Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Tuning Residual Chirality in Carbon Dots with Anti-Microbial Properties in Gram Negative Bacteria

Florence Victoria,[a,b,c] John Manioudakis,[a,b,c] Liana Zaroubi,[a] Brandon Findlay,[a]

Rafik Naccache[a,b,c,*]

[a]*Department of Chemistry and Biochemistry, Concordia University, Montreal, QC, Canada, H4B 1R6*

[b]*Centre for NanoScience Research, Concordia University, Montreal, QC, Canada, H4B 1R6*

[c]*Quebec Centre for Advanced Materials, Department of Chemistry and Biochemistry, Concordia University, Montreal, QC, H4B 1R6, Canada*

*corresponding author e-mail: rafik.naccache@concordia.ca

Table S1. The summary of reaction parameters used to synthesize cysCDs. The L CDs were synthesized using L-cysteine and the D CDs were synthesized using D-cysteine. The reaction conditions were controlled and monitored using the *CEM Discover SP microwave* synthetic and its software.

Carbon Dots	**Concentration of Cysteine**	**Concentration of Citric Acid**	**Reaction Conditions**
1 CYS: 1 CA	0.5 M	0.5 M	160°C for 5 mins 180°C for 5 mins 200°C for 5 mins 220°C for 5 mins 160°C for 10 mins 160°C for 15 mins
1 CYS: 2 CA	0.5 M	0.25 M	160°C for 10 mins
2 CYS: 1 CA	0.25 M	0.5 M	160°C for 10 mins

