ENVIRONMENTAL ANALYTICAL CHEMISTRY

A Rhodamine B–Aluminium Chloride Paper Strip for RGB-Based Quantitative Colorimetric Detection of Fluoride

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Published November 2026

Abstract

A rapid, low-cost optical method based on digital RGB analysis is developed for the quantitative determination of fluoride (F^-) in aqueous media, as a portable alternative to ion-selective electrode and spectrophotometric instrumentation. Image processing converts the colour of a spot into digital R, G and B values, enabling on-site screening around the WHO drinking water guideline value of 1.5 mg L^{-1}. The paper-based analytical device (PAD) was prepared by immobilizing Rhodamine B (RhB) and aluminium chloride ($AlCl_3$) on Whatman No. 40 filter paper by a simple dip-and-dry procedure. Sensing is proposed to rely on competitive ligand exchange, in which fluoride removes Al^{3+} from the pink $[RhB–Al]^{3+}$ species as $[AlF_6]^{3-}$ and bleaches the spot in proportion to the fluoride concentration. Under the optimized conditions (0.125% w/v RhB, 1% $AlCl_3$, 1:1 ratio, four sample drops of 0.72 µL, pH 7, 40 °C and 5 min) the green-channel effective intensity magnitude ($|A_g|$) was used as the analytical signal. Over 0.05–1000 ppm, $|A_g|$ followed a single log-linear relationship ($|A_g| = 0.0289 \log C + 0.255$, $R^2 = 0.981$) that can also be described by two linear segments: a steeper one at 0.05–0.5 ppm (three points) and a gentler one at 0.5–1000 ppm ($R^2 = 0.984$). The practical detection limit of 0.05 ppm (about 2.6 µM) is 30 times lower than the WHO guideline value and lower than that of a reported wax-patterned microfluidic paper device. Ten coexisting ions (five anions and five cations) did not interfere up to 1000 ppm.

Keywords: paper-based analytical device; fluoride; Rhodamine B; aluminium chloride; colorimetric sensor; RGB analysis

1 Introduction

Paper-based analytical devices (PADs) are lightweight, inexpensive and equipment-free platforms that give a colour read-out suitable for naked-eye screening or for digital quantification with a scanner or smartphone camera [1,2]. Coupling paper spot tests with digital image analysis turns colour changes into analytical signals, because the image of a spot can be split into red (R), green (G) and blue (B) intensities [3–5].

Fluoride in drinking water has a narrow margin between dental protection (about 1.0 mg L^{-1}) and harm. Long-term intake above the World Health Organization (WHO) guideline value of 1.5 mg L^{-1} causes dental fluorosis, and higher levels lead to skeletal fluorosis [6,7]. India sets an acceptable limit of 1.0 mg L^{-1} and a permissible limit of 1.5 mg L^{-1} where no alternative source is available [8]. Because groundwater contamination mainly affects rural communities without laboratory facilities, field-deployable methods that can measure reliably below 1.0–1.5 mg L^{-1} are needed.

Fluoride is conventionally determined with an ion-selective electrode (ISE), by UV–visible spectrophotometry or by ion chromatography [7], which require instruments, calibration buffers and trained personnel. A wax-patterned microfluidic paper device with smartphone read-out has been reported for fluoride [9], but the hydrophobic barrier printing adds a fabrication step and its linear range is a narrow window (0.76–4.75 mg L^{-1}).

Rhodamine derivatives open their ring when Al^{3+} binds, which gives intensely pink species [10,11]. Fluoride binds Al^{3+} much more strongly than the dye does, so it can remove the metal and reverse the colour; this has been used for sequential Al^{3+} and F^- sensing in solution [11]. Here we transfer this chemistry to filter paper using commercial Rhodamine B and $AlCl_3$. The strip (a) needs only two inexpensive, commercially available reagents, with no synthesis of a probe or preparation of nanomaterials (b) is made by simple dip-and-dry steps without wax or inkjet patterning and (c) works at neutral pH and mild temperature (pH 7, 40 °C), with a response over more than four orders of magnitude of concentration (0.05–1000 ppm) and a detection limit of 0.05 ppm.

2 Experimental section

2.1 Apparatus and software

ImageJ software (NIH) was used to read the mean R, G and B values from the central region of each spot. A smartphone camera under uniform diffuse illumination was used to photograph the strips, and a calibrated micropipette to apply the solutions.

2.2 Chemicals and reagents

All chemicals were of analytical grade and were used without further purification. Double-distilled water was used throughout and Whatman No. 40 filter paper was the support for the reagents.

Fluoride solutions: A 1000 ppm fluoride stock solution was prepared by dissolving 22.1 mg of sodium fluoride (NaF) in 10 mL of distilled water and was used for the optimization of the conditions. Standard solutions of 100, 10, 1, 0.5, 0.1 and 0.05 ppm (mg L^{-1}) were prepared from it by serial dilution.

