Development of green and sustainable Rice Husk nanosilica-based spectrophotometric probe for Dual Detection of Ag(I) and Fe(III) in environmental and E-waste matrices

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1 Instrumentation and Characterization

The surface morphology and internal nanostructure of RHNS and Br-Naphthophen@RHNS were characterized by High-Resolution Scanning Electron Microscopy (HR-SEM) on a JEOL JSM-7900F system (accelerating voltage: 15 kV) and Transmission Electron Microscopy (TEM) on a JEOL JEM-2100 microscope operating at 200 kv. Crystallographic phase analysis and verification of the amorphous silica framework were performed by X-ray Diffraction (XRD) on a Bruker D8 Advance diffractometer with Cu Kα radiation (λ = 1.5406 Å). Patterns were recorded over 2θ = 5–70° with a scanning step of 2° min^{-1}. Fourier-transform infrared (FTIR) spectra were recorded on a Bruker Alpha II spectrometer with an ATR unit, in the 400–4000 cm^{-1} range. Textural properties, including specific surface area and pore size distribution, were determined from N_2 adsorption–desorption isotherms at 77 K using a Micromeritics ASAP 2020 analyzer, following sample activation at 120 °C for 6 h under vacuum. Spectroscopic monitoring of the sensing mechanism was performed using a Shimadzu UV-2600 UV–vis spectrophotometer for absorbance measurements and stoichiometric analysis. The concentrations of metal ions were measured using a Seiko SPS-1500 inductively coupled plasma optical emission spectrometer (ICP-OES) produced by Seiko Instruments, located in Chiba, Japan. Theoretical insights into the electronic structure and binding energies were obtained via Density Functional Theory (DFT) calculations using the Gaussian 09 software suite, employing the B3LYP functional with the 6-31+G(d,p) basis set for light atoms and the LanL2DZ pseudopotential for metal centers.

Table S1: Tolerance concentration for interfering ions species by detection of [0.5 ppm] Ag^{+} and Fe^{3+} ions using BNP@RHNS.

Analyte	pH	Tolerance limit for foreign ions (ppm)															
		Ag^{+}	Fe^{3+}	Au^{3+}	Ca^{2+}	Cd^{2+}	Ce^{3+}	Co^{2+}	Cr^{3+}	Mg^{2+}	Hg^{2+}	Cu^{2+}	Mn^{2+}	Ni^{2+}	Pb^{2+}	Pd^{2+}	Zn^{2+}
Ag^{+}	6.2	Ref.	-	100	100	100	125	100	100	100	125	50	100	100	100	100	100
Fe^{3+}	3.6	-	Ref.	100	100	100	125	100	100	100	125	50	100	100	100	100	100

Table S2: Comprehensive tolerance limits and molar ratios for interfering ions

Interfering Ion	Tolerance Limit (ppm)	Molar Ratio (vs.0.5 ppm Ag^{+})	Molar Ratio (vs.0.5 ppm Fe^{3+})
Au^{3+}	100	110	57
Ca^{2+}	100	539	278
Cd^{2+}	100	192	99
Ce^{3+}	125	193	100
Co^{2+}	100	366	189
Cr^{3+}	100	415	214
Mg^{2+}	100	888	459
Hg^{2+}	125	135	70
Cu^{2+}	50	170	88
Mn^{2+}	100	393	203
Ni^{2+}	100	368	190
Pb^{2+}	100	104	54
Pd^{2+}	100	203	105
Zn^{2+}	100	330	170

2 Ligand Leaching Control Experiment

To evaluate the hydrolytic stability of the immobilized BNP ligand under the optimized acidic conditions required for Fe^{3+} detection, a leaching study was conducted. Approximately 20 mg of the BNP ligand sensor was suspended in 20 mL of a universal buffer adjusted to pH 3.6. The suspension was vortexed for 40 seconds, matching the maximum optimized response time of the analytical assay. Subsequently, the mixture was centrifuged at 8000 rpm for 15 minutes to isolate the solid phase. The resulting supernatant was analyzed via UV-Vis spectrophotometry within the spectral range of 200–600 nm to detect and quantify any released organic chromophores, using the free ligand calibration curve as a reference. Statistical analysis of three independent replicates (n=3) yielded a leaching percentage of 0.74 ± 0.03%. This negligible release confirms that the organic chromophore remains robustly associated with the silica framework during the analytical timeframe.

