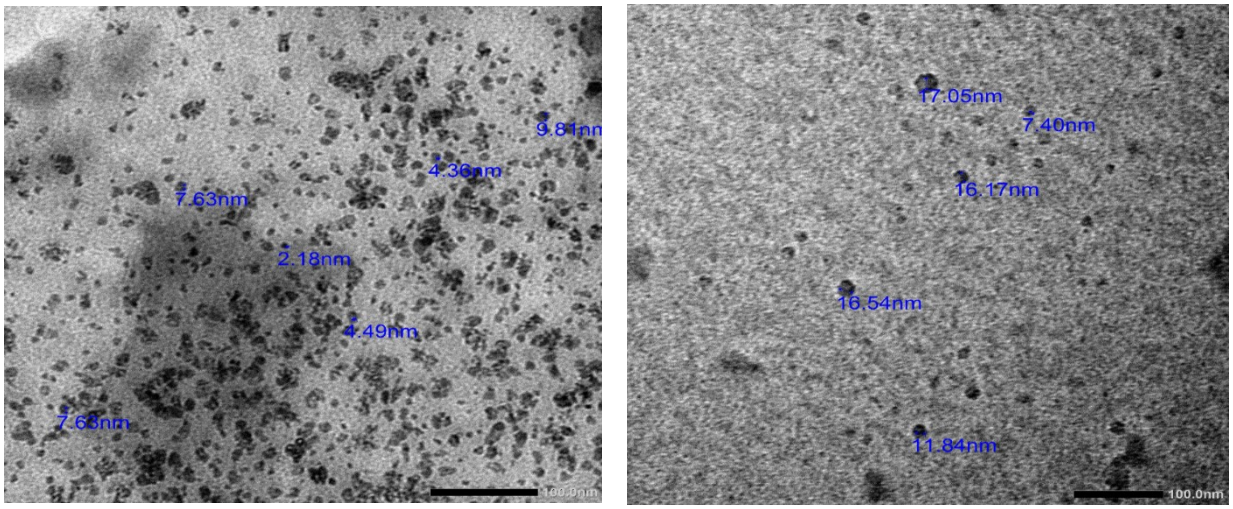
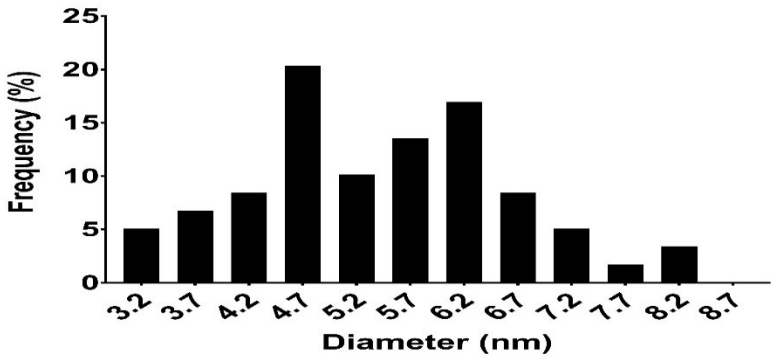
A)



B)



C)

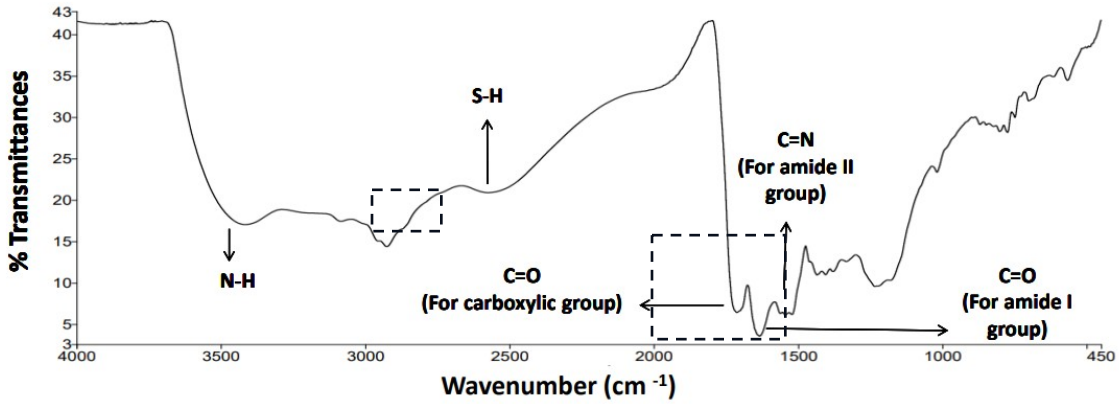


Figure S1: (A) TEM images of NSC-dots. (B)TEM size distribution diagram of NSC-dots.

(C) FTIR spectrum of the NSC-dots.