Rhodamine B solution: A 1% (w/v) Rhodamine B solution (microscopy grade) was prepared by dissolving 0.100 g in 10 mL of distilled water and was diluted successively by half to give twelve solutions from 1% to 0.00049% (w/v). The 0.125% (w/v) solution (0.0125 g in 10 mL) was used for the optimized strip.

Aluminium chloride solution: A 1% (w/v) $AlCl_3$ solution was prepared by dissolving 0.100 g in 10 mL of distilled water, 2% and 0.5% (w/v) solutions were prepared in the same way for the concentration study.

Coexisting ions and buffers: Solutions (1000 ppm of the ion each) of PO_4^{3-}, Cl^-, SO_4^{2-}, HCO_3^-, NO_3^-, Ca^{2+}, Mg^{2+}, Fe^{3+}, Cu^{2+} and Pb^{2+}, were prepared in distilled water from the anhydrous analytical-grade salts. Buffer solutions of pH 4, 7 and 9 were prepared from standard buffer tablets.

2.3 Fabrication and RGB analysis procedure

Preparation of the paper strip. Strips of Whatman No. 40 filter paper were immersed for 10 s in a 1:1 (v/v) mixture of 0.125% (w/v) Rhodamine B and 1% (w/v) $AlCl_3$ and dried at 40 °C to give uniform pink strips.

Mode of operation and RGB quantification. Four successive 0.72 µL drops (2.88 µL in total) of the standard or sample solution were applied to the strip, which was kept at 40 °C for 5 min. The spots were photographed and analysed in ImageJ against a blank strip treated with water (R = 235, G = 92, B = 230). For the optimization of the number of drops, pH, temperature and time, and for the calibration, this common water blank was used. For the series that change the reagent loading (Rhodamine B concentration, $AlCl_3$ concentration and RhB:$AlCl_3$ ratio) each strip was referenced to its own untreated blank of the same composition. The effective intensity of each channel was calculated as [5]:

$$A_r = -\log\left(R_s/R_b\right) \quad (1)$$

$$A_g = -\log\left(G_s/G_b\right) \quad (2)$$

$$A_b = -\log\left(B_s/B_b\right) \quad (3)$$

where R_s, G_s, B_s and R_b, G_b, B_b are the mean red, green and blue values of the sample spot and the blank, respectively. Fluoride bleaches the spot, so the sample values are higher than those of the blank and A is negative, its sign only shows the direction of the change. The magnitudes $|A_r|$, $|A_g|$ and $|A_b|$ are therefore used throughout the text, figures and tables. A channel value of zero in the sample gives an infinite A and is reported as a complete response.

3 Results and discussion

3.1 Sensing mechanism and RGB channel selectivity

The colour change is attributed to two equilibria on the cellulose matrix. Al^{3+} is proposed to open the ring of Rhodamine B and give the pink $[RhB–Al]^{3+}$ species (equation 4), by analogy with the Al^{3+}-induced ring opening reported for rhodamine sensors [10,11]. Fluoride competes for Al^{3+} because of the strength of the Al–F bond and removes it as hexafluoroaluminate $[AlF_6]^{3-}$, so the spot loses colour (equation 5). Charges on the dye are left out for simplicity. Partial species such as AlF^{2+} and AlF_2^{+} may form at lower fluoride levels. This mechanism is proposed from the literature and the observed colour changes, and was not confirmed spectroscopically in this work.

$$\mathrm{RhB(pale)} + Al^{3+} \rightleftharpoons \left[\mathrm{RhB-Al}\right]^{3+}\mathrm{(pink)} \quad (4)$$

$$\left[\mathrm{RhB-Al}\right]^{3+} + 6F^{-} \rightarrow \mathrm{RhB(pale)} + \left[AlF_6\right]^{3-} \quad (5)$$

In the calibration, pH and temperature series the red channel hardly changed ($|A_r|$ = 0.035) and the blue channel changed little ($|A_b| \leq 0.045$), whereas the green channel carried most of the response, with $|A_g|$ up to 2.09 in the reagent optimization. A pink dye absorbs green light, so loss of the colour increases the green reflectance, which is why $|A_g|$ was used as the analytical signal.

3.2 Optimization of assay parameters

Reagent concentrations and ratio. With $AlCl_3$ fixed at 1% (w/v), Rhodamine B was varied from 1% to 0.00049% (w/v) (Fig. 1a). The green response was high from 1% to 0.125% ($|A_g|$ = 1.415, 2.086, 1.465 and 1.898 at 1, 0.5, 0.25 and 0.125%), fell steeply to 0.461 at 0.0625% and then declined gradually to 0.092 at 0.00049%. The red and blue responses were much smaller ($|A_r|$ = 0.239 and $|A_b|$ = 0.303 at 1%, 0.088 and 0.120 at 0.125%, and below 0.01 and 0.06, respectively, at 0.0625% and lower). The 0.125% solution was chosen because it kept the response close to the highest values at a