3 Hydrolytic Stability and Leaching Analysis of BNP@RHNS Sensor

To ensure the reliability of the spectrophotometric response, particularly at the acidic conditions required for Fe^{3+} detection (pH 3.6), the hydrolytic stability of the azomethine (-C=N-) linkage was rigorously evaluated. Although Schiff bases are potentially susceptible to acid-catalyzed hydrolysis, the BNP@RHNS sensor demonstrated remarkable structural integrity during the analytical timeframe. A leaching control experiment was conducted by incubating 20 mg of the sensor in a universal buffer at pH 3.6 for the maximum optimized response time of 40 seconds. Following centrifugation at 8000 rpm, UV-Vis analysis of the supernatant revealed a negligible absorbance signal, confirming that less than 0.9% of the immobilized ligand was released into the aqueous phase. This high stability is attributed to the pore-filling mechanism and the protective microenvironment provided by the mesoporous RHNS channels, which shield the anchored BNP

molecules from direct hydrolytic attack. Furthermore, the rapid detection kinetics (< 40 seconds) effectively bypass the slower timeframe typical of imine degradation. The sensor's robustness is further corroborated by its successful regeneration and high performance over six consecutive cycles using 0.1 M HCl, demonstrating that the observed spectrophotometric signal at pH 3.6 arises from genuine Fe^{3+} coordination within the N_2O_2 cavity rather than ligand decomposition products.

4 Thermal Stability Control

To differentiate between the analytical signal and potential thermal degradation of the organic framework, a control experiment was performed on the BNP@RHNS sensor without metal ions across the temperature range of 20–50 °C. The sensor was suspended in water and equilibrated at each temperature for 10 minutes, significantly longer than the standard analytical response time. Following centrifugation, UV-Vis spectra of the supernatants were recorded. The results showed a flat baseline with no characteristic absorbance peaks associated with the BNP ligand or its components, indicating that the leaching percentage remained beneath the detection threshold even at 50 °C. This confirms that the observed enhancement in the spectrophotometric signal with increasing temperature is a direct consequence of the endothermic nature of the Ag^{+} and Fe^{3+} complexation process on the BNP@RHNS surface.

Table S3. Quantitative Reusability Data for BNP@RHNS Sensor (n=3)

Cycle Number	Ag^{+} Signal Response (%) (Mean ± SD)	Fe^{3+} Signal Response (%) (Mean ± SD)
Cycle 1	98.10 ± 1.20	96.40 ± 1.40
Cycle 2	97.60 ± 1.50	95.10 ± 1.60
Cycle 3	95.80 ± 1.80	92.30 ± 1.90
Cycle 4	92.20 ± 2.1	90.80 ± 2.20
Cycle 5	89.50 ± 2.4	87.70 ± 2.50
Cycle 6	87.10 ± 2.7	84.20 ± 2.90

5 DFT Computational Analysis

Density functional theory (DFT) calculations were performed to elucidate the electronic structure of the free ligand (BNP), the $[BNP–Ag]^{+}$ complex, and the $[BNP–Fe]^{+3}$ complex. The free BNP ligand was fully geometry-optimized at the B3LYP/6-31G(d,p) level of theory, converging after 31 optimization cycles to a stationary point confirmed by the absence of imaginary frequencies (total electronic energy: E = −3910.12096507 Hartree; ZPE correction: 0.3978 Hartree; Table S5). Single-point energy calculations with time-dependent DFT (TD-DFT, six lowest excited states) were subsequently carried out for both metal complexes at the UB3LYP/LANL2DZ level using experimentally informed input geometries. Full Cartesian coordinates for all three species are compiled in Table S4; selected geometric parameters are presented in Table S6; total energies are summarized in Table S5. The free BNP ligand exhibits a HOMO–LUMO gap of 3.36 eV (HOMO = −5.36 eV; LUMO = −1.99 eV), consistent with a moderate-gap conjugated Schiff base framework possessing π-donor and π*-acceptor character. Formation of the $[BNP–Ag]^{+}$ complex perturbs the electronic manifold into two distinct spin channels arising from the open-shell doublet ground state (S = 1/2; $\langle S^2 \rangle$ = 0.761 before annihilation, 0.750 after). The α-channel HOMO–LUMO gap widens to 3.91 eV relative to the free ligand, reflecting the stabilization of metal d-type occupied orbitals upon N,N-bidentate coordination. Crucially, the β-channel gap narrows markedly to 1.08 eV, with the singly occupied β-LUMO at −4.49 eV constituting a low-lying ligand-to-metal charge-transfer (LMCT) acceptor manifold that underpins the selective chromogenic response toward Ag^{+} (Table S5). The [BNP–Fe] complex presents a substantially compressed HOMO–LUMO gap of 1.34 eV (HOMO = −5.28 eV; LUMO = −3.94 eV), consistent with the highly polarizable electronic environment induced by Fe^{2+} and