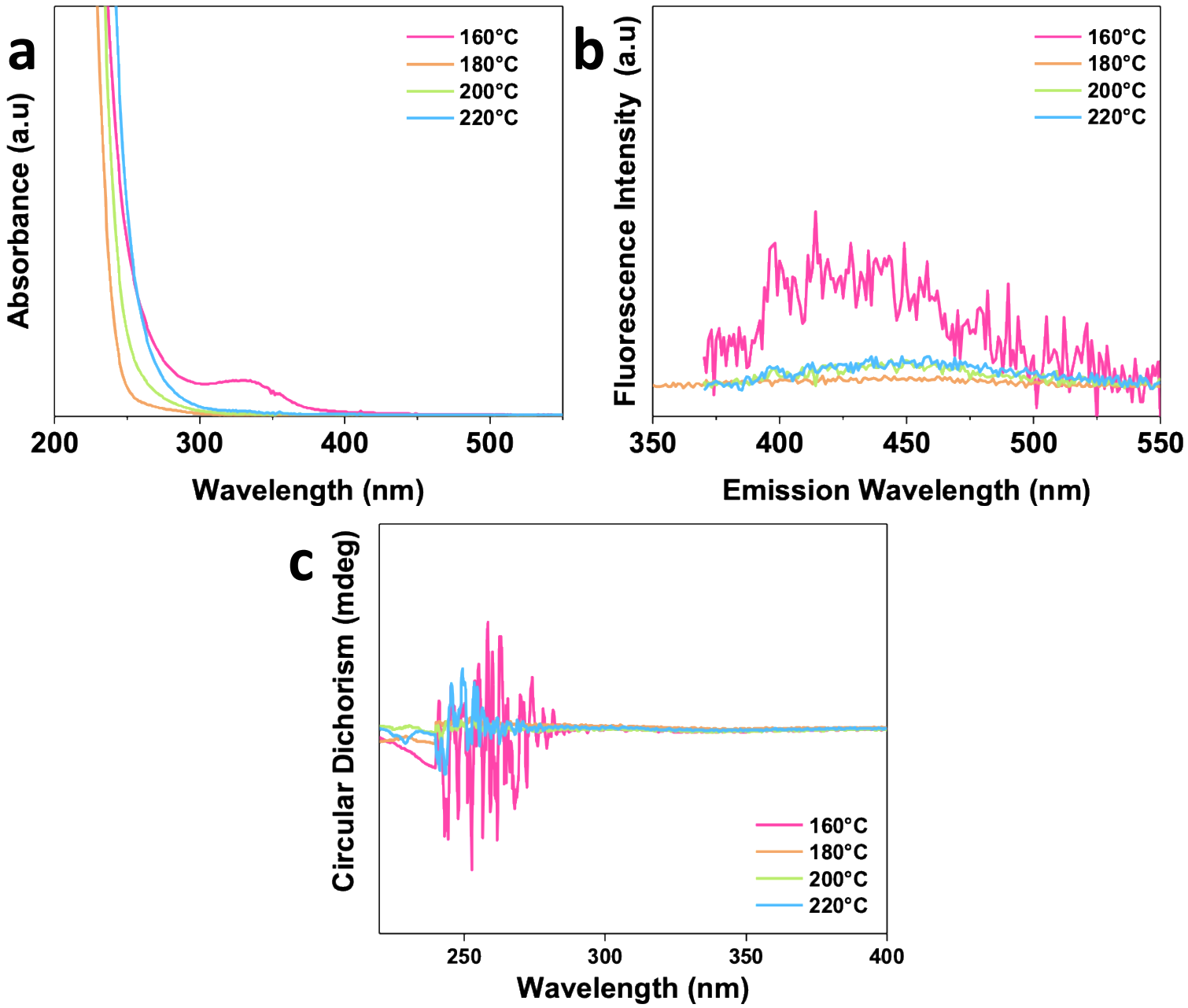

Figure S1. The optical and physical properties of unpassivated CDs synthesized at different reaction temperature at 5 minutes reaction time citric acid. (a) The unpassivated CDs synthesized at various temperatures exhibit absorbance bands at 350 nm corresponding to n → π* transitions, respectively, (b) the fluorescence intensity of the unpassivated CDs are negligible at an excitation wavelength of 350 nm, (c) the circular dichroism spectra of the unpassivated CDs indicate that there is no preferential absorbance of the polarized light.

Table S2. Summary of cysCD sizes measured following synthesis at different reaction parameters. The cysCDs seem to slightly increase in size as a function of synthesis temperature and time. The size of these nanoparticles seem unaffected by function of reaction precursor. it is difficult to conclusively interpret due to the large polydispersity values measured.

		Size (nm)
Temperature (All reactions were done at 5 mins)	**160 °C**	15.1 ± 3.7
	180 °C	18.5 ± 5.6
	200 °C	21.1 ± 5.1
	220 °C	17.1 ± 3.7
Time (All reactions were done at 160 °C)	**5 mins**	15.1 ± 3.7
	10 mins	13.3 ± 4.1
	15 mins	12.2 ± 3.3
Ratio (All reactions were done at 160 °C- 5 mins)	**1 L-CYS : 1 CA**	13.3 ± 4.1
	1 L- CYS : 2 CA	15.1 ± 3.7
	2 L-CYS : 1 CA	14.8 ± 2.7
	1 D-CYS : 1 CA	14.0 ± 4.5
	1 D-CYS : 2 CA	11.8 ± 3.0
	2 D-CYS : 1 CA	14.5 ± 4.9

