Electronic Supplementary Information

Fabrication of Paper-based Facile and Low-cost Microfluidic Device and Digital Imaging Technique for Point-of-Need Monitoring of Hypochlorite

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Experimental Section

The image capture parameter is given below, and it is carried out by Thermo iBright FL 1000 imaging system (used for molecular biology for gel imaging system).

Sl. No.	Parameter	Value
1.	Digital camera	9 megapixels
2.	Zoom Optical	1.3x
3.	Zoom Digital	1x
4.	Exposure time	25-30 ms for background 30-50 ms for samples
5.	Excitation filter	455-485 nm filter and captured at 470 nm for probe
6.	Emission filter	586-617 nm filter and captured at 592 nm for probe

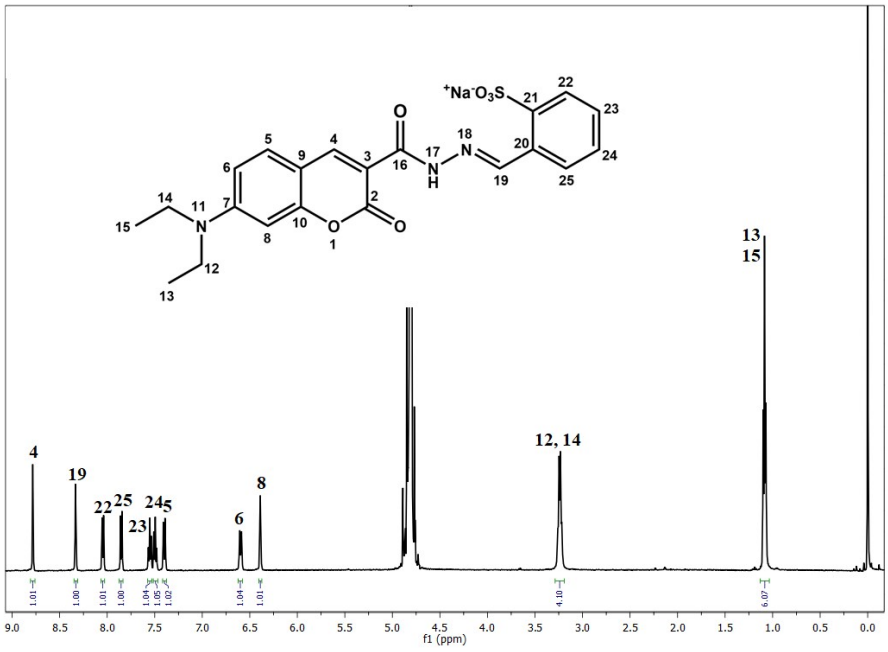


Fig. S1 ^{1}H NMR spectrum of 1 in D_2O.

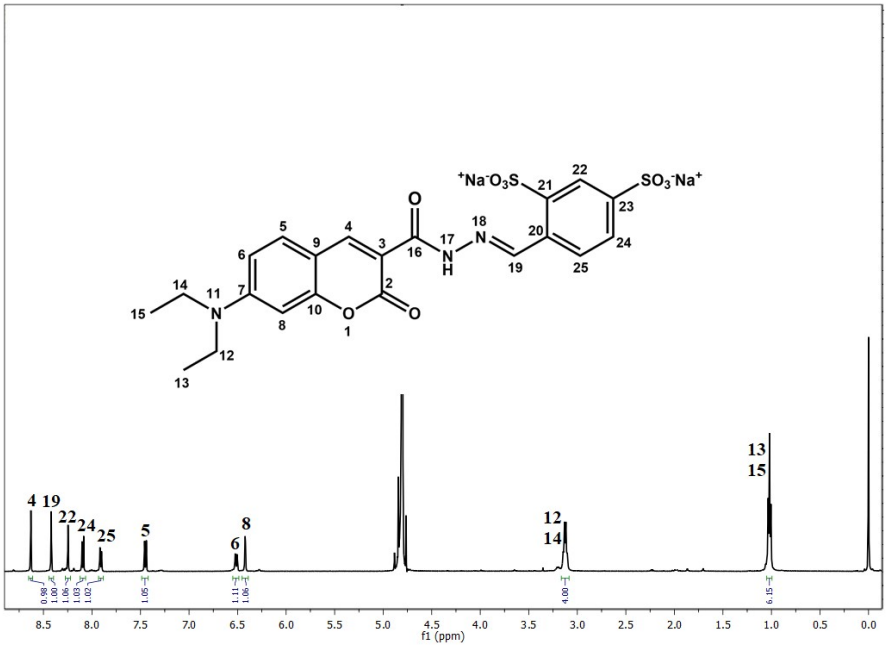


Fig. S2 ^{1}H NMR spectrum of 2 in D_2O.

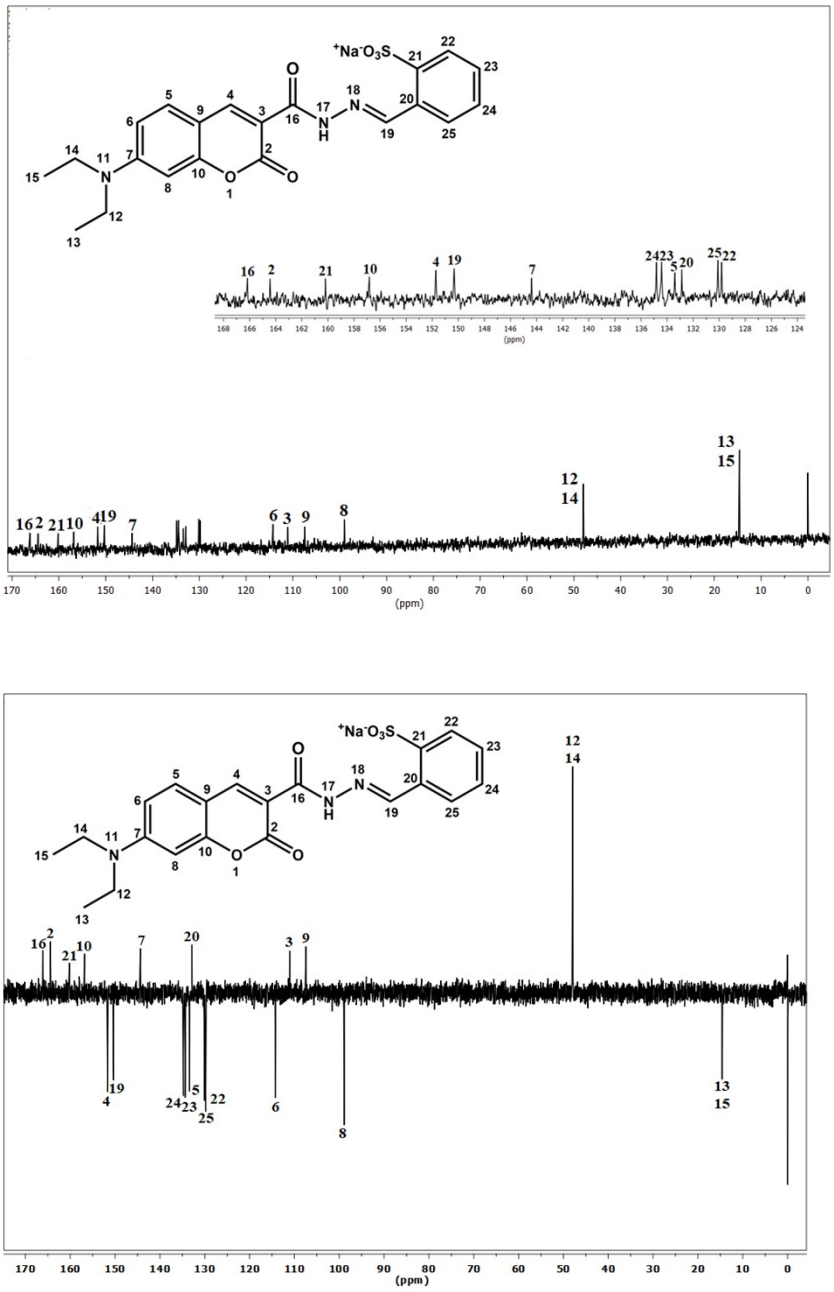


Fig. S3 ^{13}C (top) and DEPTQ NMR (bottom) spectra of 1 in D_2O.

