A New Paper-Based Microfluidic Design (μPAD) for the Determination of Total Phenols in Wastewater Using a Smartphone Sensor as a Detector

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Abstract

Phenols are well-known noxious compounds, that are often found in various water sources. In this research, a new method was used to determine the total phenol by using microfluidic paper-based (μPAD), with a smartphone Sensor. It is portable, inexpensive, and environmentally friendly. The paper was prepared using filter paper by the 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 was (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 %). The method was successfully applied for the determination of total phenols in wastewater.

Keywords: Phenol samples, microfluidic, smartphone, (Red, Green, Blue) (RGB), wastewater.

Introduction

Phenol is an aromatic chemical compound composed of a functional hydroxyl group (-OH). These phenol derivatives, which are abundantly distributed in the environment, are employed in the production of dyes, polymers, pharmaceuticals, and other organic compounds. Several different routes, including ingestion, inhalation, and skin absorption, can cause phenol poisoning. People who receive excessive dosages of phenol risk dying[1]. Phenols are classified as priority pollutants because of their toxicity and persistence in the environment[2].Phenolic compounds are present in water bodies as a result of the discharge of contaminated wastewater from industrial, agricultural, and domestic activities. They might also occur as result of a natural occurrence. These substances are known poisons that can severely and permanently harm both people and animals. They act as carcinogens even at low concentrations and harm the liver and red blood cells. Several methods have been used for the determination of phenolic compounds, such as gas chromatography-mass spectrometry[2], and HPLC[3].

Within paper-based assays, paper-based microanalyzers (μPADs) have gained significant attention as a promising analytical platform because they have many advantages such as (inexpensive production, portability, minimal reagent consumption, and liquid flow via capillary forces without external driving forces)[4],[5]. Colorimetric detection on microfluidic paper-based analytical devices (μPAD) has become a powerful alternative: colorimetric reactions are fairly easy to implement on paper; furthermore, they are compatible with easy-to-use imaging devices for analyzing samples and recording results on-site[6],[7].

The integration of cell phones with sensors is a potential method for achieving quick, portable, and simple detection[8]. Further integration of μPAD with a smartphone app provides an excellent strategy for smart on-site analysis[9]. Making sensors compatible with smartphones is becoming more common in the industry in order to take advantage of its mobility, and connectivity[10]. 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[11], sensors of environmental[12], and detection of enzymatic urea hydrolysis in microfluidic systems[13]. In this study, a new method was included for total phenol determination in wastewater by basic color analysis (RGB) of samples taken by a smartphone.

Materials and Methods

Chemicals: all solutions of chemicals used in water were prepared according to 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[14].

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

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

This 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 Fig. 1.

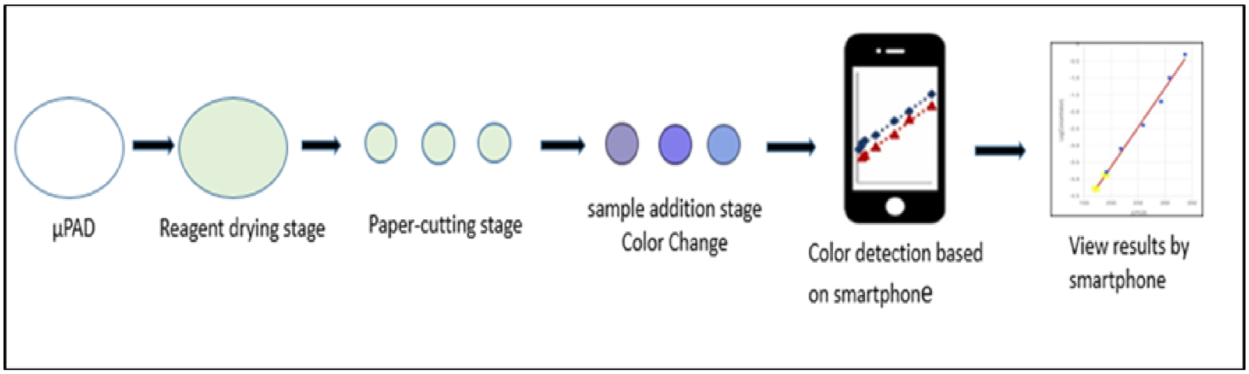

Figure (1) : Show using a smartphone with μPAD.