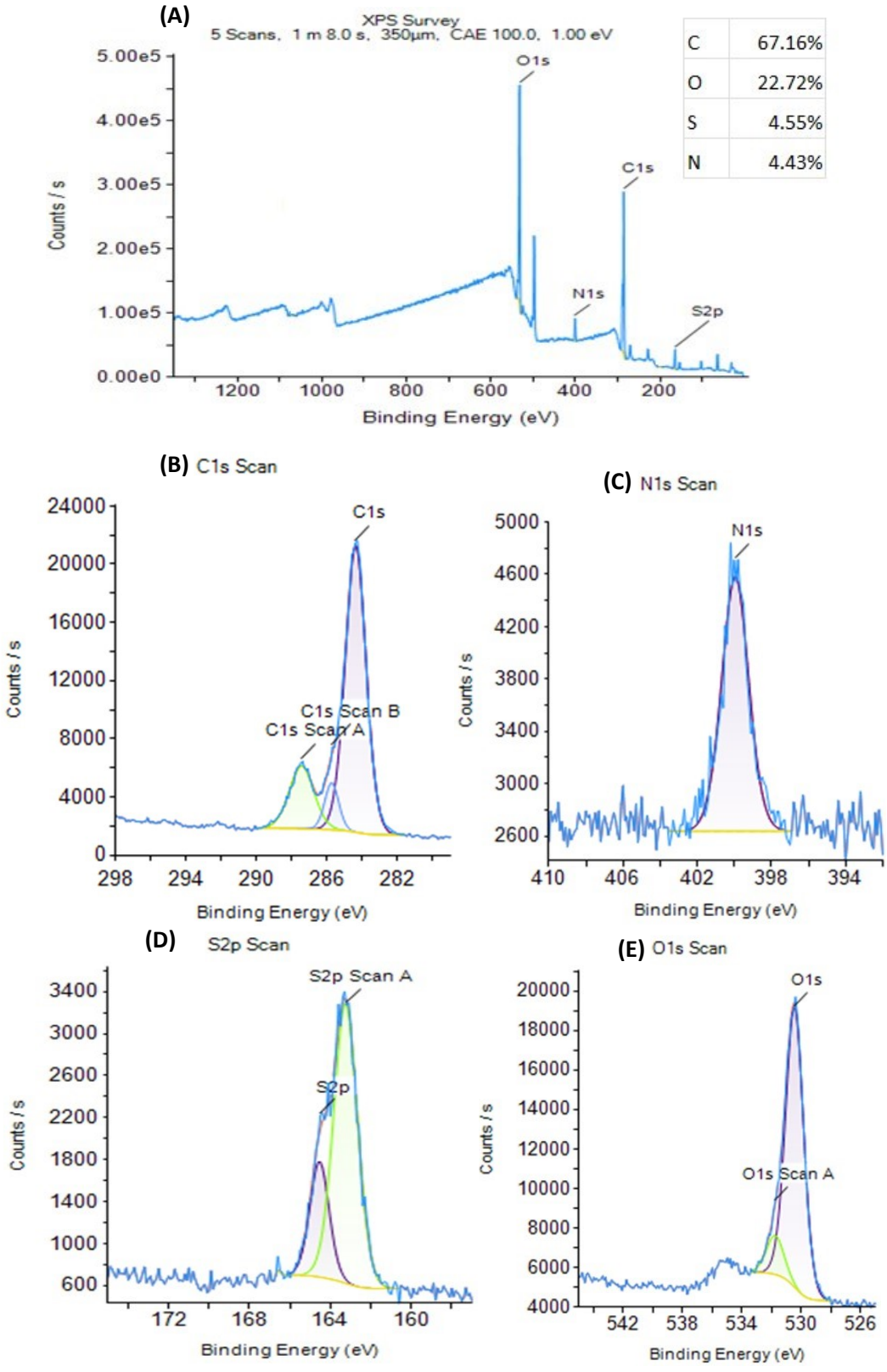

(A)
XPS Survey
5 Scans, 1 m 8.0 s, 350µm, CAE 100.0, 1.00 eV
C 67.16%
O 22.72%
S 4.55%
N 4.43%
O1s
C1s
N1s
S2p
Counts / s
Binding Energy (eV)
(B) C1s Scan
C1s
C1s Scan B
C1s Scan A
(C) N1s Scan
N1s
(D) S2p Scan
S2p Scan A
S2p
(E) O1s Scan
O1s
O1s Scan A

Figure S2: XPS spectrum of NSC-dots with atomic content (A). High resolution C 1s (B), N 1s (C), S2p (D), and O 1s (E) XPS spectra of NSC-dots.