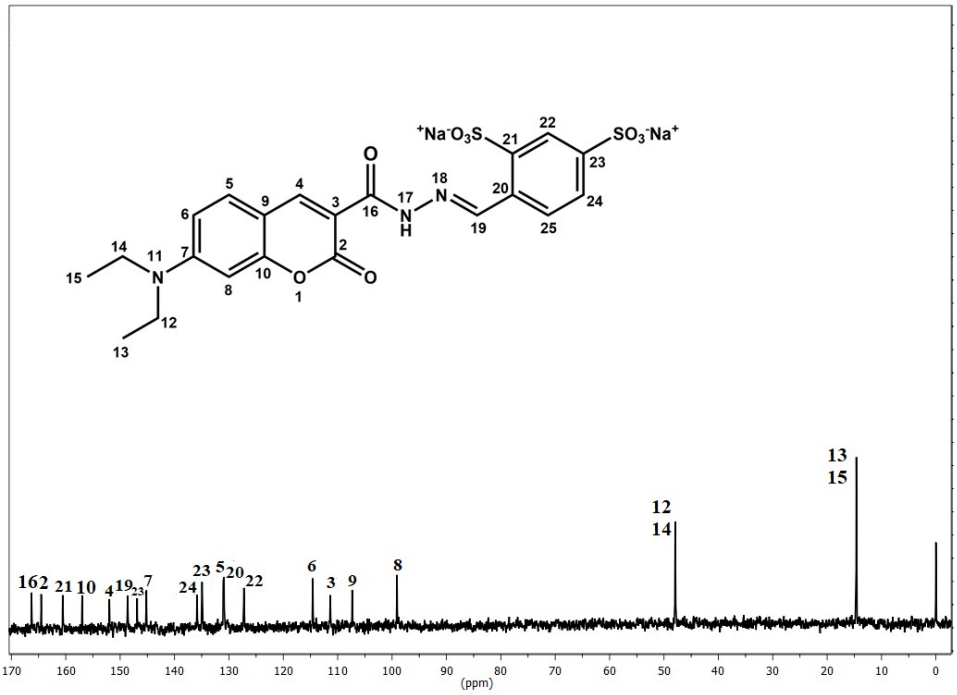


Fig. S4 ^{13}C NMR spectrum of 2 in D_2O.

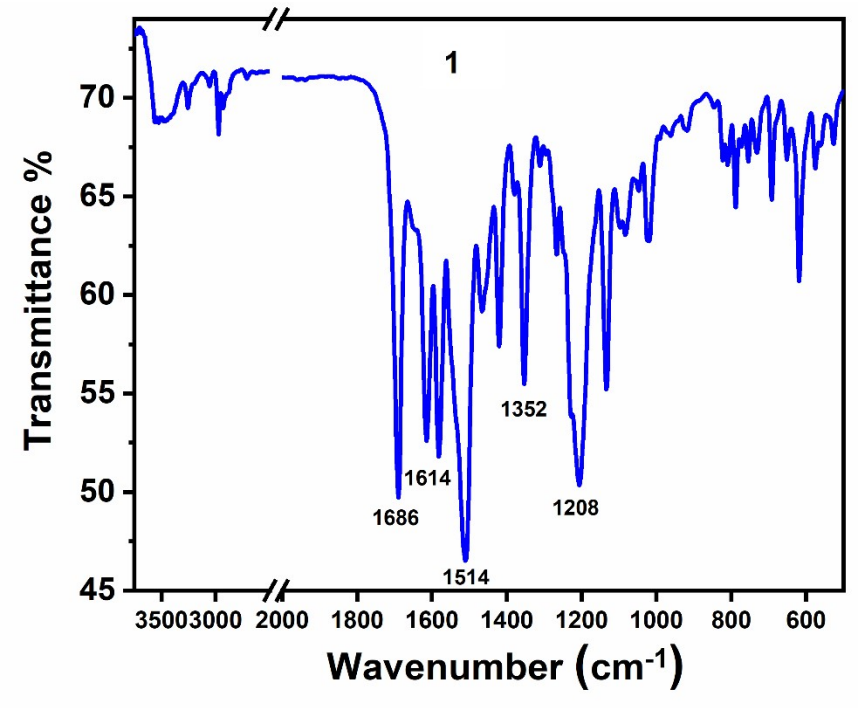


Fig. S5 FT-IR spectrum of 1.

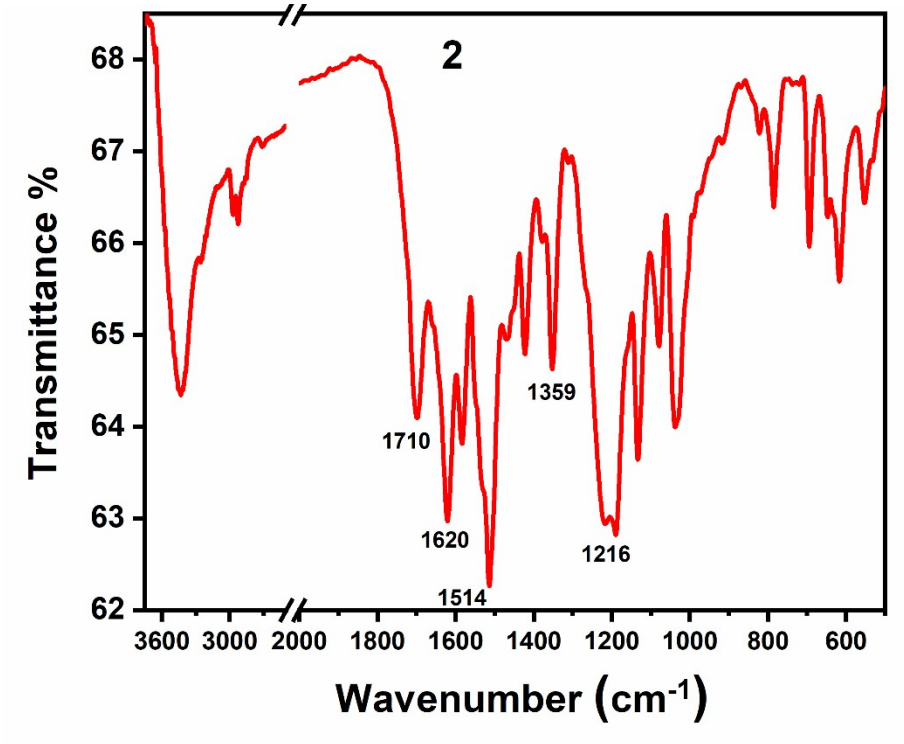


Fig. S6 FT-IR spectrum of 2.

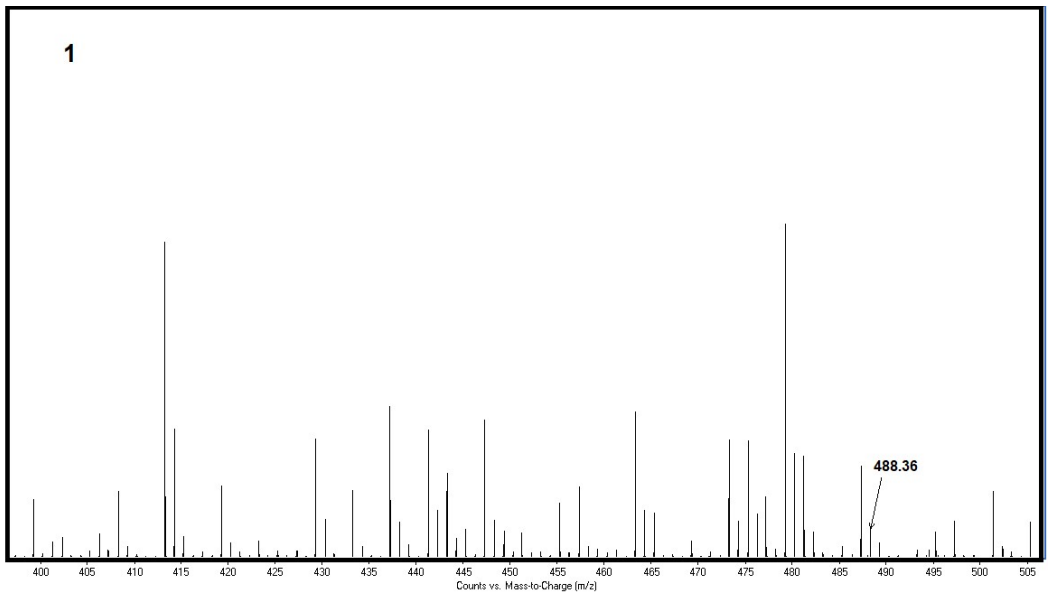


Fig. S7 ESI-MS of 1.

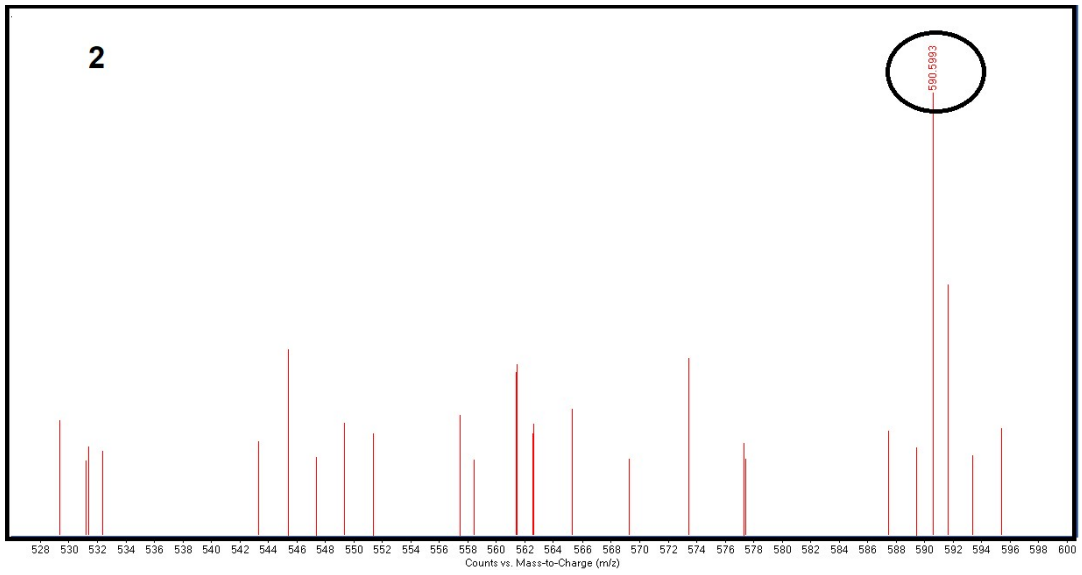


Fig. S8 ESI-MS of 2.