lower dye loading. $AlCl_3$ was varied at 0.5%, 1% and 2% (w/v) with the selected 0.125% Rhodamine B (Fig. 1b). 1% $AlCl_3$ gave the highest response ($|A_g|$ = 0.223, against 0.130 at 2% and 0.129 at 0.5%), while the red and blue responses remained small at all three concentrations ($|A_r| \leq 0.009$ and $|A_b| \leq 0.030$). Of the RhB:$AlCl_3$ volume ratios 2:1, 1:1 and 1:2 (Fig. 2), 1:1 gave the largest response in all three channels ($|A_r|$ = 0.099 and $|A_b|$ = 0.101, against 0.012 and 0.076 at 2:1 and 0.085 and 0.081 at 1:2). In the green channel the G value of the fluoride spot fell to zero at 1:1, which is taken as a complete response, against $|A_g|$ = 0.439 at 2:1 and 0.345 at 1:2.

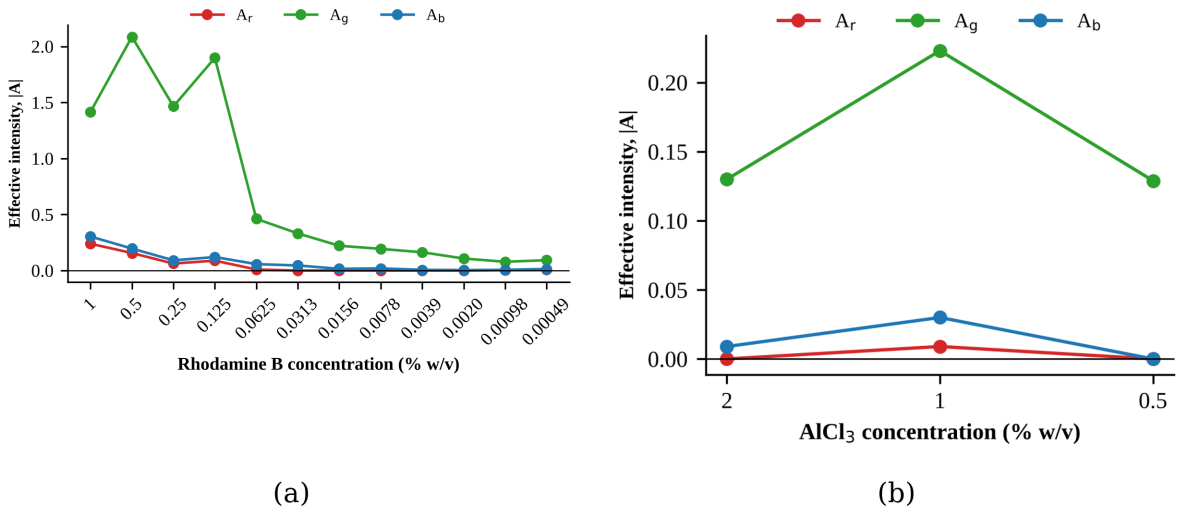

Figure 1. Effect of (a) Rhodamine B concentration (1% AlCl3) and (b) AlCl3 concentration (0.125% Rhodamine B) on the effective intensities.