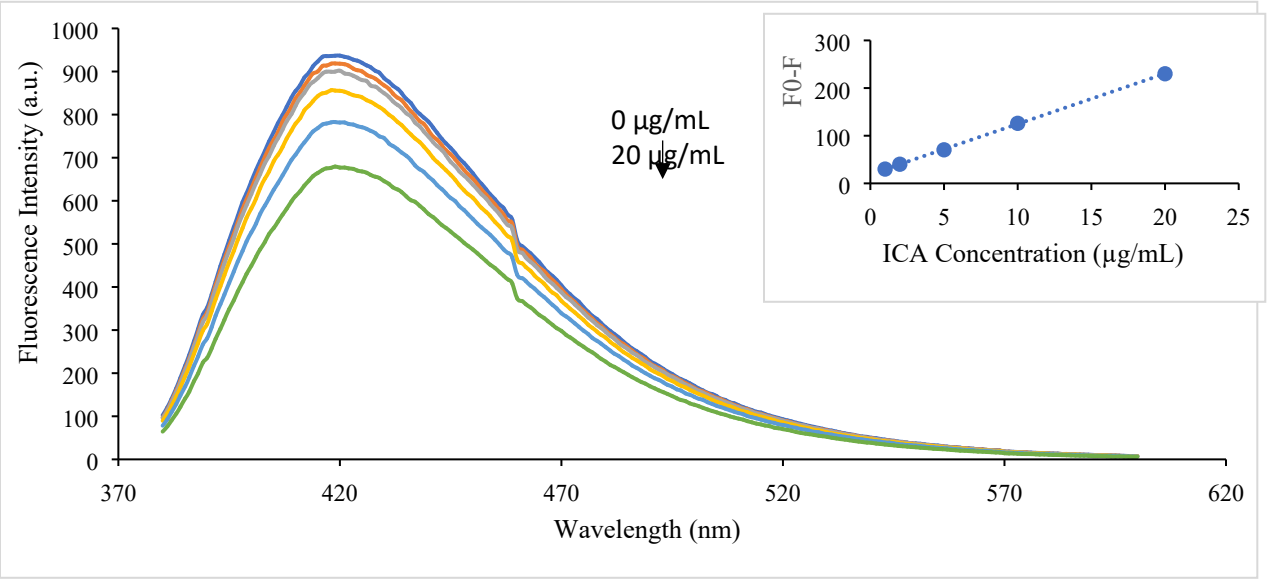


Figure S3: Fluorescence emission spectra of 0.1 mL NSC-dots in aqueous solution upon addition of various ICA concentrations (0.0-20 µg/mL) at λex = 370 nm. The inset represents the corresponding calibration curve.

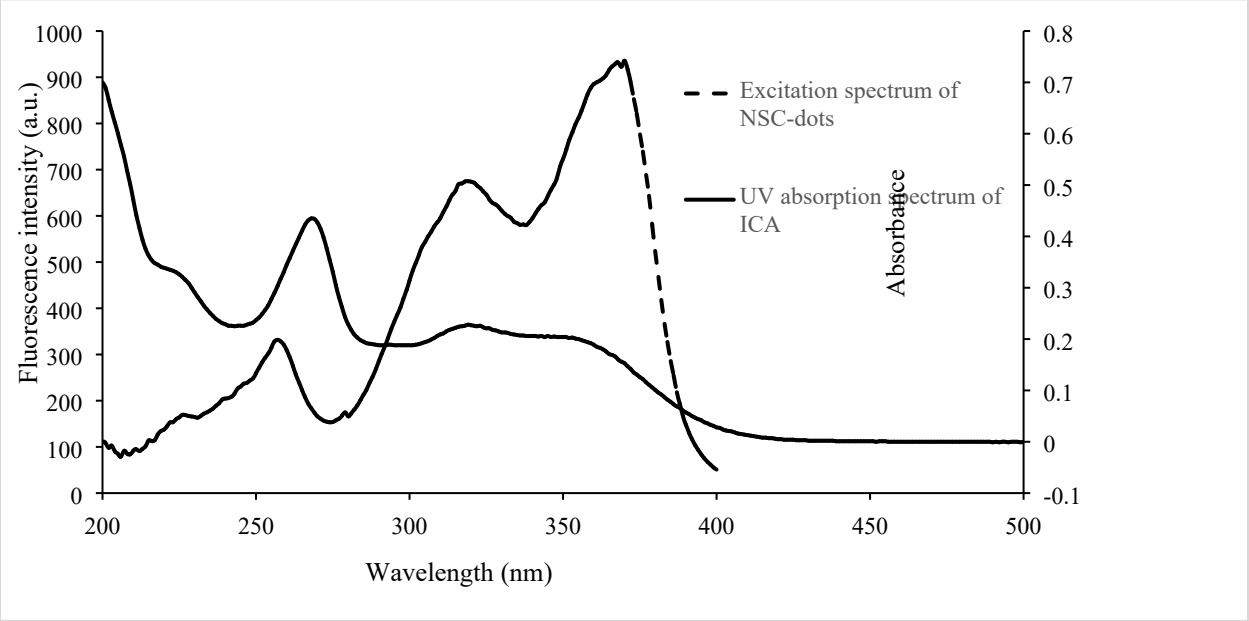


Figure S4: Plot showing overlap between UV-vis absorption spectrum of ICA (20 µg/mL) and fluorescence excitation scan of NSC-dots.