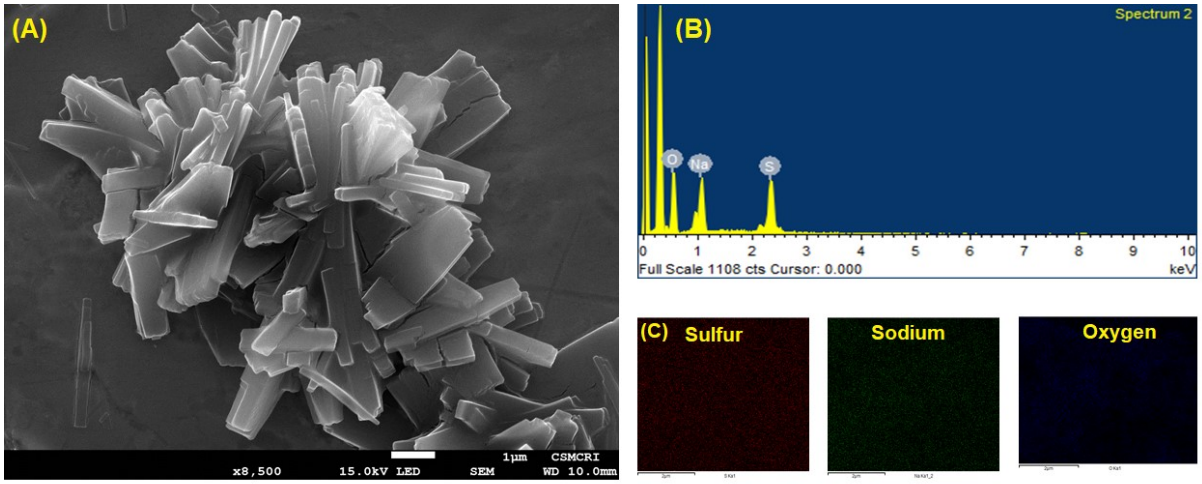


Fig. S9 (A) SEM image profile of 1. (B) SEM-EDX profile, and (C) elemental mapping of 1 showing traces of sulfur, sodium, and oxygen.

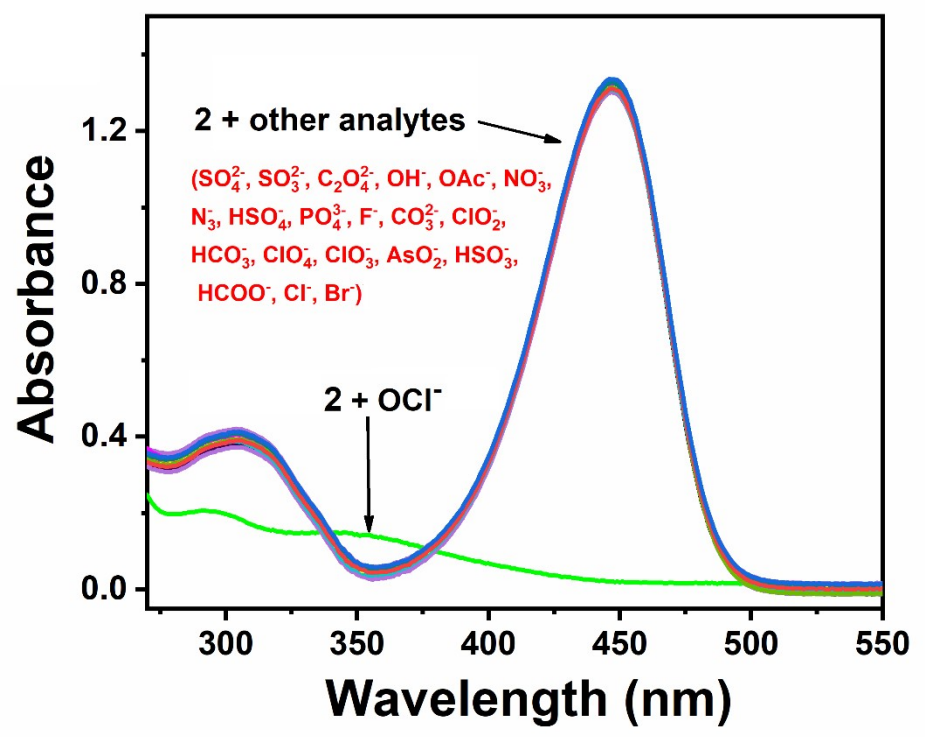


Fig. S10 UV-vis selectivity profile of 2 (B) in the presence of different anionic species (250 µM) in 10 mM aqueous PBS buffer at 7.4 pH.

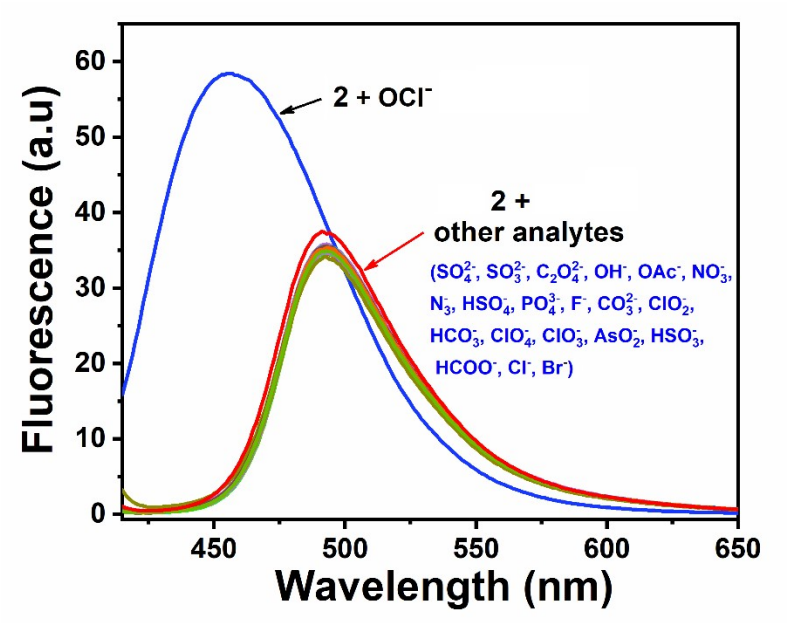


Fig. S11 Fluorometric response of 20 μM 2 towards 12.5 equivalents of different anionic species in 10 mM aqueous PBS buffer at 7.4 pH. The excitation wavelength was set at 400 nm.

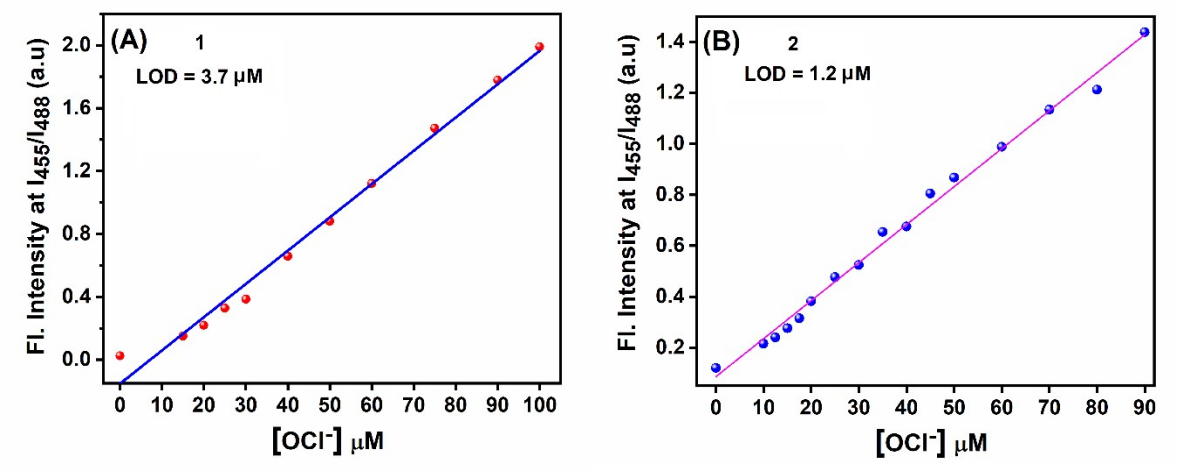


Fig. S12 Linear fitting plots of the fluorescence titration data points of 1 (A) and 2 (B) with varying concentrations of OCl^-. The excitation wavelength was set at 400 nm.