Results and Discussion Chemistry.

design of (μPAD) and the 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 as a phenol detector.

5- 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.

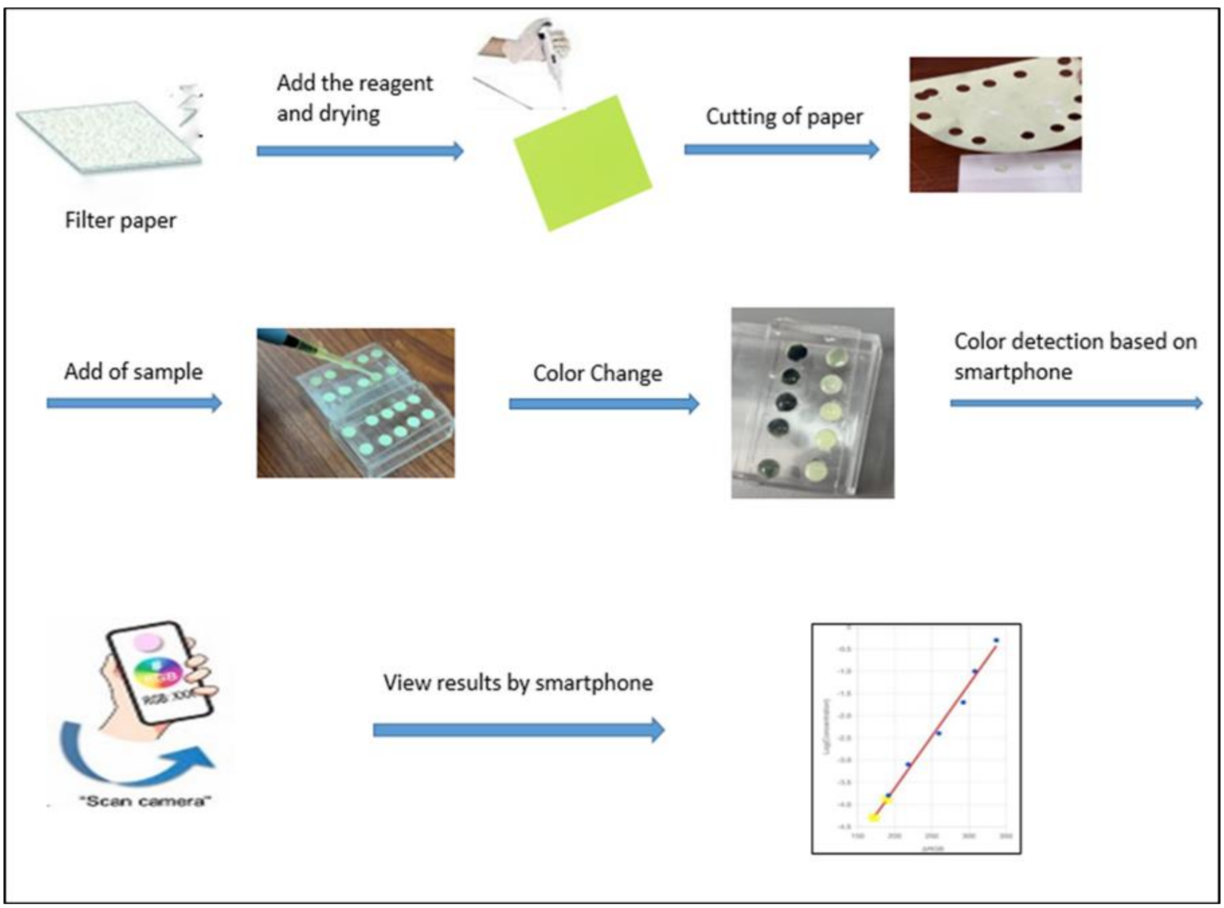

Figure (2) : The design stages of a microfluidic paper-based analytical device µPAD.