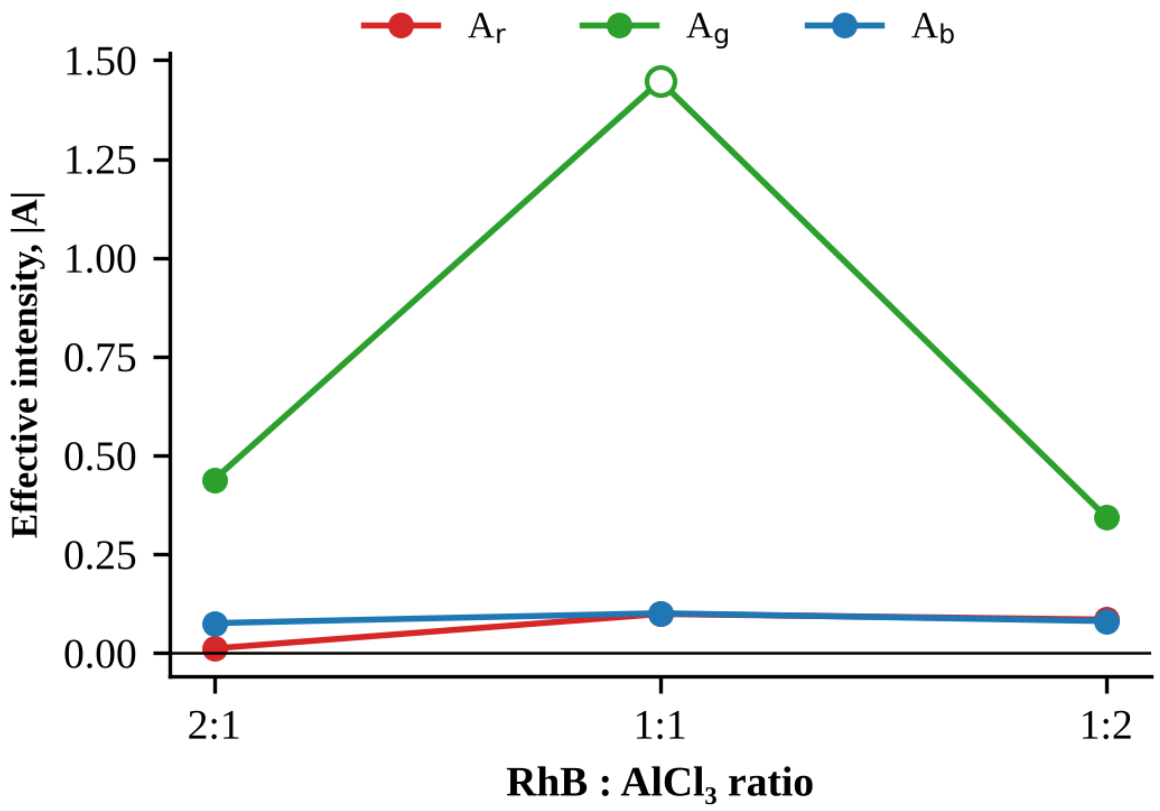

Figure 2. Effect of the Rhodamine B to AlCl3 ratio on the effective intensities (open marker: sample G value zero, response complete).

Sample volume. One to eight drops (0.72 µL each) of fluoride solution were applied (Fig. 3). The green response was highest at four drops ($|A_g|$ = 1.362) and was lower at one, two, three, five, six, seven and eight drops (1.265, 0.517, 0.760, 1.061, 0.733, 0.472 and 0.445, respectively). The red and blue responses were also highest at four drops ($|A_r|$ = 0.083, $|A_b|$ = 0.121; ranges 0.017–0.057 and 0.088–0.109 for the other drop numbers). The lower response with more drops may be due to spreading and dilution of the reagent over a larger spot area.

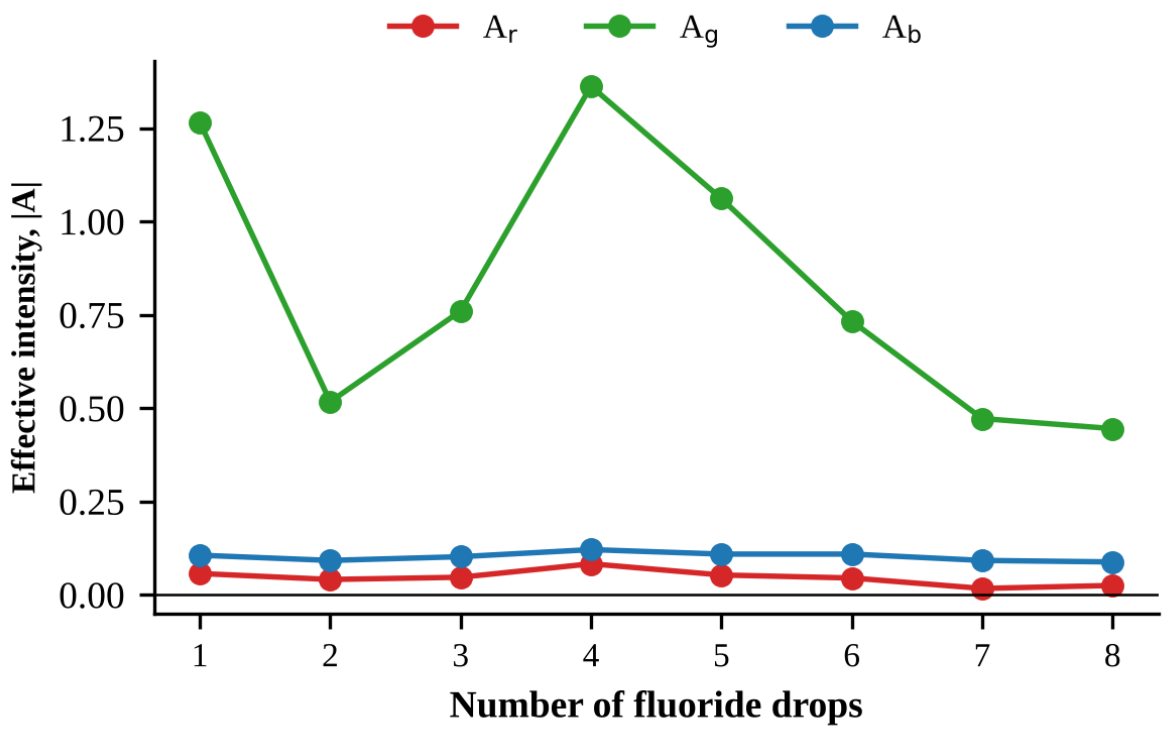

Figure 3. Effect of the number of fluoride drops on the effective intensities.

pH. The responses at pH 4 ($|A_g|$ = 0.173) and pH 7 (0.160) were similar, and that at pH 9 was lower (0.089) (Fig. 4), possibly because hydroxide competes for Al^{3+} in alkaline medium. The red channel was the same at the three pH values ($|A_r|$ = 0.035) and the blue channel changed little ($|A_b|$ = 0.043, 0.036 and 0.043). pH 7 was chosen so that drinking water samples need no pH adjustment.