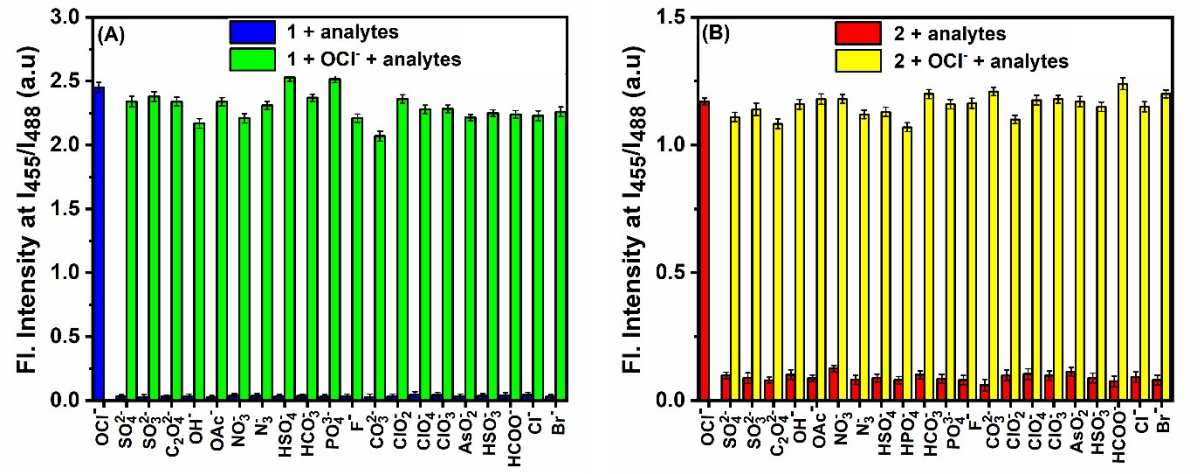


Fig. S13 Fluorescence-based interference data during OCl^- detection by 1 (A) and 2 (B) in the presence of excess competing analytes in 10 mM aqueous PBS buffer at 7.4 pH. The excitation wavelength was set at 400 nm.

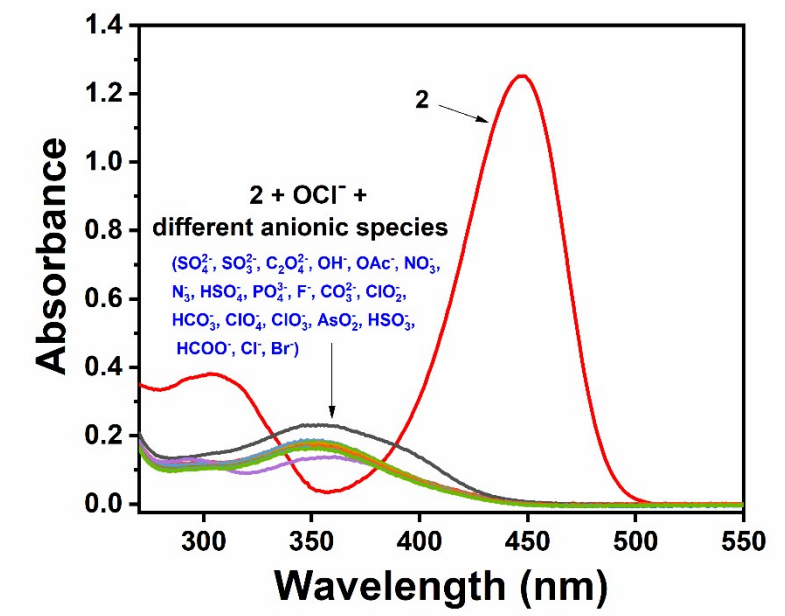


Fig. S14 UV-vis-based interference data for OCl^- detection by 2 in the presence of excess competing analytes in 10 mM aqueous PBS buffer at 7.4 pH.

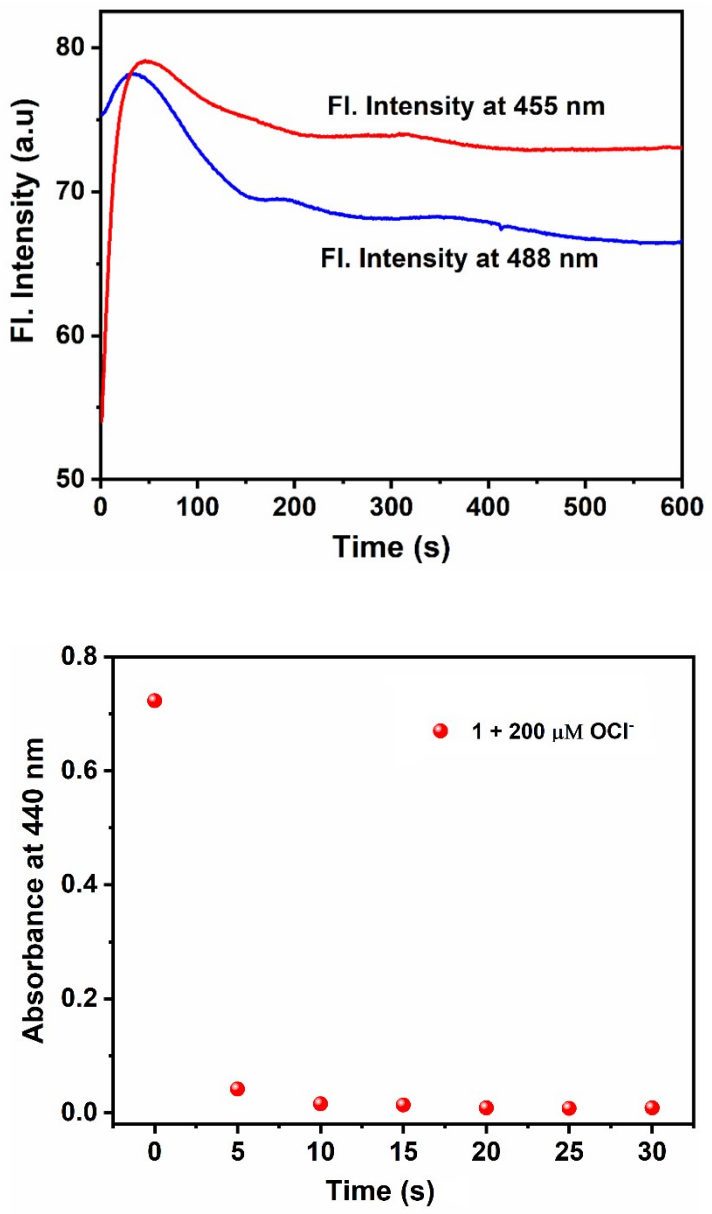


Fig. S15 Time-dependent fluorescence intensity (top) and UV-vis (bottom) changes of 1 (20 μM) in the presence of 200 μM OCl^- in PBS buffer solution (10 mM, pH = 7.4).

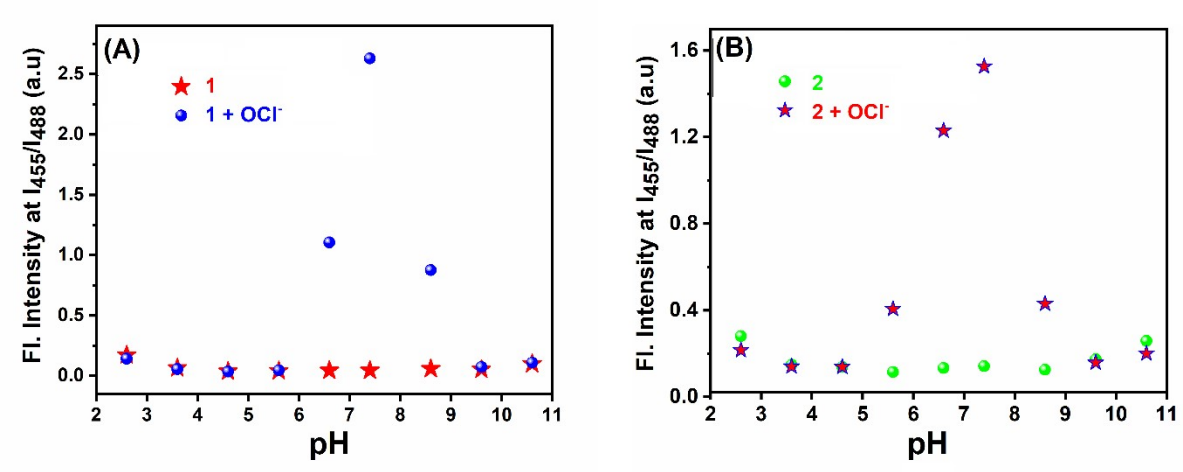


Fig. S16 Change in fluorescence intensities of 1 (A) and 2 (B) in the presence and absence of 200 μM OCl^- in PBS buffer solution (10 mM, pH = 7.4) under variable pH conditions. The excitation was set at 400 nm.