The Reproducibility 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 Fig. 3 below.

Table 1: the congruence results of the RGB method.

Value B		Concentrations measured using the 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		

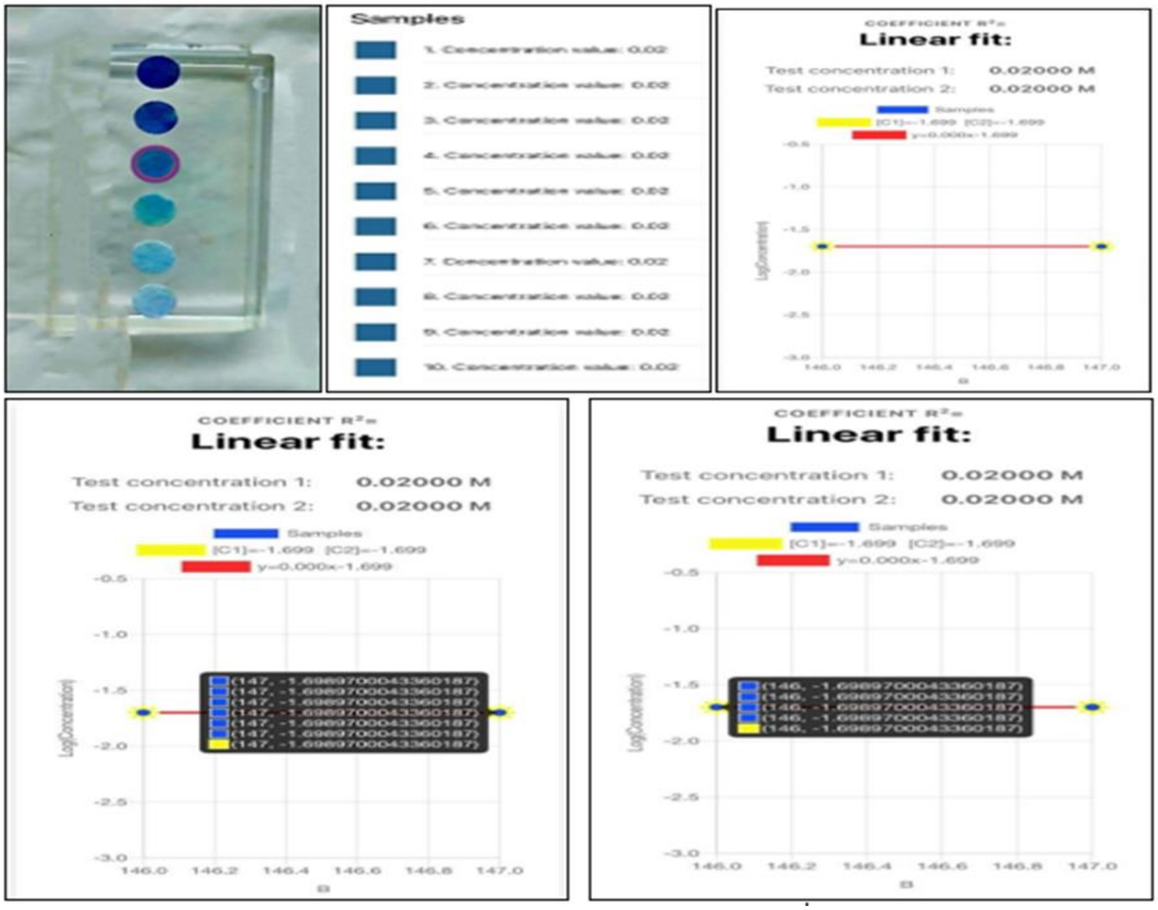

Figure (3) : Pictures of the reproducibility study of the concentration (0.02).

