0003242	0.0010668	0.9753683	-13.19547	5.8254995	0.0003349	0.0010308	0.9760249	4.2786621	-18.32648	7.3295986
19	LOG(C) vs B	0.0009479	0.0017201	0.9753218	-0.051758	5.8273169	0.0009207	0.0016317	0.9759795	6.588E-05	-0.071904	7.3333083
20	C vs B	-0.009582	0.0206148	0.5812949	-0.006039	1.023157	-0.056187	-0.063872	0.9726217	0.0002429	-0.080318	6.5757256
21	C vs V	-0.06395	-0.003594	0.5812996	-1.539704	1.0229268	-0.011722	-0.058672	0.9725967	15.787384	-20.47207	6.5727438
22	C vs H	-0.245806	-0.185525	0.7601109	3.6168753	-1.953775	0.2872917	0.1666764	0.9699558	53.635122	-58.78623	16.087155
23	LOG(C) vs G	0.0001451	0.0002676	0.9562225	-0.026583	0.9465553	6.004E-05	0.0001552	0.9697563	-0.000104	-0.004713	0.0088691
24	C vs G	-0.121994	-0.088407	0.6682044	-0.003359	0.4825767	0.1446951	0.0762202	0.9551252	7.265E-05	-0.018579	1.1351442
25	C vs RGB	-0.105516	-0.064541	0.5302376	-0.090873	0.0568628	0.0747478	-0.00171	0.9169991	0.0659996	-0.03654	-0.070692
26	C vs ΔRGB	-0.139059	-0.09474	0.5076195	0.002542	-0.576268	0.1697722	0.0575048	0.8898045	5.481E-05	-0.02625	3.0630709
27	LOG(C) vs R	1.425E-05	5.12E-05	0.7997532	-0.039686	0.1144237	0.0002074	0.0001719	0.8263148	0.0004346	-0.096743	1.6186036
28	LOG(C) vs H	5.516E-05	0.0001325	0.6924063	22.839092	-15.04348	0.0010484	0.0009273	0.7199947	128.66673	-126.8614	28.235395
29	LOG(C) vs S	1.01E-05	2.251E-05	0.6965718	8.3879201	-7.559039	2.03E-06	7.397E-06	0.7025988	-9.024957	19.420323	-10.78442
30	C vs R	-0.114695	-0.071235	0.2142034	-0.003104	0.2733459	0.2057239	0.07364	0.3024425	0.0001197	-0.018823	0.6877272
31	C vs S	-0.085423	-0.063031	0.1261654	0.5395581	-0.250312	-0.230752	-0.163851	0.1376438	-1.882501	2.8407882	-0.923088

Fig 4: This table shows the statistical analysis of reproducibility by smartphone.

Studying the Detection Limit for the RGB Method:

The detection limit represents the least analytical quantity in the substance that can be detected when measuring the detection limit of the chromatic density (RGB) method. Results and the (0.000032 M) concentration was the detection limit value of the RGB method with the smartphone because it is the lowest value that has been detected by this method in practice, as shown in **(**Figs. 5 and 6**).**