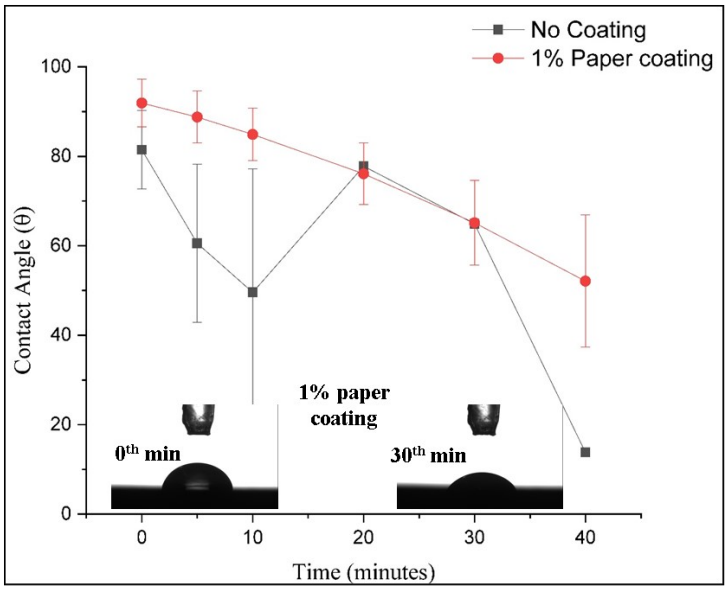


Fig. S17 Contact angle vs. time plot of the paper without coating and with 1 % paper coating.

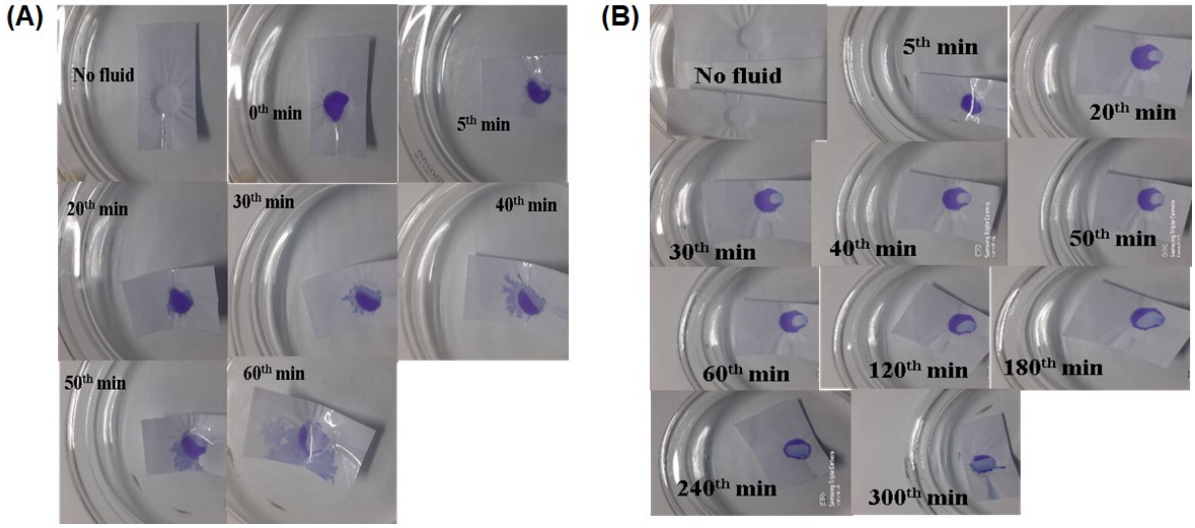


Fig. S18 Fluid holding images of a paper chip with time tested by methylene blue without coating (A) and with 1% polystyrene coated paper (B).

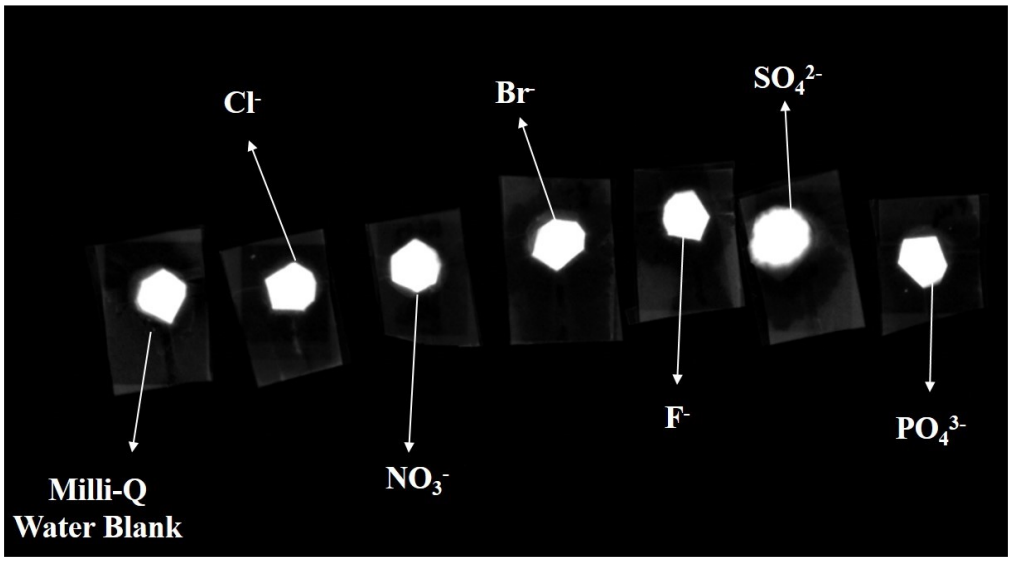


Fig. S19 Fluorescent images of 1@μPADs toward various anions such as Cl^-, NO_3^-, Br^-, F^-, SO_4^{2-} and PO_4^{3-} at 250 μM solution captured by gel doc imaging system.

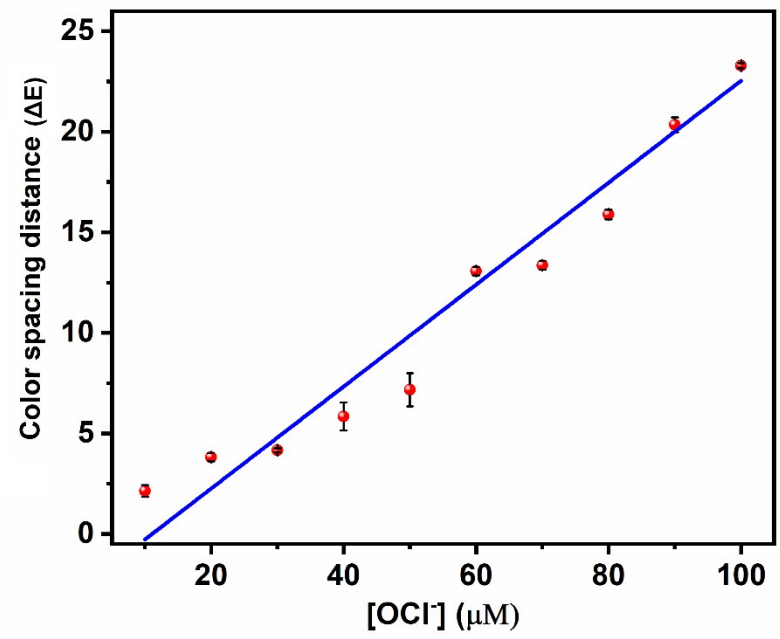


Fig. S20 Linear calibration plot of 1@μPAD via fluorescence technique with colour spacing distance vs. various concentrations of OCl^-.