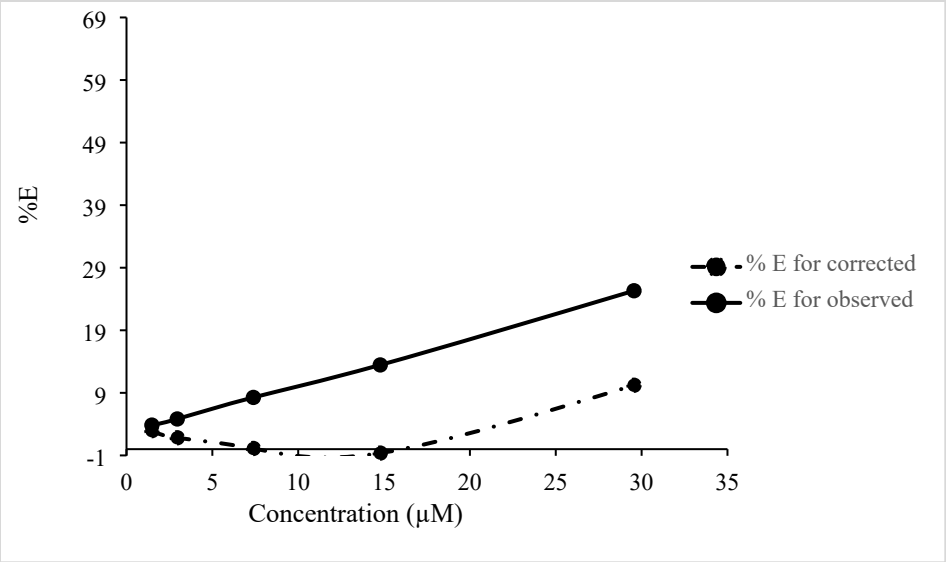


Figure S5: Suppressed efficiency (% E) of observed and corrected fluorescence of NSC-dots after addition of different molar concentrations of ICA.