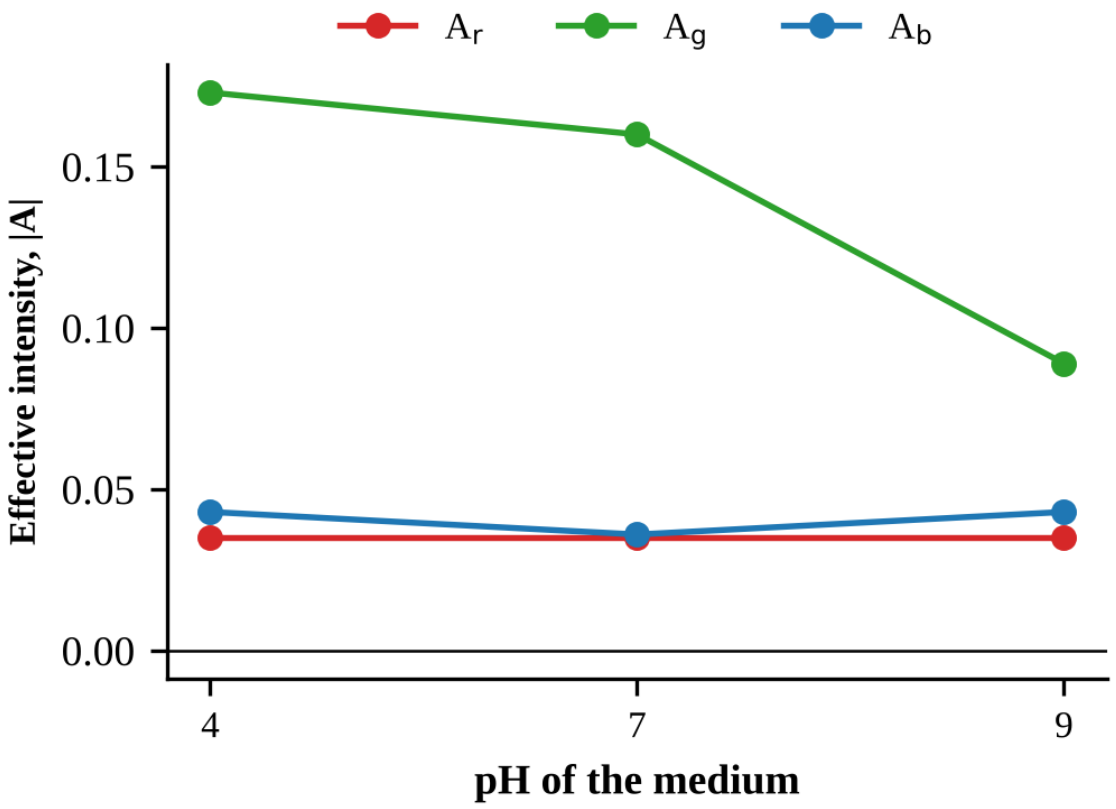

Figure 4. Effect of the pH of the medium on the effective intensities.

Temperature and time. The response was followed at room temperature, 40, 45 and 50 °C (Fig. 5). At room temperature $|A_g|$ rose from 0.137 (instant reading) to 0.185, 0.212 and 0.238 at 5, 10 and 15 min, and then stayed at 0.224–0.232 up to 30 min. At 40 °C the instant reading was low (0.049), the response reached 0.218 after 5 min, about 92% of the room-temperature maximum, and then remained at 0.198–0.215 up to 25 min. At 45 °C (instant 0.089; 0.212 at 5 min, 0.198–0.212 afterwards) and 50 °C (instant 0.093; 0.192 at 5 min, 0.189–0.195 afterwards) the signals were similar to or slightly lower than at 40 °C. A temperature of 40 °C and a time of 5 min were therefore chosen, as they give a stable signal in a third of the time needed at room temperature.