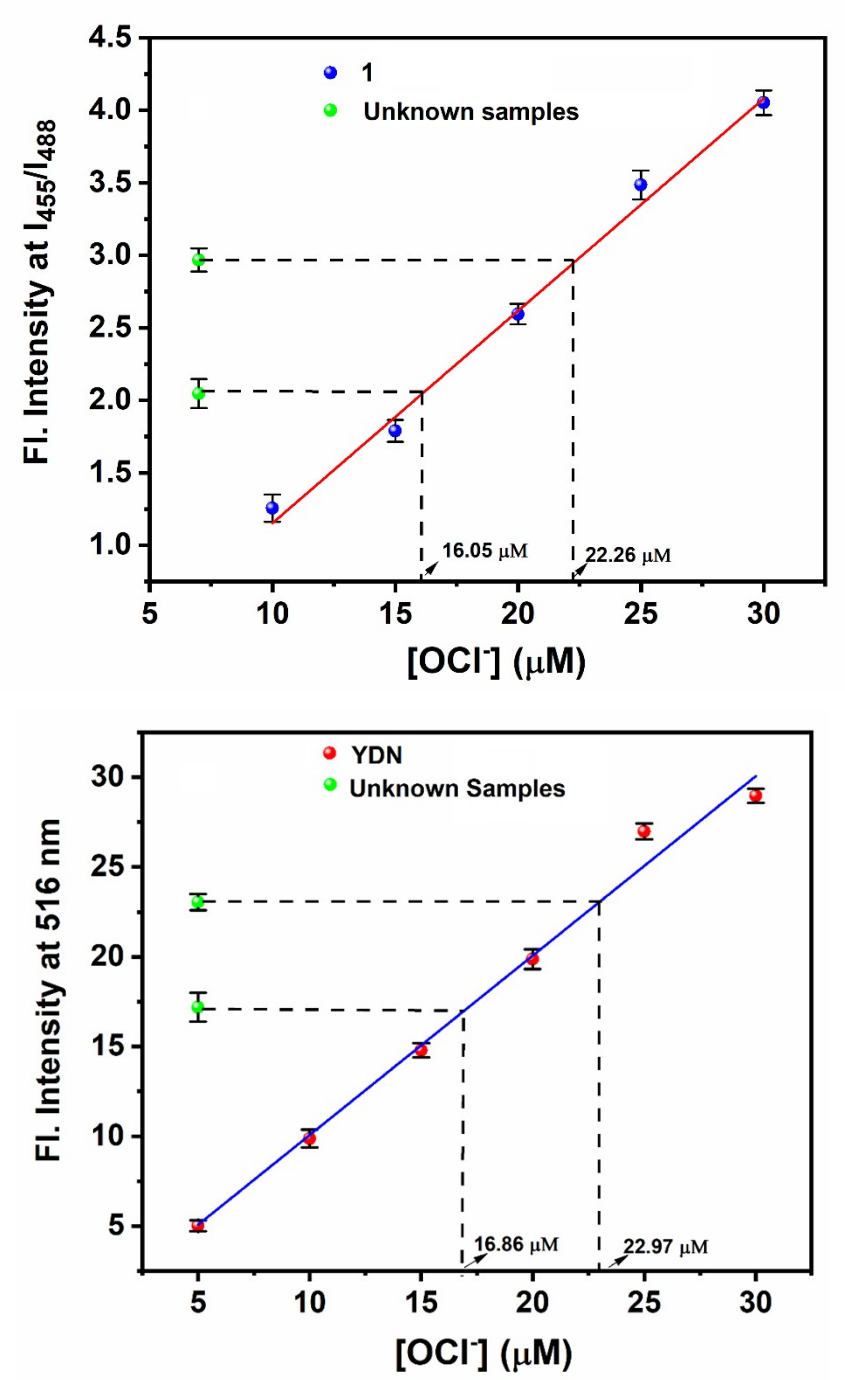


Fig. S21 Unknown concentration measurements of OCl^- using (a) compound 1 and (b) YDN. Fluorimetric linear calibration plot in PBS buffer solution (10 mM, pH = 7.4) was used.

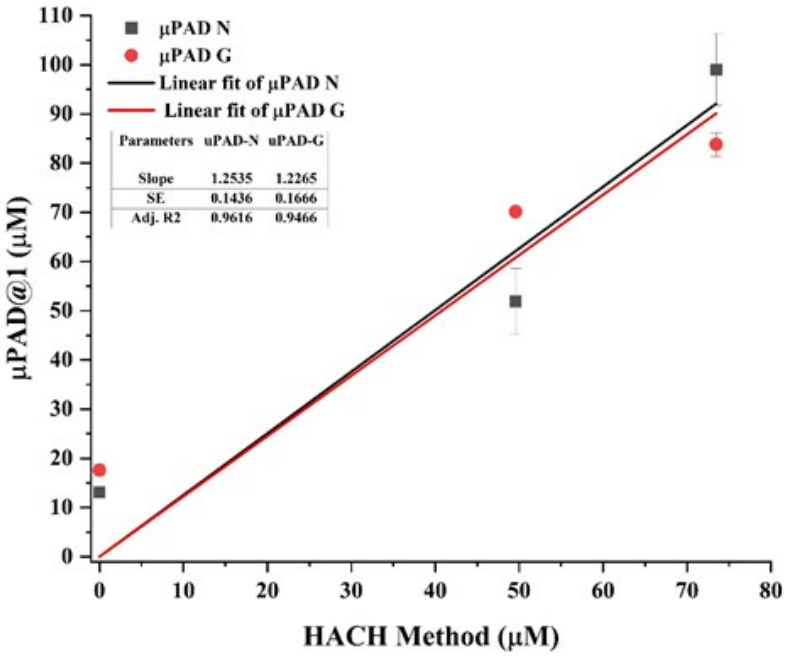


Fig. S22 1:1 Analytical validation of various concentrations of OCl^- between 1@µPAD (µPAD –N (Narmada) & µPAD-G (Ground water)) and HACH method.

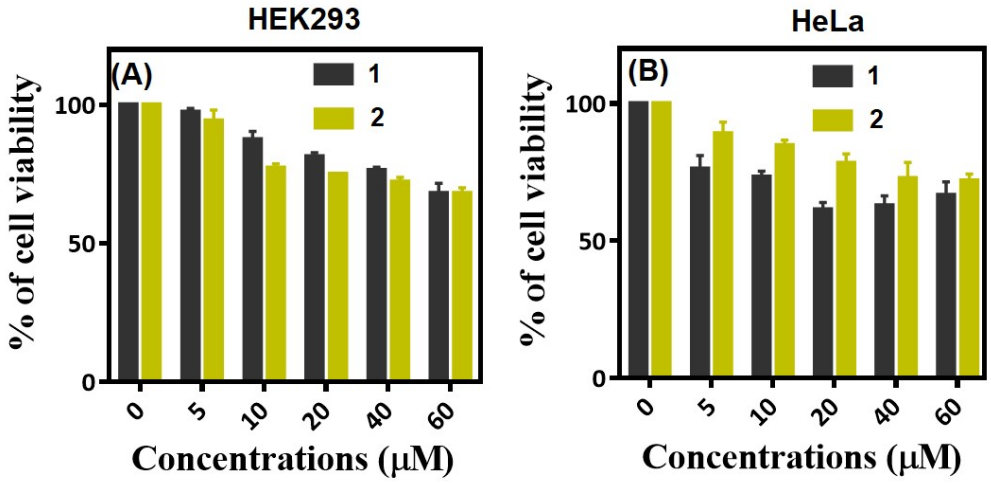


Fig. S23 Cytotoxicity profiles of 1 and 2 with HEK293 (A) and HeLa (B) cells.

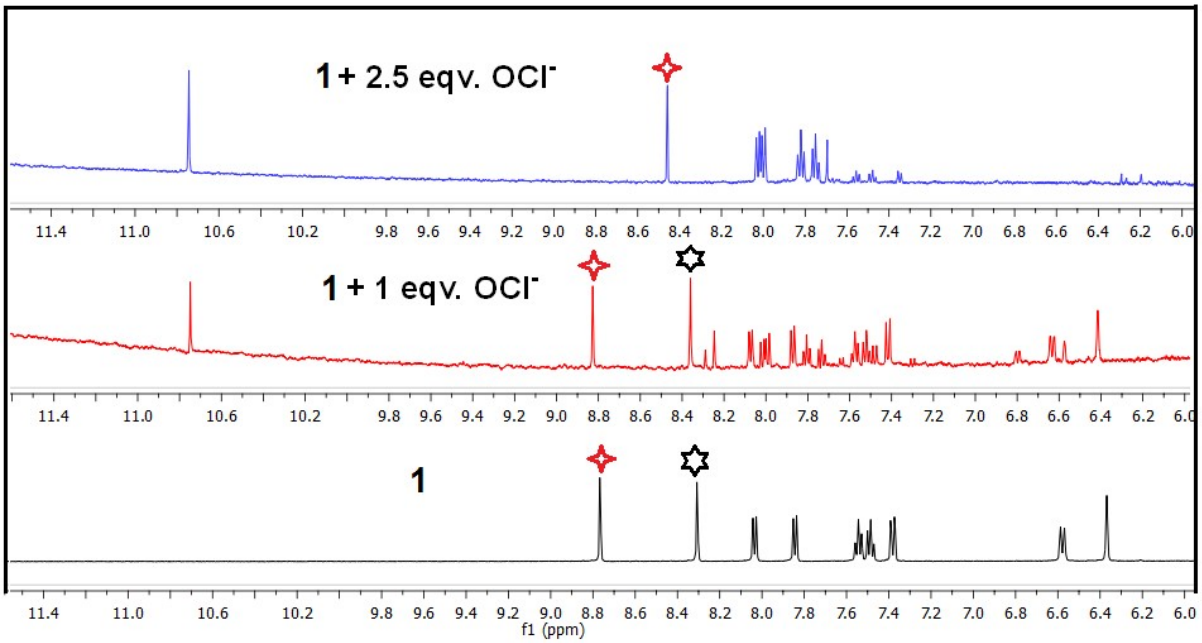


Fig. S24 ^{1}H NMR profiles of 1 in the absence and presence of different equivalents of OCl^- in D_2O solution (Red astericks indicate imine proton peak, black astericks indicate proton peak of the adjacent position of the coumarin acetyl moiety).