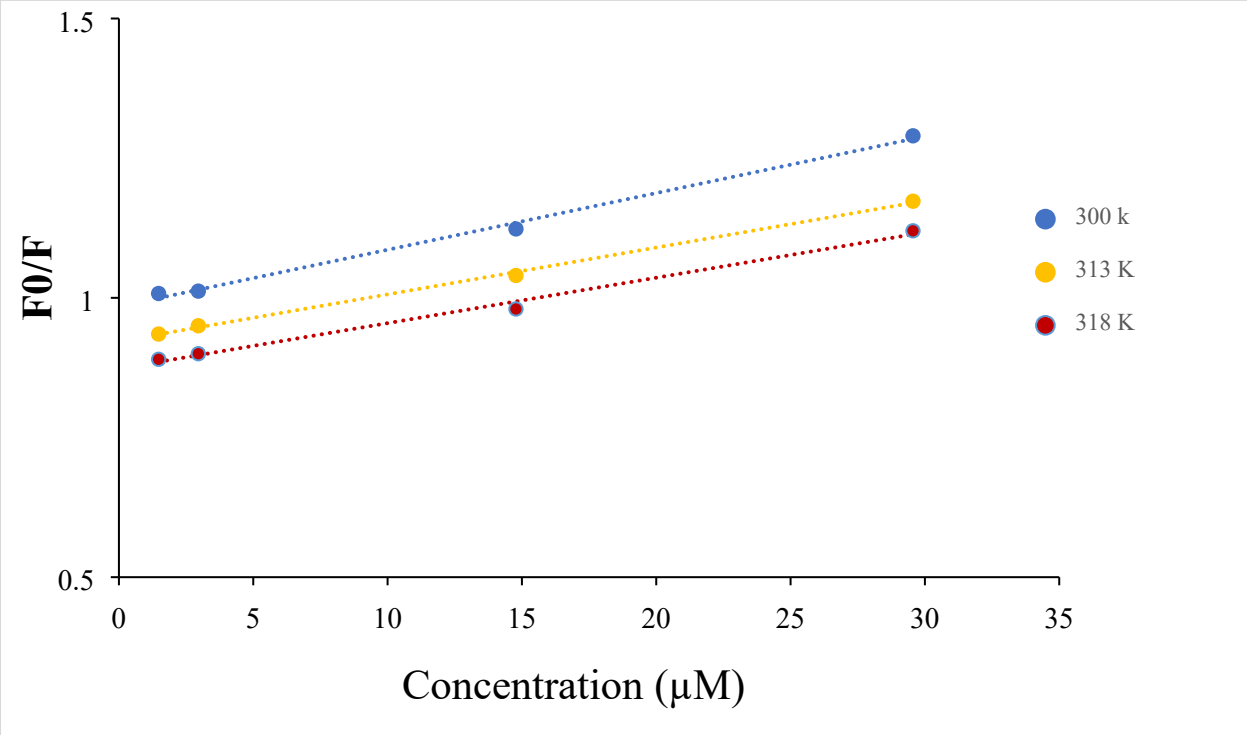
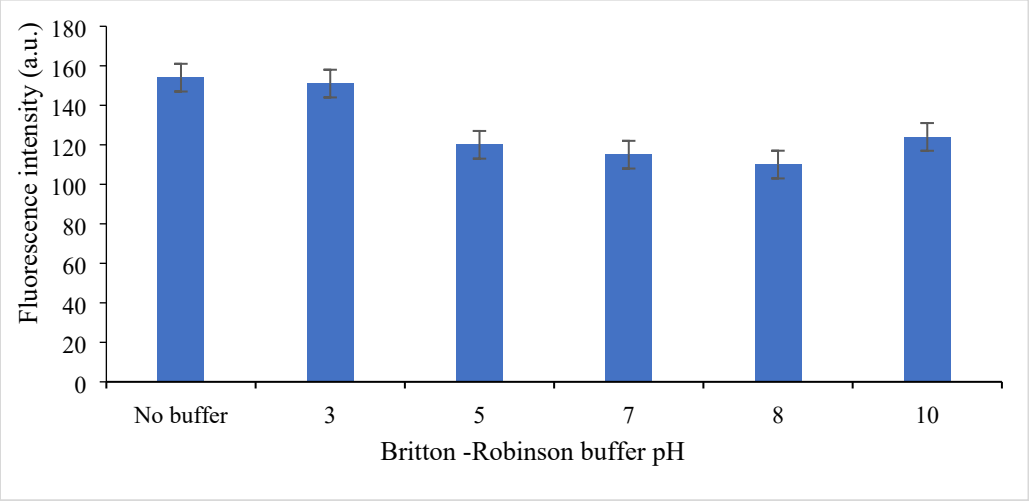
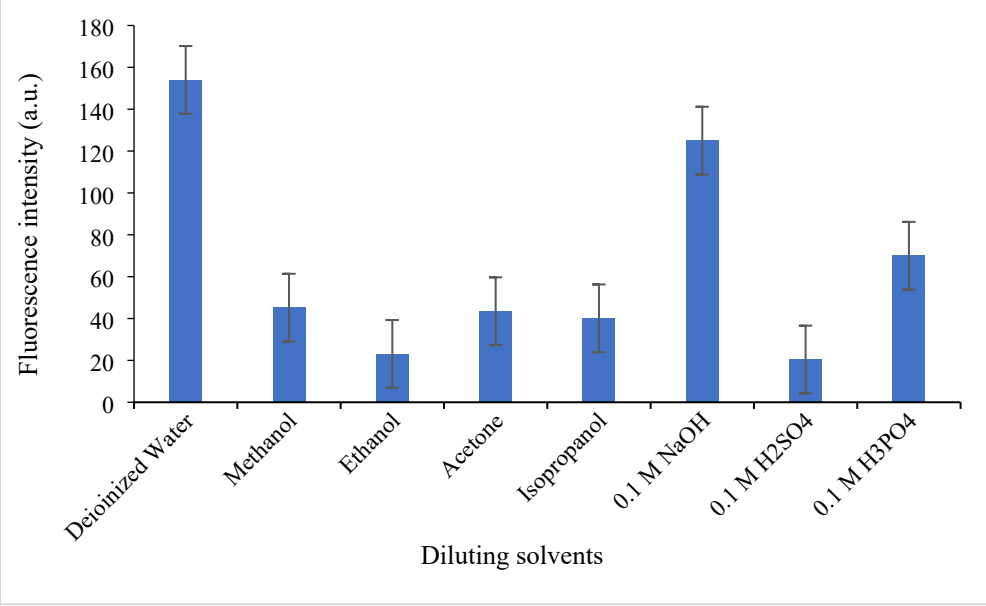


Figure S6: Stern– Volmer plot between F_0/F and concentration (μM) of ICA at different temperatures.