with the observed quench-type optical response. Complete frontier orbital energy data are collected in Table S6.

Table S4; Optimized Cartesian coordinates for the (a) free BNP ligand, (b) $[BNP–Ag]^{+}$ Complex, and (c) $[BNP–Fe]^{+3}$ Complex.

(a) Free BNP Ligand

No.	Elem.	X (Å)	Y (Å)	Z (Å)
1	**C**	1.335179	3.739574	0.221397
2	**C**	1.760251	2.423028	0.359635
3	**C**	0.818548	1.386500	0.466380
4	**C**	-0.568207	1.700471	0.428822
5	**C**	-0.957393	3.042198	0.299977
6	**C**	-0.018713	4.066018	0.193953
7	**Br**	2.640006	5.134544	0.098234
8	**N**	1.181968	0.058755	0.675256
9	**C**	2.140737	-0.474136	0.008761
10	**C**	2.592313	-1.856749	0.169494
11	**C**	3.856045	-2.272250	-0.393730
12	**C**	4.229097	-3.656701	-0.362147
13	**C**	3.351716	-4.592252	0.245491
14	**C**	2.170472	-4.188721	0.809636
15	**C**	1.783940	-2.824405	0.785576
16	**C**	4.785770	-1.364906	-0.975021
17	**C**	5.978001	-1.800859	-1.513290
18	**C**	6.325100	-3.169460	-1.497158
19	**C**	5.462893	-4.075003	-0.923950
20	**O**	0.612996	-2.447748	1.339449
21	**O**	-2.073012	-1.650129	1.553512
22	**C**	-7.123002	-1.383380	-0.807738
23	**C**	-7.519088	-0.323201	-1.592399
24	**C**	-6.624166	0.745159	-1.808069
25	**C**	-5.363633	0.739187	-1.245464
26	**C**	-3.263712	-1.511723	0.967648
27	**C**	-4.193737	-2.567309	1.167297
28	**C**	-5.433522	-2.517256	0.592977
29	**C**	-5.834742	-1.417692	-0.218781
30	**C**	-4.917676	-0.338420	-0.434390
31	**C**	-3.607194	-0.392574	0.176829
32	**C**	-2.637473	0.664000	-0.015180
33	**N**	-1.476976	0.645107	0.562176
34	**H**	2.817386	2.194040	0.423136
35	**H**	-2.012846	3.294349	0.324252
36	**H**	-0.333377	5.099899	0.116272
37	**H**	2.647215	0.100612	-0.777259
38	**H**	3.634615	-5.641156	0.266170
39	**H**	1.509791	-4.910011	1.285118
40	**H**	4.575798	-0.302230	-0.982065
41	**H**	6.662790	-1.077785	-1.946763
42	**H**	7.267672	-3.497973	-1.923709
43	**H**	5.715591	-5.131539	-0.886931
44	**H**	0.072764	-3.215829	1.569389
45	**H**	-1.511790	-0.844432	1.323054
46	**H**	-7.796653	-2.216888	-0.625612

47	**H**	-8.507975	-0.306008	-2.039726
48	**H**	-6.928886	1.586526	-2.423794
49	**H**	-4.713607	1.585037	-1.436483
50	**H**	-3.882205	-3.401379	1.787015
51	**H**	-6.138976	-3.328783	0.751785
52	**H**	-2.899146	1.485654	-0.687104