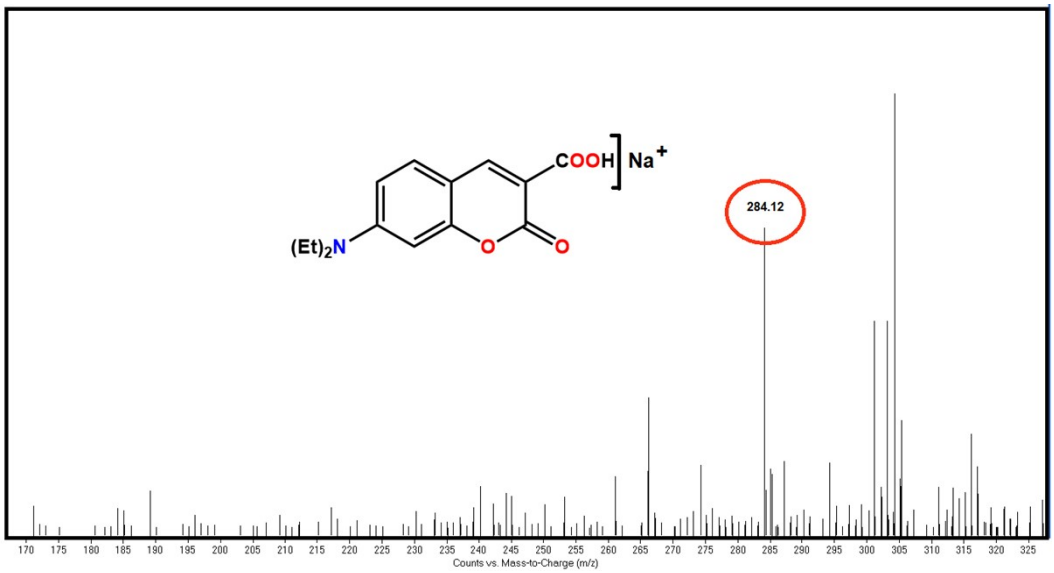


Fig. S25 ESI-MS spectrum of 1 upon addition of OCl^-.

Table S1. Comparison of the current embossing μPAD technique with the existing printing techniques.

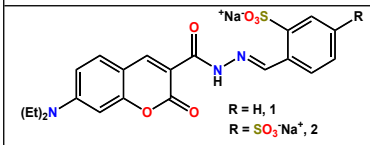
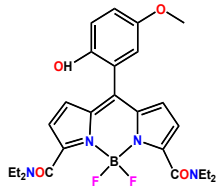
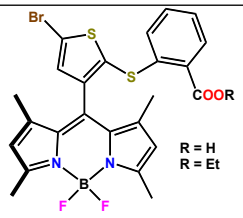
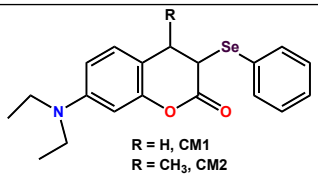
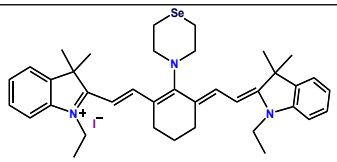
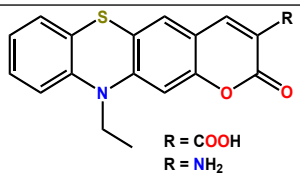
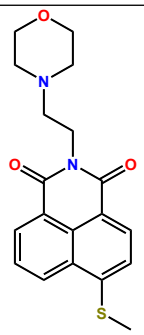
Sl. No.	Type of Fabrication	Advantage	Disadvantage
1	Laser Printing	1. Commercial tonner, high resolution. 2. Simple to print using commercial devices. 3. Fabricate to print the hydrophobic walls in 2D.	1. Printing equipment is required. 2. Additional heating step. 3. Limited to the use of specific materials for printing purpose. 4. Ability to do 3D with extensive process. 5. Not compatibility for all types analytical reagents. 6. Ability to retain the fluid in the capillary of the substrate <10 minutes.
2	Wax Printing	1. Requires wax printer, hotplate, and solid wax. 2. Simple and fast fabrication process. 3. Fabricate to print the hydrophobic walls in 2D.	1. Low resolution and requires heating step. 2. Wax spreads on edges of the barriers. 3. Ability to do 3D with extensive process. 4. Ability to retain the fluid in the capillary of the substrate <10 minutes. 5. Not compatible for all types analytical reagents.
3	Embossing	1. Fabrication by plastic mold for 2D and adhesive tapes helps for 3D in paper substrate. 2. Flexible, foldable, lightweight, portable,	1. Low resolution. 2. Susceptible to contamination. 3. Requires polymer coating on the substrate.

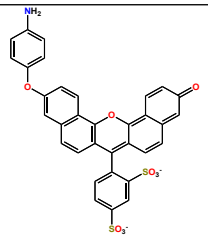
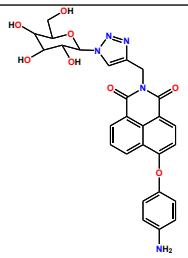
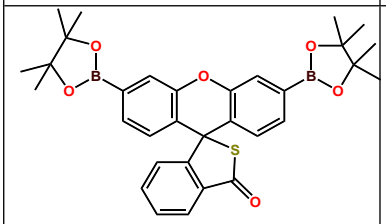
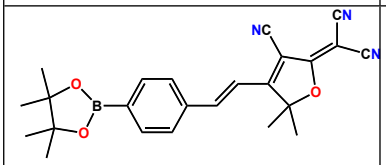
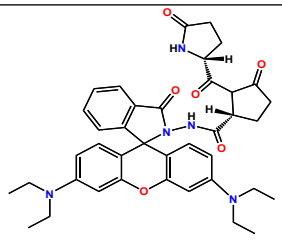
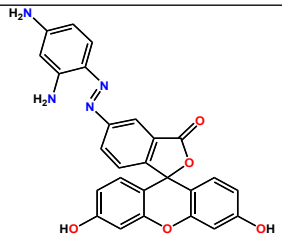
		and disposable. 3. Low cost. 4. Possibility of mass production. 5. Compatible for all types' analytical reagents.	
3	3D printing	1. Requires 3D printer and 3D printer resin. 2. Fast and accessible to mass production.	1. Resolution depends on 3D printer equipment. 2. High cost
4	Craft cutting	1. Requires digital craft cutter. 2. Lightweight, flexible, portable, and disposable.	1. Requires external pumping mechanism. 2. Low resolution 3. Devices are difficult to manipulate and usually require a polymer backing.
5	Screen-printing	1. Requires for mask for patterning and wax. 2. Hydrophobic patterning like UV curable ink, carbon, silver/silver chloride ink, etc. 3. Low cost and simple fabrication process. 4. Fabricate to print the hydrophobic walls in 2D.	1. Low resolution 2. Unadaptable to mass production. 3. New screen required for each design. 4. Ability to retain the fluid in the capillary of the substrate <10 minutes. 5. Not compatible for all types analytical reagents. 6. Ability to do 3D with extensive process.
6	Flexographic Printing	1. Requires customized printing equipment. 2. Hydrophobic chemicals like polystyrene and PDMS for applicable	1. High cost 2. Complex preparation and cleaning. 3. Printing quality depends on surface to roughness of the

		roll-to-roll process. 3. No heating step. 4. Ability to fabricate in 3D.	paper.
7	Inkjet Printing	1. Requires printing equipment. 2. Requires hydrophobic chemical and UV curable acrylate ink. 3. High resolution 4. Fabricate to print the hydrophobic walls in 2D.	1. Requires customised inkjet printer. 2. Requires solvent treatment of the paper. 3. Not compatible for all types analytical reagents. 4. Ability to do 3D with extensive process. 5. Ability to retain the fluid in capillary of the substrate for < 10 minutes.
8	Current embossing technique µPAD	1. Same as embossing technique. Coating with 1 % polystyrene in paper helps in hydrophobic conditions and ability to retain the fluid for > 60 min as shown in Fig. S18B. 2. Probe was coated on the filter paper and sealed with adhesive tape to react with the analyte uniformly and avoid of susceptible to contamination. 3. Surface to roughness of the paper didn't affect the flow due to external	1. Low resolution. 2. Requires polymer coating of paper.

		pumping (micropipette pressure). 4. Possibility of mass production by systematic process. 5. Easy and low cost fabrication process of testing new probe. 6. No heating process.	

Table S2. Comparative literatures on 100 % aqueous soluble OCl^- specific optical probes.