(A)



(B)



(C)

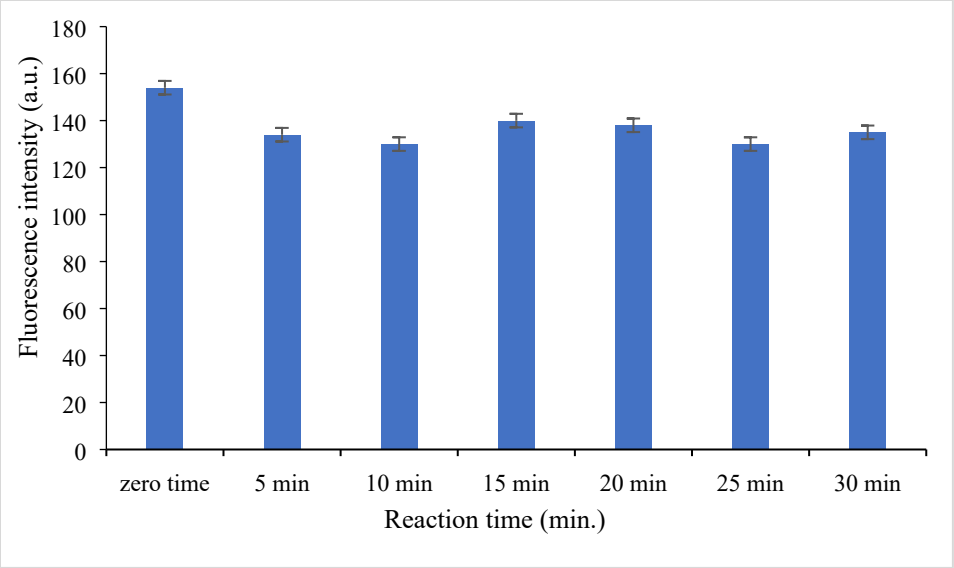


Figure S7: (A) Effect of Britton Robinson buffer pH (B) Effect of the diluting solvents. (C) Effect of incubation time on fluorescence quenching of NSC-dots by 10 µg mL^{-1} ICA.

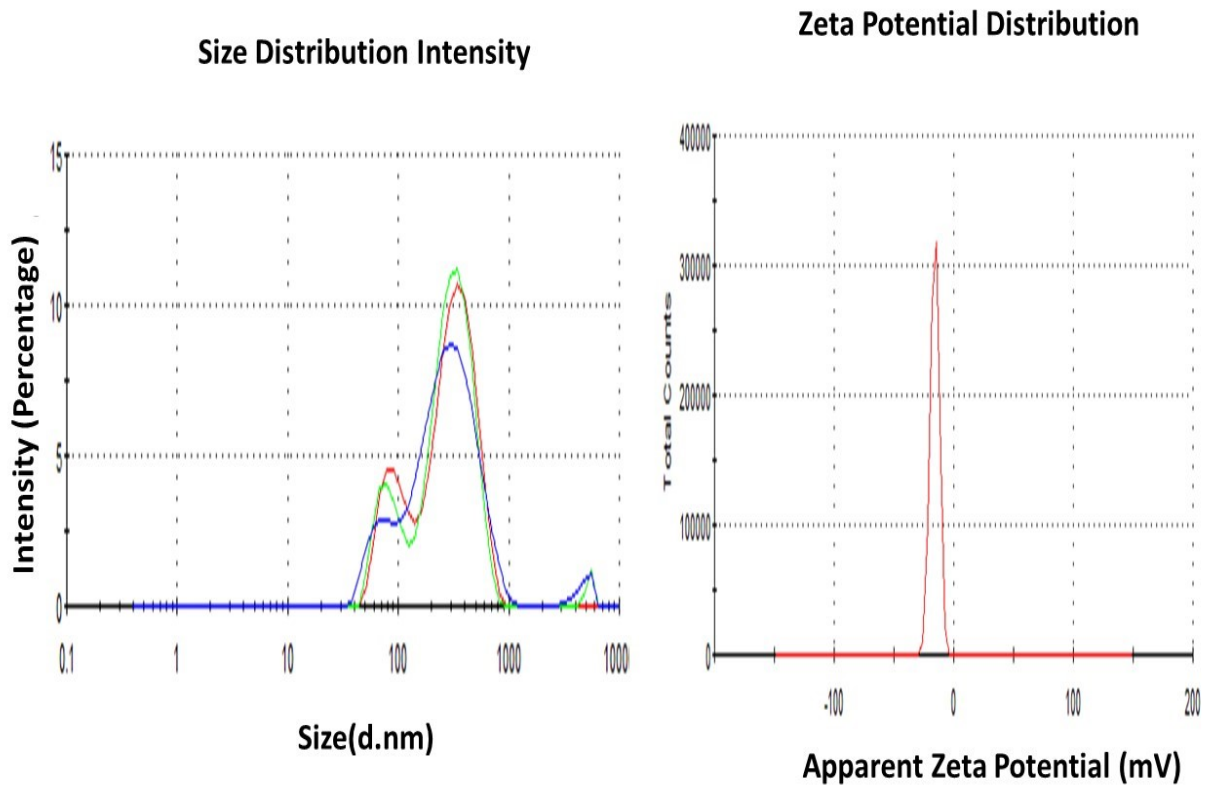


Figure S8: Particle size distribution, and zeta potential distribution of Icariin loaded whey protein nanoparticles.