	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	31	102	147	291.974314	0.64701636	0.56388889	0.7891	0.5765	0.02		
3	Calibration s	29	101	146	294.402785	-2.0176973	0.56388889	0.8014	0.5725	0.02		
4	Calibration s	31	102	147	291.974314	0.64701636	0.56388889	0.7891	0.5765	0.02		
5	Calibration s	33	103	147	289.91723	2.24052459	0.56388889	0.7755	0.5765	0.02		
6	Calibration s	31	102	147	291.974314	0.64701636	0.56388889	0.7891	0.5765	0.02		
7	Calibration s	33	103	147	289.91723	2.24052459	0.56388889	0.7755	0.5765	0.02		
8	Calibration s	29	101	146	294.402785	-2.0176973	0.56388889	0.8014	0.5725	0.02		
9	Calibration s	29	101	146	294.402785	-2.0176973	0.56388889	0.8014	0.5725	0.02		
10	Calibration s	32	102	147	291.20783	1.03643995	0.56388889	0.7823	0.5765	0.02		
11	Calibration s	29	101	146	294.402785	-2.0176973	0.56388889	0.8014	0.5725	0.02		
12	Test sample	31	102	146								
13	Test sample	32	102	147								
14												
15												
16												
17												
18		Linear adjustment y=a*x+b					Cuadratic adjustment y=a*x^2+b*x+c					
19	Name	Result for te	Result for te	R2	a	b	Result for te	Result for te	R2	a	b	c
20	C vs G	0.02	0.02	0	0	0.02	0.02	0.02	-1.305E+18	-1.14E-13	0	0.02000001
21	C vs B	0.02	0.02	0	0	0.02	0.45025635	0.45472717	-1.56E+34	1.5259E-05	0	0.125
22	C vs V	0.02	0.02	0	0	0.02	0.01936699	0.01934837	-3.45E+28	-0.03125	0.03125	0.01171875
23	C vs ΔRGB	0.02	0.02	0	0	0.02	0.02000001	0.02000001	-4.163E+18	5.68E-14	-2.91E-11	0.02000001
24	C vs RGB	0.02	0.02	0	-4.85E-19	0.02	0.02	0.02	-8	0	-4.34E-19	0.02
25	LOG(C) vs R	0.02	0.02	null	-3.77E-15	-1.69897	0.02	0.02	null	2.84E-13	-1.82E-11	-1.69897
26	LOG(C) vs G	0.02	0.02	null	0	-1.69897	0.02	0.02	null	1.46E-11	0	-1.6989702
27	LOG(C) vs B	0.02	0.02	null	0	-1.69897	1.24E-47	5.00E-48	null	-0.0004883	-0.25	0
28	LOG(C) vs H	0.02	0.02	null	-2.6666667	-0.1952663	0.02859134	0.02859134	null	-8	0	1
29	LOG(C) vs S	0.02	0.02	null	-2.85E-12	-1.69897	0.02	0.02	null	-2.98E-08	1.19E-07	-1.69897
30	LOG(C) vs V	0.02	0.02	null	-3.70E-11	-1.69897	0.06725991	0.06797547	null	1	0	-1.5
31	LOG(C) vs ΔI	0.02	0.02	null	2.40E-14	-1.69897	0.01999998	0.01999998	null	-1.09E-11	7.45E-09	-1.6989717
32	LOG(C) vs R(	0.02	0.02	null	-3.10E-17	-1.69897	0.02	0.02	null	0	-5.55E-17	-1.69897
33	C vs H	0.02	0.02	-3	0.02083333	0.00825231	0.02868393	0.02868393	-6.26E+30	0.0625	0.015625	0
34	C vs R	0.02	0.02	-176.5	2.95E-17	0.02	0.02	0.02	-1.452E+11	-2.66E-15	1.14E-13	0.02
35	C vs S	0.02	0.02	-4096.9	-2.22E-14	0.02	0.02	0.02	-1.197E+14	9.31E-10	-1.40E-09	0.02

Figure (4) : This table shows the statistical analysis of reproducibility using a 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 Fig.5 and Fig. 6.