Probe	λ_{ex} and λ_{em}	Buffer pH	Technique	Response time	Reference
	400 and 455/488	PBS buffer	Ratiometric fluorescence, and colorimetry	10 s (UV vis) and 60 s (Fl.)	Present work
	520 and 541	Sodium phosphate buffer 7.4	Fluorescence	2 min	1
	542 and 560	Water 7	Fluorescence and colorimetry	5 min	2
	405 and 480	PBS buffer 7.4	Fluorescence	Within sec	3
	690 and 786	PBS buffer 7.4	Fluorescence	100 sec	4
	400 and 503	PBS buffer 7.4	Fluorescence and UV-vis	Within secs	5
	405 and 505	PBS buffer 7.4	Fluorescence (off) and UV-vis ratiometric	150 s	6

	614 and 676	PBS buffer (7.4)	Fluorescence		7
	470 and 558	PBS buffer (7.4)	Fluorescence and colorimetry	3 s	8
	450 and 550	PBS buffer (7.4)	Fluorescence and UV-vis	60 s	9
	498 and 523	KH_2PO_4 buffer 7.4	Fluorescence		10
	560 and 610	PBS buffer 7.4	Fluorescence and colorimetry	Within 1 min	11
	520 and 580	HEPES buffer solution 7.4	Ratiometric fluorescence, and colorimetry	Within 2 min	12
	485 and 516	PBS buffer 7.4	Fluorescence and colorimetry	2 min	13

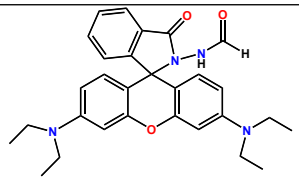
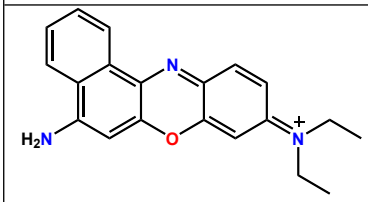
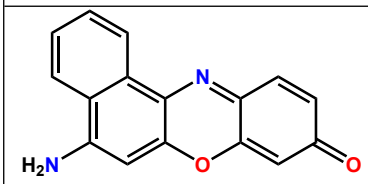
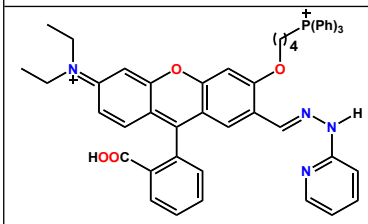
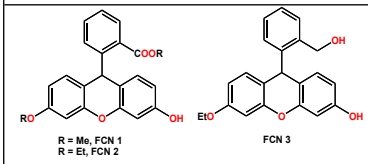
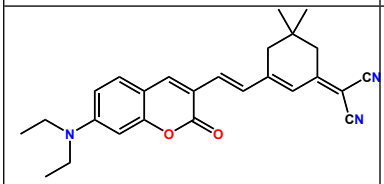
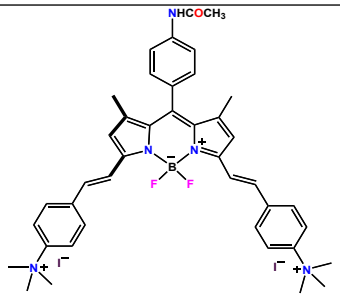
	520 and 580	PBS buffer (7.4)	Fluorescence and colorimetry	Within 3 sec	14
	600 and 672	PBS buffer (7.4)	Fluorometry off and colorimetry	5 sec	15
	580 and 626	PBS buffer (7.4)	Fluorometry off and colorimetry	10 min	16
	505 and 580	PBS buffer 7.4	Fluorescence and UV-vis ratiometric	4 s	17
	415 and 485	HEPES buffer 7.4	Fluorescence	20 min	18
	488 and 496/713	PBS buffer 7.4	Ratiometric fluorescence and UV-vis	NA	19
	500 and 567/629	PBS buffer 7.4	Fluorometric on ratiometric and UV-vis	10 s	20

Table S3. Fluid comparison table between paper chips fabricated by laser toner printed vs. embossed using micropipette vs. embossed using capillary driven**.**

Sl. No.	**Type of paper chip**	**Fluid insertion timing**	**Fluid insertion**
1	Embossed paper chip using capillary based fluid insertion	14 s	Improper
2	Laser wax printed paper using capillary based fluid insertion	3 min 51 s	Proper with longer time
3	Embossed paper chip using Micropipette based fluid insertion	20 s	Proper with shorter time like mini paper based tubes.

The total volume of methylene blue used in the experiment is 40 µL.

Table S4. Colour standard chart for 1 (20 µM) towards OCl^- via visible spectrophotometer and digital camera colour variation taken in a cuvette.

Colour Standard chart from Digital Camera					**Colour Standard chart from Visible spectrophotometer**				**Colour Differences between Digital Camera and Visible Spectrophotometer (ΔE)**
NaOCl Std. Conc.	**L (R)**	**a (G)**	**b (B)**	**C. chart (ΔE)**	**L (R)**	**a (G)**	**b (B)**	**C. chart (ΔE)**	
Blank	74.35 (164)	-28.05(195)	56.50 (72)	**0**	93.98 (244)	-17.76 (245)	66.60 (102)	**0**	14.84
10 µM	74.30 (164)	-27.48 (194)	55.59 (74)	0.6 ± 0.4	94.02 (244)	-17.51 (245)	65.16 (106)	0.41 ± 0.2	14.79
20 µM	73.89 (164)	-26.33 (193)	52.05 (81)	1.4 ± 0.5	94.14 (244)	-16.95 (245)	60.88 (116)	1.5 ± 0.3	15.01
30 µM	73.67 (164)	-25.14 (192)	48.79 (87)	2.5 ± 0.9	94.30 (244)	-16.33 (245)	56.74 (125)	2.6 ± 0.7	15.10
40 µM	74.07 (167)	-23.02 (192)	43.72 (99)	4.1 ± 1.1	94.45 (243)	-15.18 (245)	50.14 (140)	4.6 ± 1.07	14.67
50 µM	73.60 (168)	-19.81 (189)	36.40 (112)	6.7 ± 1.8	94.72 (243)	-13.53 (245)	42.14 (157)	7.2 ± 1.5	14.91
60 µM	73.07 (170)	-15.28 (186)	27.01 (129)	10.6 ± 2.5	95.02 (243)	-11.14 (245)	32.39 (178)	10.7 ± 1.9	15.20
70 µM	72.86 (172)	-10.04 (183)	17.04 (147)	15.6 ± 3.8	95.28 (243)	-8.05 (245)	22.19 (198)	15.1 ± 3.03	15.35
80 µM	72.90 (175)	-6.20 (182)	9.90 (161)	19.4 ± 3	95.50 (243)	-5.65 (244)	14.90 (213)	18.5 ± 2.6	15.39
90 µM	72.70 (176)	-2.92 (180)	4.34 (170)	23.1 ± 2.5	95.57 (243)	-3.32 (244)	8.54 (226)	21.9 ± 2.2	15.52
100 µM	73.03 (178)	-1.45 (180)	1.89 (176)	24.7 ± 1.6	95.70 (243)	-2.12 (244)	5.32 (232)	23.7 ± 1.6	15.30

Note: All the samples were analyzed in the cuvette was repeatability and reproducibility of n=10 to demonstrate the probe performance.

Table S5. Colour standard chart for 1@μPAD towards OCl^- via fluorescence detection in the imaging system.

Concentration of NaOCl (μM)	Colour Representation for Fluorescent intensity					
	R	G	B	Luminosity (L)	delta E	Colour Chart
0	127	127	127	53.18 ± 0.3	0	
10	124	124	124	51.00 ± 0.6	2.14 ± 0.2	
20	116	116	116	49.33 ± 0.3	3.82 ± 0.2	
30	137	137	137	48.99 ± 0.1	4.16 ± 0.1	
40	111	111	111	47.33 ± 0.5	5.84 ± 0.7	
50	109	109	109	46.00 ± 0.5	7.17 ± 0.82	
60	94	94	94	39.67 ± 0.6	13.07 ± 0.21	
70	92	92	92	39.33 ± 0.6	13.36 ± 0.22	
80	85	85	85	36.33 ± 0.5	15.89 ± 0.24	
90	73	73	73	30.67 ± 0.5	20.35 ± 0.37	
100	64	64	64	26.67 ± 0.1	23.29 ± 0.13	

Note: All the samples were analyzed in μPAD was repeatability and reproducibility with n=3 to demonstrate the probe performance.

Table S6. Comparison table of analytical limits in various detection systems.

Sl. No.	Type of analysis	Fitting method	Detection Limit (µM)	Limit of quantification (µM)	Fitting Equation	R value
1	Digital Camera image colour analysis	Linear	16.83	168.33	0.27308x-3.74194	0.94
2	Visible spectrophotometer colour analysis	Linear	13.73	137.28	0.26083x-3.33861	0.95
3	Visible spectrophotometer absorption analysis	Linear	4.52	45.25	0.01215x-0.04029	0.98
4	Paper chip-based fluorescence camera image analysis	Linear	13.34	133.41	0.2056x	0.99

Table S7. Comparative table showing concentration of measured OCl^- using a reported probe YDN and current probe 1.

Quantitative analysis of OCl^-			
Samples	[OCl^-] measured using a reported fluorescent probe YDN	[OCl^-] measured using probe 1	Error %
Unknown Sample - 1	16.86 µM	16.05 µM	5 %
Unknown Sample - 2	22.97 µM	22.26 µM	3 %

Table S8. Colour standard chart for 1@µPAD for the sample analysis via fluorescence detection in the imaging system.

Sample	**Water samples with added NaOCl (µM)**	**Digital camera image colour analysis via fluorescence technique**		**Color representation for fluorescent intensity**
		Obtained (µM)	**Recovery %**	
R1B N	-	13.09 ± 0.4	-	
R1B G		17.59 ± 1.1	-	
S1R N	69	78.88 ± 2.7	96.1 ± 0.3	
S1R G		87.73 ± 1.4	101.6 ± 0.2	
S2R N	96	112.12 ± 1.3	102.7 ± 0.2	
S2R G		111.42 ± 2.4	98.1 ± 0.3	

Note: G means groundwater samples collected from the CHARUSAT University campus, and N means Narmada river water samples collected from Kevadia, Gujarat, India. All the samples were analyzed in µPAD was repeatability and reproducibility with n=3 to demonstrate the probe performance.

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