(b) $[BNP–Ag]^+$ Complex

No.	Elem.	X (Å)	Y (Å)	Z (Å)
1	**C**	0.122846	4.091400	0.168875
2	**C**	0.089523	3.071615	-0.693945
3	**C**	0.037183	1.723579	-0.165481
4	**C**	0.047856	1.492022	1.132122
5	**C**	0.070440	2.575047	2.094145
6	**C**	0.107800	3.830160	1.637171
7	**Br**	0.191260	5.877896	-0.475644
8	**N**	0.052983	0.532961	-0.900324
9	**C**	1.518176	0.207284	-1.209770
10	**C**	2.199147	-1.194751	-0.199201
11	**C**	3.533499	-1.384630	-0.248799
12	**C**	4.178916	-2.498350	0.500866
13	**C**	3.512551	-3.374362	1.258424
14	**C**	2.073961	-3.327081	1.423803
15	**C**	1.365546	-2.393383	0.818251
16	**C**	4.432417	-0.510788	-1.055014
17	**C**	5.745738	-0.736722	-1.085528
18	**C**	6.355697	-1.840798	-0.335625
19	**C**	5.619517	-2.663057	0.407005
20	**O**	-0.081877	-2.376665	0.991574
21	**O**	-0.047753	-1.959287	-1.367572
22	**C**	-5.760260	-2.130083	-0.866147
23	**C**	-6.459622	-1.574314	0.119495
24	**C**	-5.800215	-0.826848	1.196421
25	**C**	-4.476590	-0.672955	1.233605
26	**C**	-1.494842	-1.958131	-1.197746
27	**C**	-2.244989	-2.589882	-2.079946
28	**C**	-3.685333	-2.615879	-1.928122
29	**C**	-4.312404	-2.018442	-0.910679
30	**C**	-3.616775	-1.263823	0.169225
31	**C**	-2.273862	-1.138670	0.175866
32	**C**	-1.529779	-0.205577	1.599649
33	**N**	-0.044189	0.124592	1.411062
34	**Ag**	-0.029939	-0.908404	0.035876
35	**H**	0.103088	3.244652	-1.739860
36	**H**	0.056450	2.376492	3.135604
37	**H**	0.126225	4.642151	2.319574
38	**H**	1.596620	-0.088359	-2.264154
39	**H**	2.156538	1.094365	-1.092200
40	**H**	4.057766	-4.137210	1.753045
41	**H**	1.587543	-4.048017	2.030229
42	**H**	4.055368	0.295243	-1.613198
43	**H**	6.365694	-0.102754	-1.666723
44	**H**	7.404611	-1.987094	-0.387205
45	**H**	6.089518	-3.452905	0.935958
46	**H**	-6.265717	-2.665605	-1.629285
47	**H**	-7.515260	-1.674559	0.127253
48	**H**	-6.391790	-0.398405	1.964899

49	**H**	-4.063347	-0.127409	2.030517
50	**H**	-1.790925	-3.084173	-2.900776
51	**H**	-4.264787	-3.133882	-2.649126
52	**H**	-2.117179	0.702757	1.795424
53	**H**	-1.638714	-0.837443	2.490702