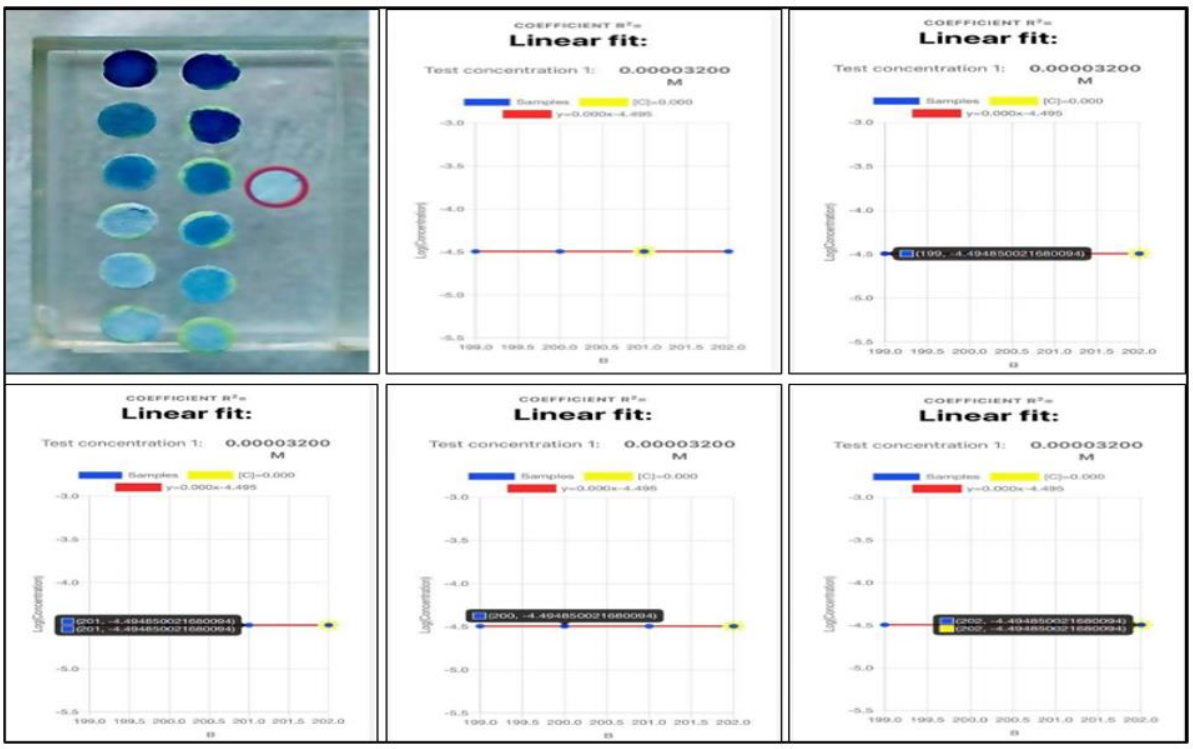

Fig 5: shows the images taken to study the concentrations of the detection limit.

	A	B	C	D	E	F	G	H	I	J
1	Name	R	G	B	ΔRGB	RGB	H	S	V	Concentration
2	Calibration s	131	183	202	152.869225	1.47311188	0.54444444	0.3515	0.7922	0.000032
3	Calibration s	131	181	199	154.880599	-1.9184025	0.54444444	0.3417	0.7804	0.000032
4	Calibration s	130	182	201	154.499191	0.20593901	0.54444444	0.3532	0.7882	0.000032
5	Calibration s	131	182	201	153.691249	0.07978387	0.54444444	0.3483	0.7882	0.000032
6	Calibration s	131	181	200	154.521843	-1.3135441	0.54722222	0.345	0.7843	0.000032
7	Test sample	131	183	202						
8										
9										
10										
11										
12		Linear adjustment y=a*x+b			Cuadratic adjustment y=a*x^2+b*x+c					
13	Name	Result for te	R2	a	b	Result for te	R2	a	b	c
14	C vs R	3.2E-05	null	3.47E-18	3.2E-05	-9.303E-05	null	-5.59E-09	9.54E-07	-0.0001221
15	C vs G	0.000032	null	0	0.000032	3.2E-05	null	-8.88E-16	4.55E-13	3.2E-05
16	C vs B	3.2E-05	null	1.07E-18	3.2E-05	3.2E-05	null	2.22E-16	0	3.2E-05
17	C vs H	3.2E-05	null	-1.76E-15	3.2E-05	3.9684E-05	null	9.1553E-05	-6.104E-05	4.5776E-05
18	C vs S	0.000032	null	0	0.000032	3.2E-05	null	3.64E-12	1.82E-12	3.2E-05
19	C vs V	3.2E-05	null	2.70E-16	3.2E-05	3.2E-05	null	-4.37E-11	5.82E-11	3.2E-05
20	C vs ΔRGB	0.000032	null	0	0.000032	3.2E-05	null	-8.88E-16	2.27E-13	3.2E-05
21	LOG(C) vs R	0.000032	null	0	-4.49485	237137371	null	0	0.125	-8
22	LOG(C) vs G	0.000032	null	0	-4.49485	3.1999E-05	null	3.49E-10	-1.79E-07	-4.4948425
23	LOG(C) vs B	0.000032	null	0	-4.49485	3.2E-05	null	-5.82E-11	1.49E-08	-4.4948511
24	LOG(C) vs H	3.2E-05	null	-2.30E-10	-4.49485	1.88E-07	null	-8	-8	0
25	LOG(C) vs S	0.000032	null	0	-4.49485	3.2E-05	null	-1.431E-06	7.15E-07	-4.4948502
26	LOG(C) vs V	0.000032	null	0	-4.49485	3.2E-05	null	3.8147E-06	0	-4.4948473
27	LOG(C) vs Δ	0.000032	null	0	-4.49485	3.2001E-05	null	2.33E-10	0	-4.4948425
28	C vs RGB	0.000032	null	-7.54E-22	0.000032	0.000032	null	0	3.39E-21	0.000032
29	LOG(C) vs R	0.000032	null	1.98E-16	-4.49485	3.2E-05	null	8.88E-16	4.44E-16	-4.49485

Fig 6: This table shows the statistical analysis of the detection limit value of the method by smartphone.