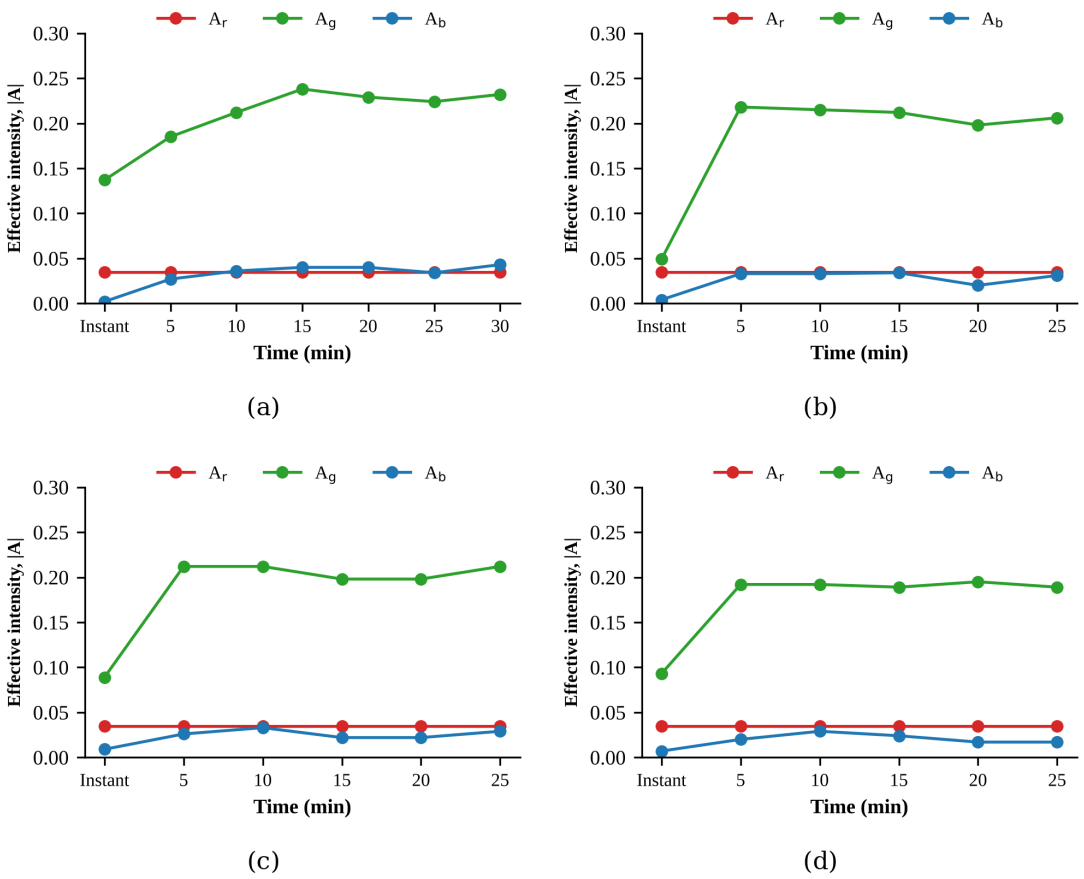

Figure 5. Effect of temperature and time on the effective intensities: (a) room temperature, (b) 40 °C, (c) 45 °C and (d) 50 °C.

3.3 Calibration curve and detection limit

Under the optimized conditions, standard fluoride solutions from 0.05 to 1000 ppm progressively bleached the pink strip, and the G value rose from 92 (blank) to 149 at 0.05 ppm and 202 at 1000 ppm (Table 1). Over the whole range, $|A_g|$ followed a single log-linear calibration (Fig. 6):

$$|A_g| = 0.0289 \log C + 0.255 \left(R^2 = 0.981\right)$$

The data can also be described by two linear segments (Fig. 7; Table 2): a steeper segment between 0.05 and 0.5 ppm ($|A_g|$ from 0.209 to 0.256; slope 0.0469, three points) and a gentler one between 0.5 and 1000 ppm ($|A_g|$ from 0.256 to 0.342; slope 0.0258, R^2 = 0.984). The steeper segment suggests a larger change in signal per decade of concentration in the region below the guideline value. The segmented fits are given as a description of the data, since the first segment has only three points. The last column of Table 2 gives the number of calibration standards used in each fit: all seven standards for the full range, 0.05, 0.1 and 0.5 ppm for the first segment, and 0.5, 1, 10, 100 and 1000 ppm for the second (the 0.5 ppm standard belongs to both).

The practical detection limit was 0.05 ppm (0.05 mg L^{-1}, about 2.6 µM), the lowest concentration tested, which gave a clear 57-unit shift in the green channel above the blank (G = 149 against 92; $|A_g|$ = 0.209). This is 30 times below the WHO guideline value of 1.5 mg L^{-1} [6] and 20 times below the Indian acceptable limit of 1.0 mg L^{-1} [8].

Table 1. RGB values and effective intensity magnitudes (|A|) at different fluoride concentrations (blank R = 235, G = 92, B = 230).