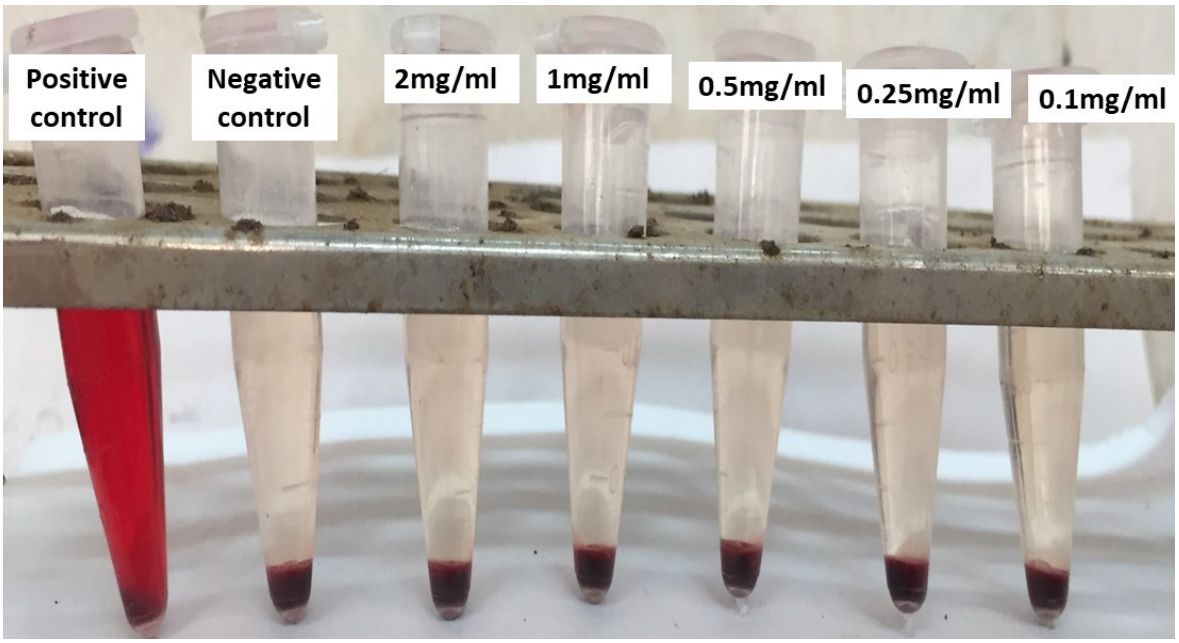


Figure S9: In vitro hemolysis image of ICA loaded whey protein nanoparticles

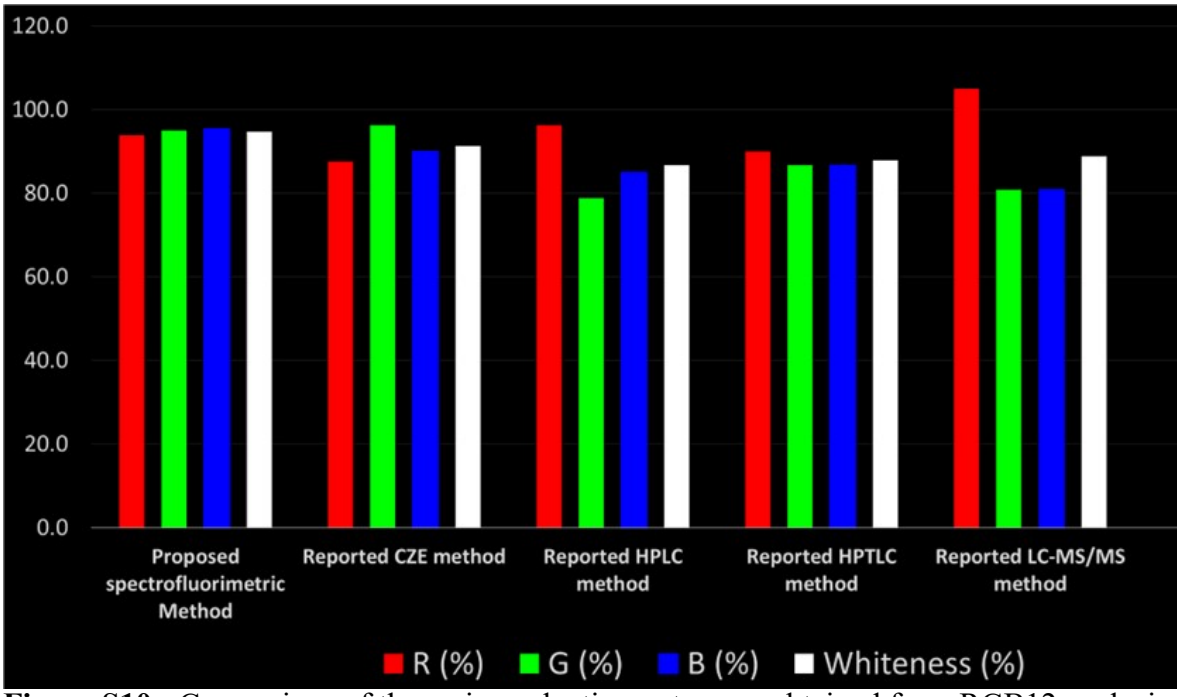


Figure S10: Comparison of the main evaluation outcomes obtained from RGB12 analysis of the proposed spectrofluorimetric method for determination of ICA with other reported methods. The white bar (whiteness %) indicates the arithmetic mean of the three other bars (red, green and blue).