Standard Calibration Curve Of The Chromatic Intensity Method (RGB) by Smartphone:

Under the optimal conditions studied, the calibration curve was obtained for the concentration of phenol in the samples. (Fig 7) is a graph showing the linearity of the application of (Beer Lambert's Law) within the range (0.00016-0.5 M) between LOG (Concentration) and Blue color (B) as shown in Table 2, the linear graph has a correlation coefficient (R^2) equal to (0.9753), and the value of the relative standard deviation coefficient (RSD%) for the concentration of (0.02) M for ten repeated assays is (0%), and the value of Recovery % is equal to (100%).

Table 2: shows the results of the calibration curve for the RGB method.

No	LOG Concentration M	B
1	0.5	118
2	0.1	136
3	0.02	146
4	0.004	152
5	0.0008	176
6	0.00016	185

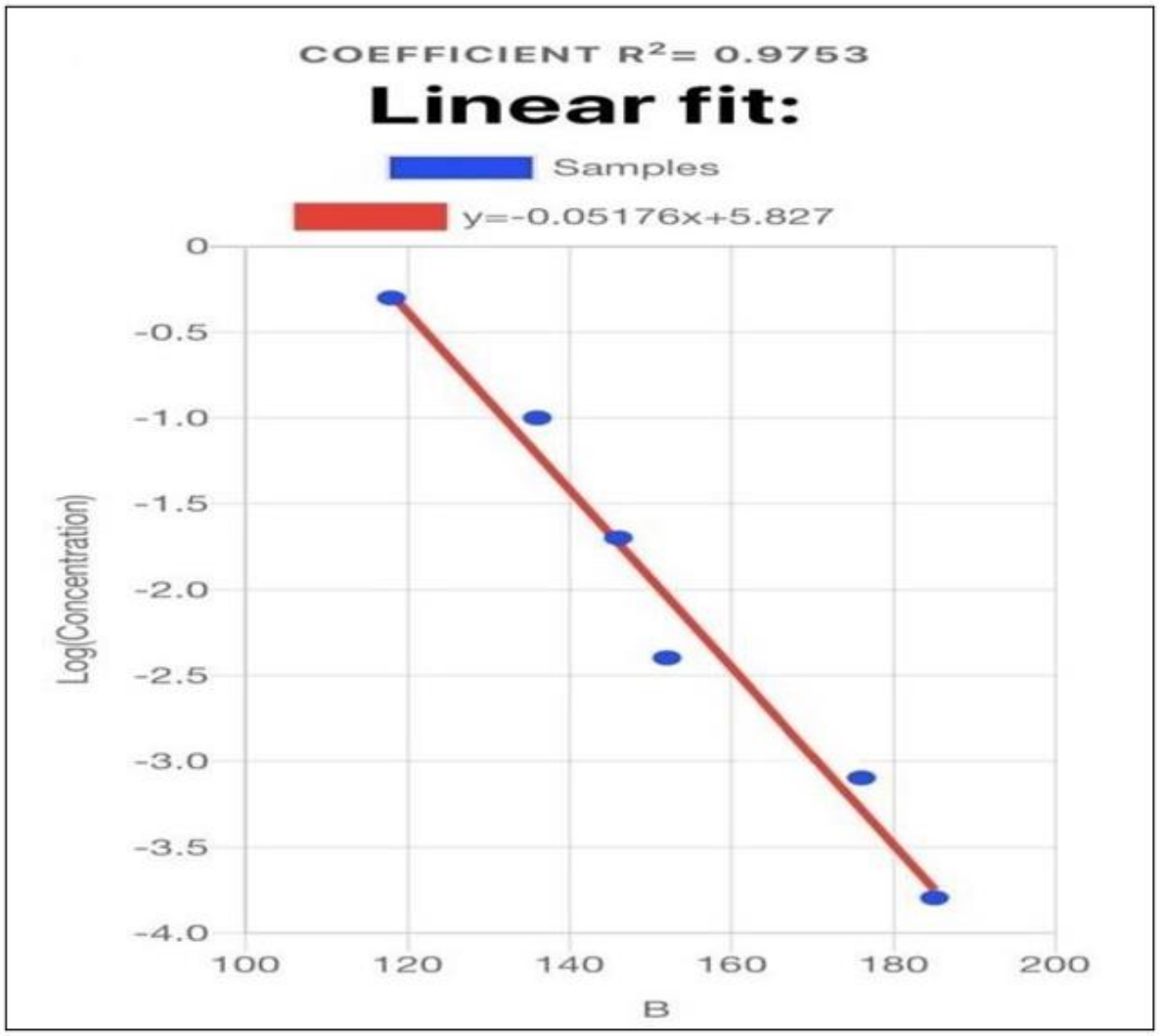

Fig 7: Graph of calibration of phenol concentrations with a blue color value using the (RGB) method.