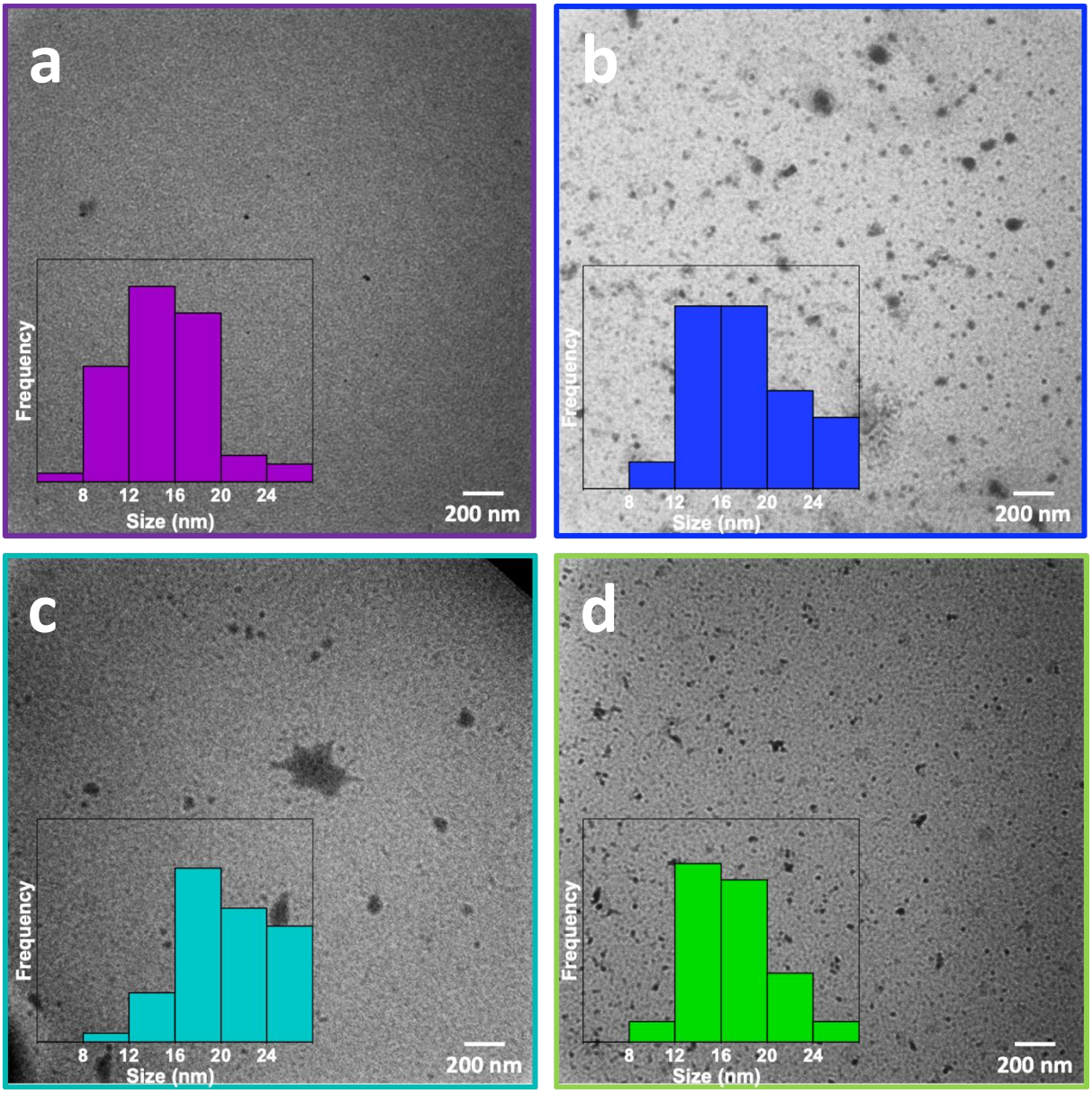

Figure S2. TEM images of cysCDs at different reaction temperatures. TEM images of cysCDs synthesized at 5mins different reaction temperature at 160 °C (a), 180 °C (b), 200 °C (c), and 220 °C (d). The cysCDs seem to slightly increase in size as a function of synthesis temperature.