F^- (ppm)	log C	R	G	B	$\lvert A_r \rvert$	$\lvert A_g \rvert$	$\lvert A_b \rvert$
Blank (0)	—	235	92	230	0.000	0.000	0.000
0.05	−1.301	255	149	248	0.035	0.209	0.033
0.1	−1.000	255	154	250	0.035	0.224	0.036
0.5	−0.301	255	166	248	0.035	0.256	0.033
1	0	255	168	248	0.035	0.262	0.033
10	1.000	255	175	249	0.035	0.279	0.034
100	2.000	255	188	250	0.035	0.310	0.036
1000	3.000	255	202	255	0.035	0.342	0.045

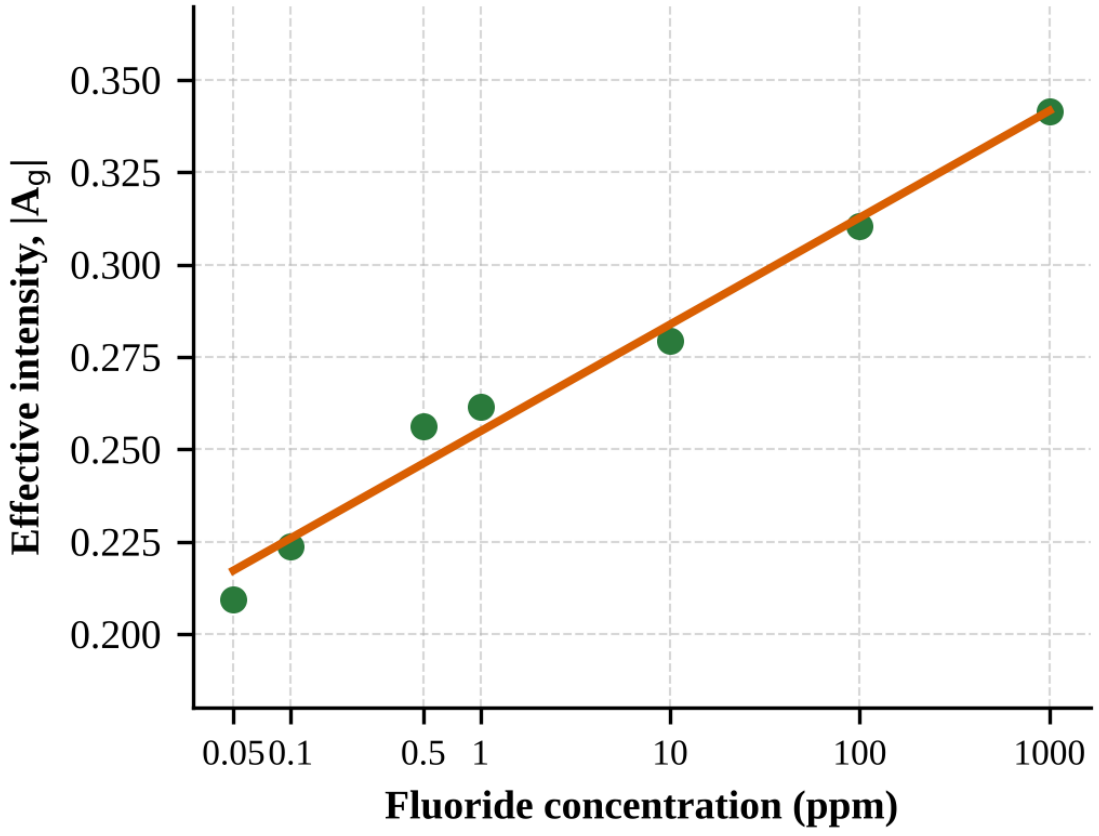

Figure 6. Calibration plot of the green-channel effective intensity (|Ag|) against fluoride concentration over 0.05–1000 ppm.