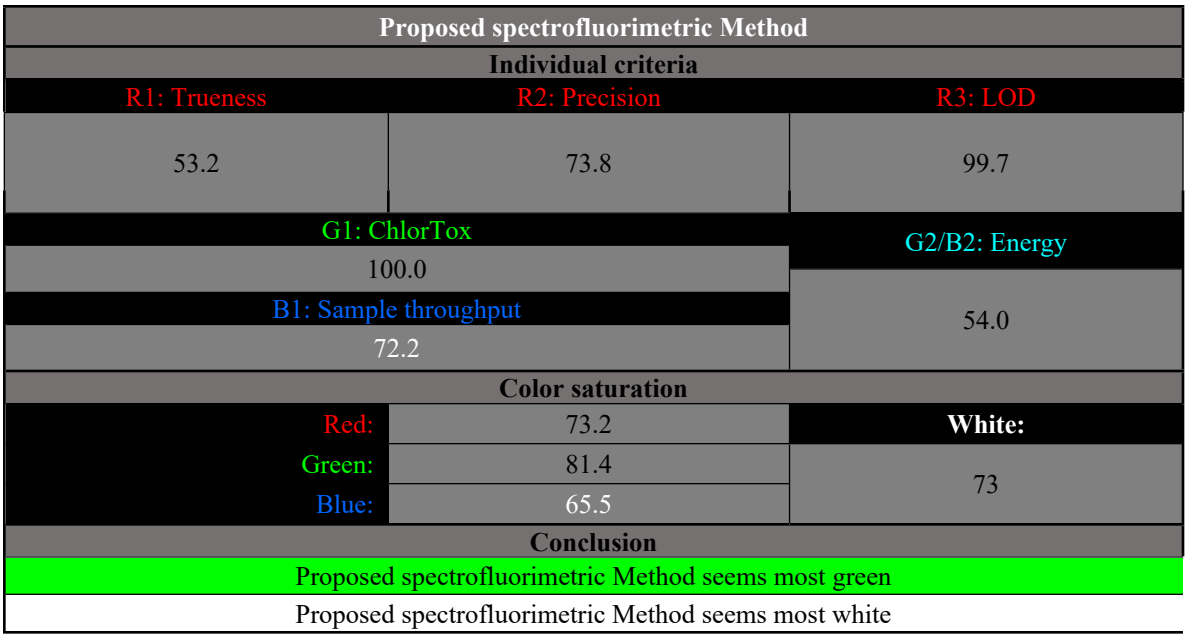


Figure S11: Comparison of the proposed spectrofluorimetric method for determination of ICA with other reported methods in terms of the saturation of red, green, blue colors, and resulting whiteness as a holistic assessment using RGBfast.

Table S1: Analytical eco-scale for assessment of greenness of the proposed spectrofluorimetric method compared to other reported methods.

The penalty points (PPs) to calculate Analytical Eco -scale					
Reagent	**Proposed spectrofluorimetric method**	**Reported capillary zone electrophoresis method (CZE)**	**Reported HPLC method**	**Reported HPTLC Method**	**Reported LC-MS/MS**
Deionized water	0				
L-cysteine	1				
Citric acid	1				
Sodium Bicarbonate	1				
Borate		4			
Glacial acetic acid				4	
Ethyl acetate				8	
Formic acid				6	6
Trifluoroacetic acid			4		

Acetonitrile		4	4		4
Instrument	**Penalty points**				
Energy	0	0	1	1	2
Waste	3	1	5	3	3
Occupational hazards	0	0	0	0	0
Final total penalty points	6	9	14	22	15
Analytical eco-scale score	94	91	86	78	85

Table S2: RGB12 profiles of the proposed spectrofluorimetric method and other reported methods:

Method number	Method name	R (%)	G (%)	B (%)	Whiteness (%)
1	**Proposed spectrofluorimetric Method**	93.8	95.0	95.4	94.7
2	**Reported CZE method**	87.5	96.3	90.0	91.3
3	**Reported HPLC method**	96.3	78.8	85.0	86.7
4	**Reported HPTLC method**	90.0	86.7	86.7	87.8
5	**Reported LC-MS/MS method**	105.0	80.8	80.8	88.9

Table S3: NQS final scores of the proposed spectrofluorimetric method

Need	**100**
Quality	**95**
Sustanability	**76**
NQS Index	**90**