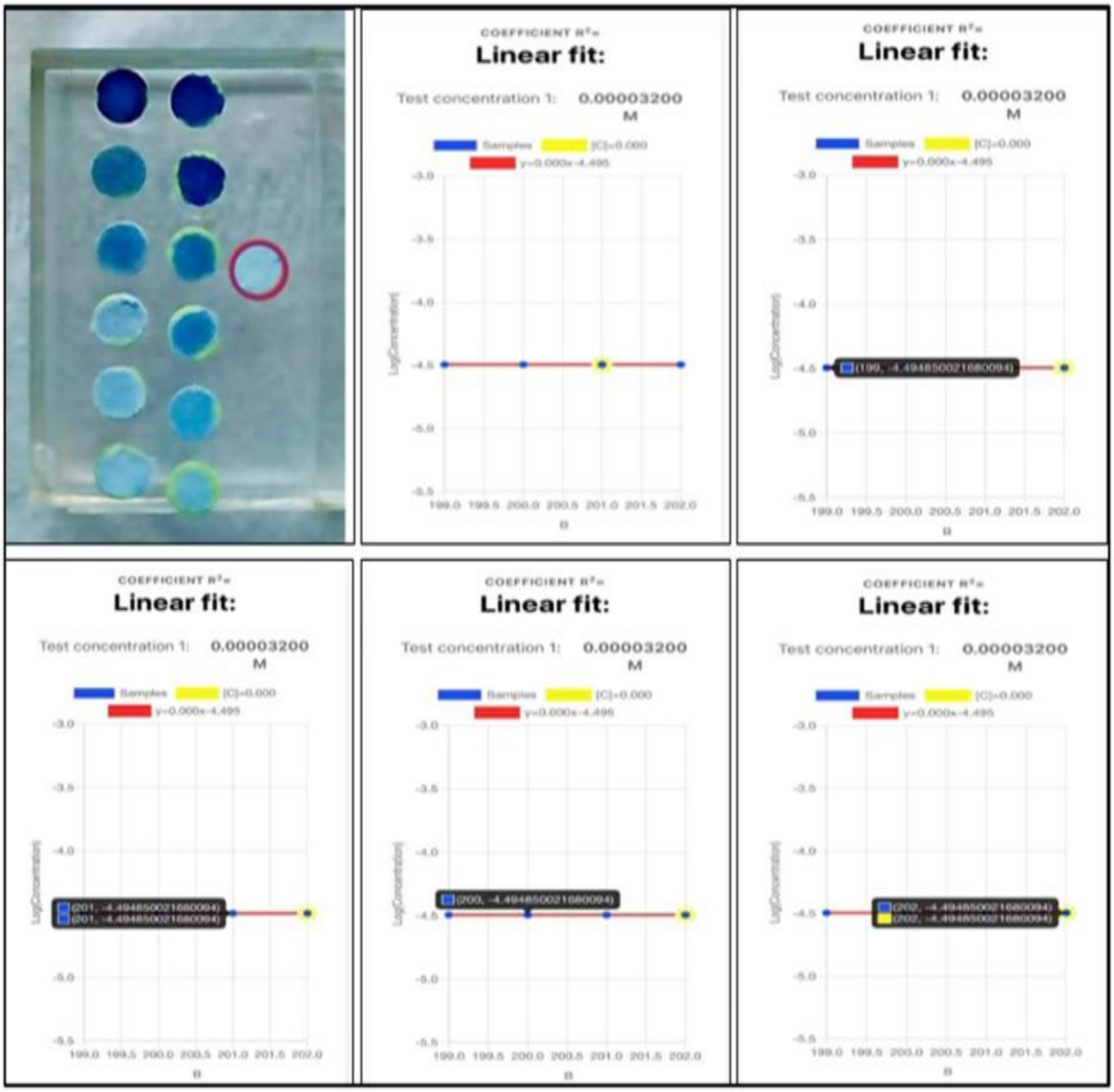

Figure (5) : shows the images taken to study the concentrations of the detection limit.