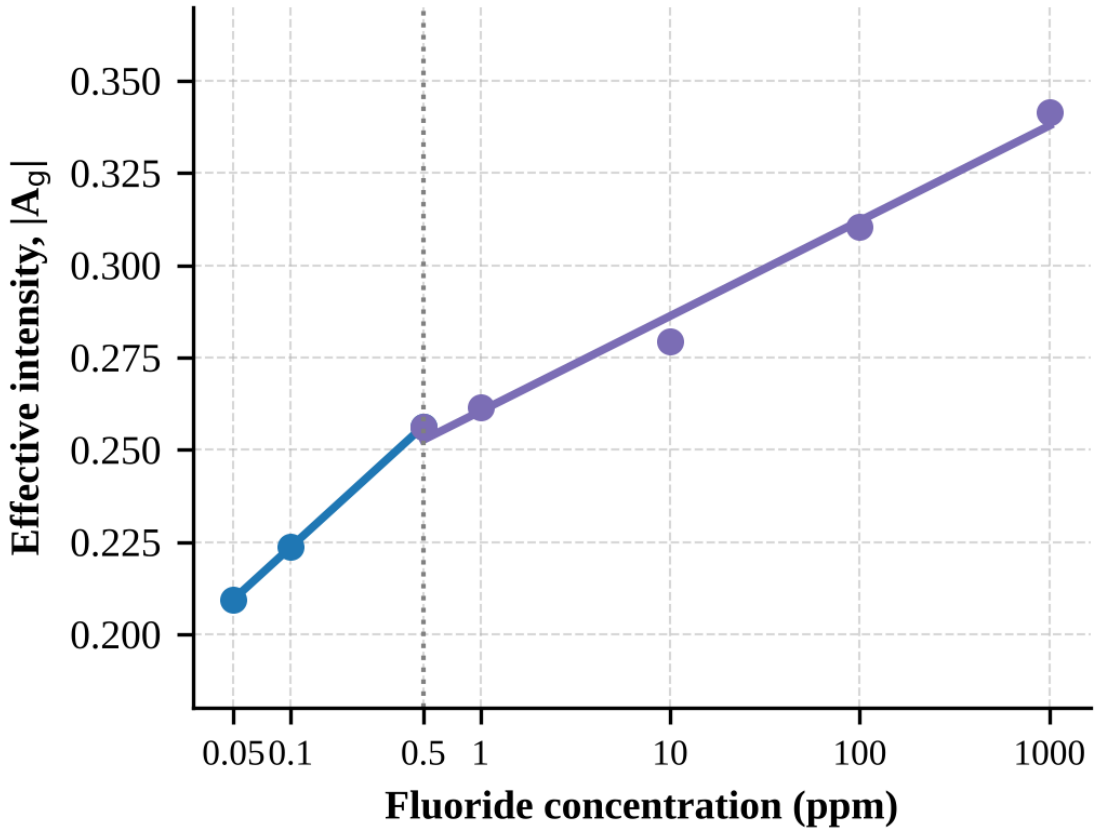

Figure 7. Calibration plot of |Ag| against fluoride concentration showing two linear segments, 0.05–0.5 ppm and 0.5–1000 ppm.

Table 2. Linear regression parameters for the full-range and segmented calibrations.

Fit	Range (ppm)	Equation	R^2	No. of data points
Full range (Fig. 6)	0.05–1000	$\|A_g\| = 0.0289 \log C + 0.255$	0.981	7
Segment I (Fig. 7)	0.05–0.5	$\|A_g\| = 0.0469 \log C + 0.270$	1.000	3
Segment II (Fig. 7)	0.5–1000	$\|A_g\| = 0.0258 \log C + 0.260$	0.984	5

3.4 Interference studies

The selectivity of the strip was tested with ten coexisting ions, namely the anions PO_4^{3-}, Cl^-, SO_4^{2-}, HCO_3^- and NO_3^- and the cations Ca^{2+}, Mg^{2+}, Fe^{3+}, Cu^{2+} and Pb^{2+}, at 1000 ppm under the optimum conditions without any masking agent. None of them interfered with the fluoride response (Table 3), which is consistent with the strong affinity of F^- for Al^{3+}.

Table 3. Tolerance limit of coexisting ions in the determination of fluoride.

Serial no.	Coexisting ions	Tolerated up to (ppm)	Interference
1	PO_4^{3-}, Cl^-, SO_4^{2-}, HCO_3^-, NO_3^-	1000	None observed
2	Ca^{2+}, Mg^{2+}, Fe^{3+}, Cu^{2+}, Pb^{2+}	1000	None observed

3.5 Comparison with an existing paper-based fluoride sensor

As summarized in Table 4, a wax-patterned microfluidic paper device with smartphone read-out has been reported with a detection limit of 5 μM (about 0.095 mg L^{-1}) and a linear range of 0.04–0.25 mM (about 0.76–4.75 mg L^{-1}) [9]. The present strip detected fluoride down to 0.05 ppm (about 2.6 μM), a lower concentration, and gave a log-linear response from 0.05 to 1000 ppm, a range of more than four orders of magnitude, from commercial reagents by dip-and-dry without wax patterning or probe synthesis.

Table 4. Analytical performance compared with a reported paper-based colorimetric fluoride sensor.

Sensing platform	Read-out	Range (ppm)	LOD (ppm)	Features
Wax-patterned μPAD [9]	Smartphone RGB	0.76–4.75	0.095	Wax-printed channels
RhB–$AlCl_3$ strip (this work)	Smartphone RGB (ImageJ)	0.05–1000	0.05	Commercial reagents, dip-and-dry, >4 decades

4 Conclusion

A Whatman No. 40 paper strip carrying Rhodamine B and $AlCl_3$, prepared by dip-and-dry, changes colour on contact with fluoride and the change can be read from a photograph. Fluoride-induced loss of the pink $[RhB–Al]^{3+}$ colour raises the green-channel value, and $|A_g|$ gave a single log-linear calibration over 0.05–1000 ppm (R^2 = 0.981), which can also be described by two linear segments. The best response was obtained with 0.125% Rhodamine B, 1% $AlCl_3$ at a 1:1 ratio, four drops of sample, pH 7, 40 °C and 5 min. The practical detection limit of 0.05 ppm is 30 times below the WHO guideline value (1.5 mg L^{-1}), and ten common anions and cations did not interfere up to 1000 ppm.

Acknowledgement

The authors are grateful to the Director and the Department of Chemistry, UIT, Barkatullah University, Bhopal, and to the Chairman, Director and Department of Chemistry, Career College (Autonomous), Bhopal, for providing laboratory and instrumentation facilities.

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