(c) [BNP–Fe] $^{+3}$ Complex

No.	Elem.	X (Å)	Y (Å)	Z (Å)
1	**C**	-0.753442	3.396002	-0.198706
2	**C**	-1.115861	2.310365	0.493894
3	**C**	-0.473396	1.052311	0.209672
4	**C**	0.551958	0.982210	-0.857769
5	**C**	0.918022	2.181710	-1.568090
6	**C**	0.304207	3.330552	-1.262913
7	**Br**	-1.592557	5.059032	0.176021
8	**N**	-0.787607	-0.382478	1.006627
9	**C**	-2.148234	-0.763974	1.307144
10	**C**	-2.929270	-1.392852	0.182180
11	**C**	-4.140975	-1.936140	0.405889
12	**C**	-4.884998	-2.519132	-0.699929
13	**C**	-4.386242	-2.511152	-1.929316
14	**C**	-3.085839	-1.921086	-2.175462
15	**C**	-2.388027	-1.395107	-1.172990
16	**C**	-4.775788	-1.967411	1.748904
17	**C**	-5.977368	-2.523256	1.907829
18	**C**	-6.705012	-3.115733	0.772120
19	**C**	-6.187267	-3.111521	-0.454917
20	**O**	-1.179518	-0.868812	-1.445610
21	**O**	1.592884	-0.085511	1.312450
22	**C**	6.960389	-1.551232	0.967472
23	**C**	7.513476	-1.852675	-0.205969
24	**C**	6.730118	-1.801623	-1.452587
25	**C**	5.441646	-1.458199	-1.449640
26	**C**	2.886713	-0.434269	1.193862
27	**C**	3.633660	-0.469063	2.293614
28	**C**	5.028647	-0.854077	2.216530
29	**C**	5.563373	-1.164513	1.042655
30	**C**	4.764195	-1.117174	-0.172273
31	**C**	3.465613	-0.766911	-0.103447
32	**C**	2.620117	-0.686489	-1.349335
33	**N**	1.197533	-0.534066	-1.145297
34	**Fe**	0.206699	-0.473764	-0.066721
35	**H**	-1.861648	2.375737	1.245008
36	**H**	1.659446	2.152635	-2.325629
37	**H**	0.568415	4.214251	-1.786191
38	**H**	-2.162230	-1.444830	2.168956
39	**H**	-2.702933	0.128836	1.628992
40	**H**	-4.937375	-2.937310	-2.728317
41	**H**	-2.684688	-1.916712	-3.156792
42	**H**	-4.290915	-1.554711	2.593204
43	**H**	-6.418072	-2.539134	2.872052
44	**H**	-7.657691	-3.551974	0.934163
45	**H**	-6.728855	-3.542284	-1.257977
46	**H**	7.543185	-1.592984	1.852046

47	**H**	8.535348	-2.132763	-0.243833
48	**H**	7.200447	-2.043345	-2.371650
49	**H**	4.919517	-1.435700	-2.368821
50	**H**	3.203847	-0.224356	3.231286
51	**H**	5.620277	-0.890183	3.095406
52	**H**	2.984566	0.170772	-1.932778
53	**H**	2.815768	-1.571166	-1.969678

Table S5: Total electronic energies (Hartree) and zero-point energy (ZPE) corrections for all computed structures.

Species	**E_{al} (Hartree)**	**ZPE (Hartree)**	**E + ZPE (Hartree)**
Free Ligand (BNP)	-3910.12096507	0.397764	-3909.72320107
$[BNP–Ag]^{+}$ Complex	-1496.27776109	N/A [a]	—
$[BNP–Fe]^{+3}$ Complex	-1474.20641363	N/A [a]	—

[a] ZPE unavailable for metal complexes

Table S6: Selected bond lengths (Å), bond angles (°), and HOMO–LUMO gaps (eV) at key structural and coordination sites

Parameter	**Free Ligand**	**$[BNP–Ag]^{+}$**	**$[BNP–Fe]^{+3}$**
Bond Lengths (Å)			
C=N (imine, C9–N1)	1.2835	1.5325	1.4447
C–O (phenolate, C15–O1)	1.3490	1.4579	1.3460
N1···N2 (bite distance)	2.7252	2.3492	2.4318
M–N1 (imine N)	—	2.1–2.3	2.0–2.2
M–N2 (amine N)	—	2.1–2.3	2.0–2.2
M–O1 (phenolate O)	—	—	1.9947
M–O2	—	—	1.9936
Bond Angles (°)			
N1–M–N2	—	86.12	178.76
N1–M–O1	—	—	92.70
N2–M–O2	—	—	92.70
O1–M–O2	—	—	179.81
HOMO–LUMO Gap (ΔE, eV)			
ΔE (HOMO–LUMO)	3.3644	3.9119 (α) / 1.0770 (β)	1.3445
HOMO (eV)	−5.3577	−5.6447 (α)	−5.2834
LUMO (eV)	−1.9932	−1.7328 (α)	−3.9389

Table S7: Total electronic energies for $[BNP–Ag]^{+}$ and $[BNP–Fe]^{3+}$.

Complex	E(complex) (Ha)	Level
$[BNP–Ag]^{+}$	-1496.27776109	UB3LYP/LANL2DZ
[BNP–Fe]	-1474.20641363	UB3LYP/LANL2DZ