	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

Figure (6) : This table shows the statistical analysis of the detection limit value of the method using a 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: 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

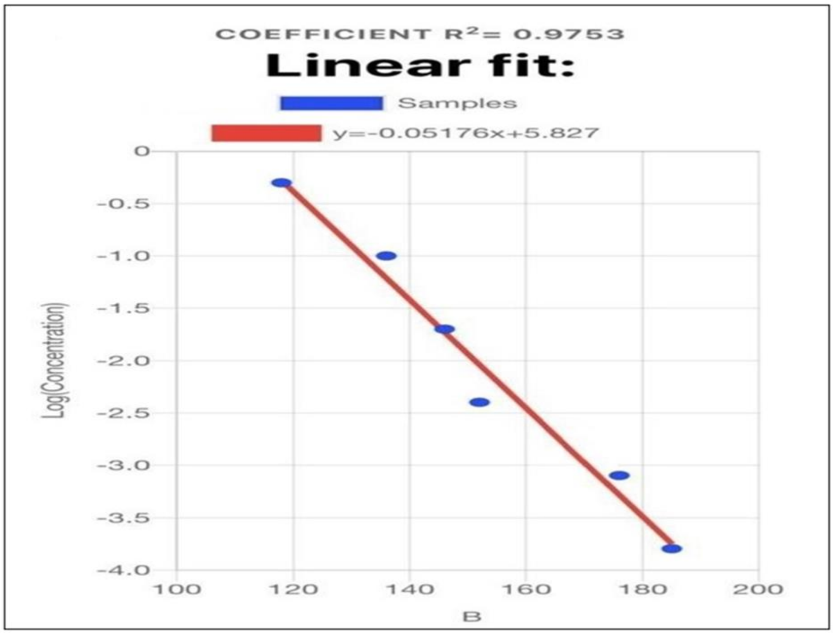

Figure (7) : Graph of calibration of phenol concentrations with a blue value using the (RGB) method.

Fig. 8 shows the color gradient of the concentrations, Fig. 9 shows the statistical analysis of the standard calibration curve for the method, and Table 3 shows the optimal conditions for the method.

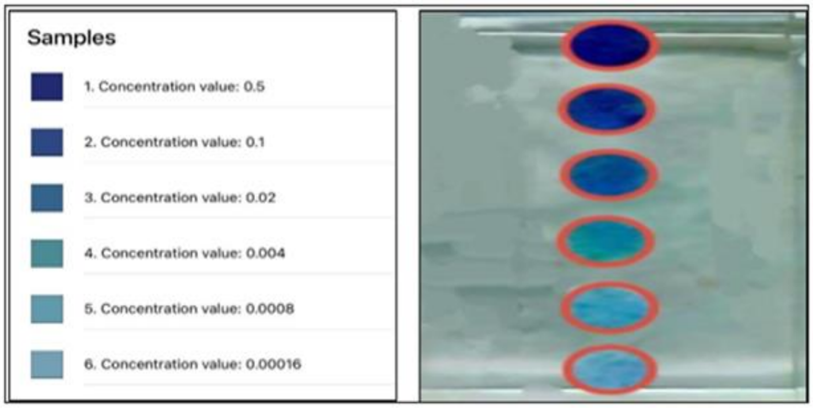

Figure (8) : The color gradient of the concentrations of the calibration curve measured using the chromatic density method (RGB) using smartphone.

	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

Figure (9) : This table shows the statistical analysis of the standard calibration curve for the method by smartphone.

Table 3: 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

Application of the (RGB) method to measure total phenols in wastewater

A wastewater sample was obtained from an environmental monitoring station. The sample was measured by the spectral method using the flow injection technique[15] and the (RGB) method. The value of (RSD%) for the measured sample (0.58 %), which is a good result compared to the above method for measuring the amount of total phenol in wastewater.

Conclusion

A new form of microfluidic paper-based analytical device (μPAD) is designed with a smartphone as a sensor. The method was used to measure the concentration of total phenols in wastewater samples and analyze the basic colors (RGB) using a smartphone 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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