Figure 8 shows the color gradient of the concentrations. Table 3 shows the antibacterial activity of phenol samples, and Table 4 shows the optimal conditions for the method

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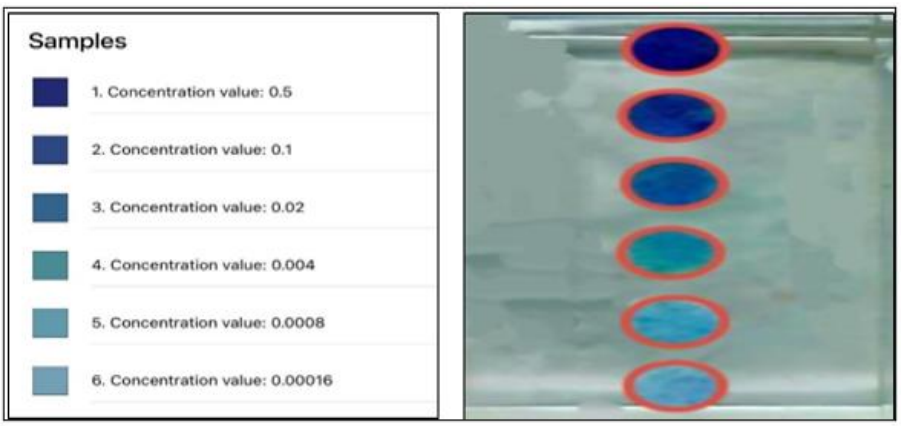

Fig 8: The color gradient of the concentrations of the calibration curve measured by the chromatic density method (RGB) by smartphone.

Table 3: Antibacterial activity of phenol samples (inhibition region, mm), including three separate phenol samples Oral suspension toward four types of multidrug-resistant bacteria. Staphylococcus aureus, Enterococcus faecalis, Enterobacter cloacae and Proteus mirabilis.

No. of Samples	*S. aureus*	*E. faecalis*	*E. cloacae*	*P. mirabilis*
Sample 1	26	28	25	23
Sample 2	29	22	24	26
Sample 3	27	25	24	27

Table 4: Optimal conditions for the (RGB) chromatic intensity method.

Analytical values	Values
Limits of Applicability of the (Beer-Lambert) Law (M)	0.00016-0.5
(Detection limit)((M)	0.000032
(Recovery%) of congruence for a concentration of (0.02 M) for10 assays	100 %
(RSD%) of congruence for concentration (0.02 M) for 10 assays	0 %
Correlation coefficient (R^2)	0.9753
(Slope)	0.05176

Accuracy of RGB Method:

In order to demonstrate the accuracy and sensitivity of the RGB method in determining the concentration of phenol using (µPAD) with a smartphone. Analytical sample number (2) was prepared with a concentration of (0.0026 M) and a concentration of (0.0053 M), and the samples were measured by method the traditional method using the (spectrophotometer)(Ilaybi, Hussien *et al.*, 2023), and the chromatic density method (RGB) using the (µPAD). The average recovery was three. Measurements in the range (99.9 –100.1 %). Good results were obtained, as shown in Table 5, which clearly indicates that the RGB method is very suitable as a new method for the determination of phenol.

Table 5: the results of the examination of analytical samples by the traditional method and the RGB method with a smartphone.

Analytical samples M	Traditional method M	*RGB Method M	RSD% Traditional Method	RSD% (RGB) mothed	Recovery % Traditional method	Recovery % (RGB) mothed
0.0026	0.0027	0.0025	0.62%	0.4%	98%	99.9%
0.0053	0.0052	0.0061	0.53%	0.6%	98.8%	100.1%

* The average of three assays was extracted for both methods.

DISCUSSION

After completing the design. The method was used to measure the concentration of phenol in the prepared samples and analyze the basic colors (RGB) using a smartphone. After the analysis, the following results were obtained: The calibration curve for this method was in the range of M (0.00016–0.5), the correlation coefficient (R2) was equal to (0.9753), the limit of detection was in the amount of (0.000032) M, the relative standard deviation (RSD%) for the concentration was (0.02) M, was equal to (0 %), and the recovery value (Recovery%) was equal to (100%).

CONCLUSION

A new form of the microfluidic paper-based analytical device (µPAD) is designed with the smartphone as a sensor. The method was used to measure the concentration of

phenol in the prepared samples and analyze the basic colors (RGB) using a smartphone. Considering the (µPAD) method for the determination of total phenol for the prepared samples and measuring the biological activity as a new method compared to the traditional measurement methods, it is characterized as an easy-to-use and low-cost method, and it can be used in work sites far from the laboratory.

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Declaration of Interests: The authors declare that they have no conflict of interest.

funding: none

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