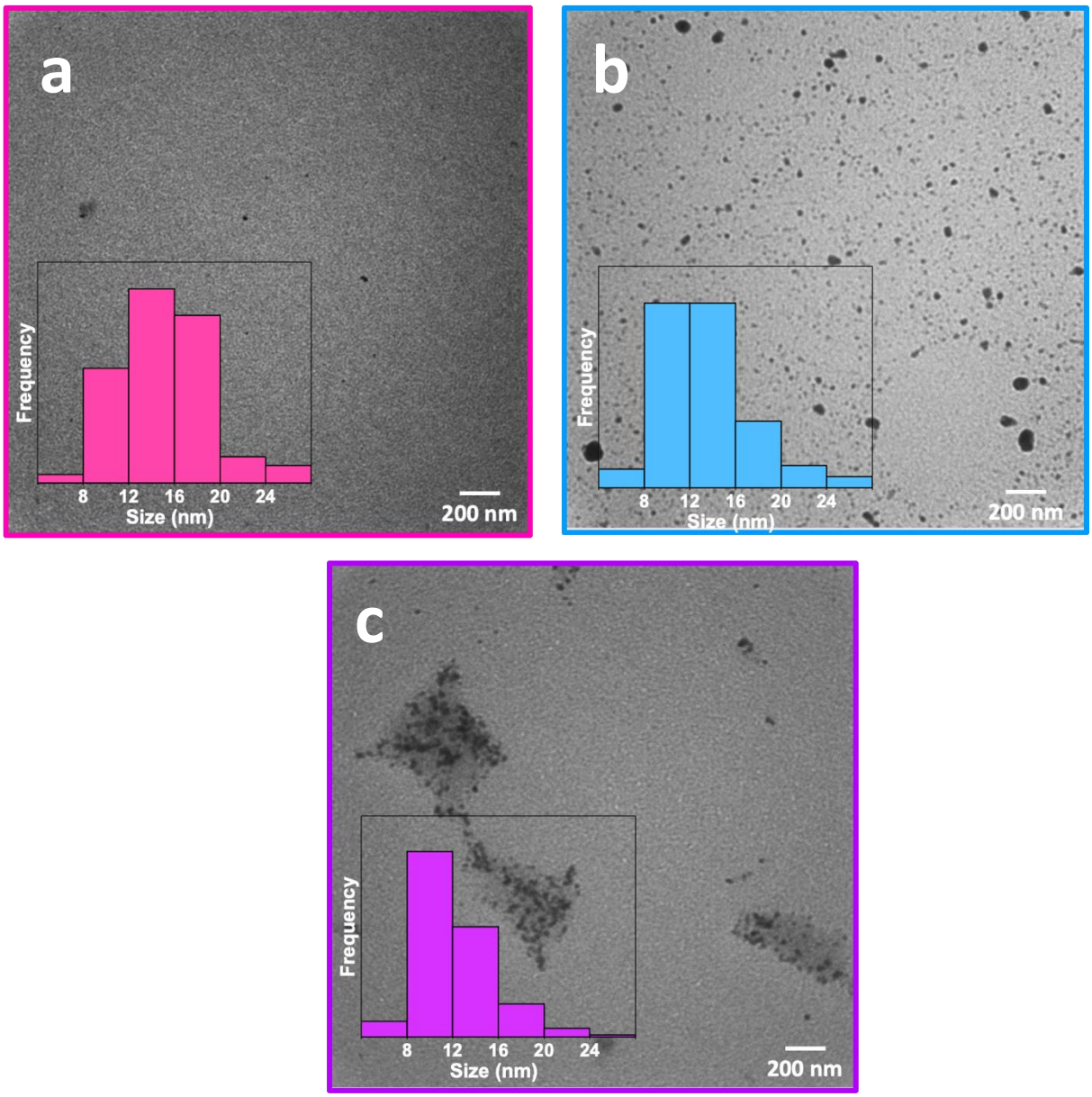

Figure S3. TEM images of cysCDs at different reaction times. TEM images of cysCDs synthesized at 160 °C and different reaction times at 5 mins (a), 10 mins (b), and 15 mins (c).The cysCDs seem to slightly increase in size as a function of synthesis time.

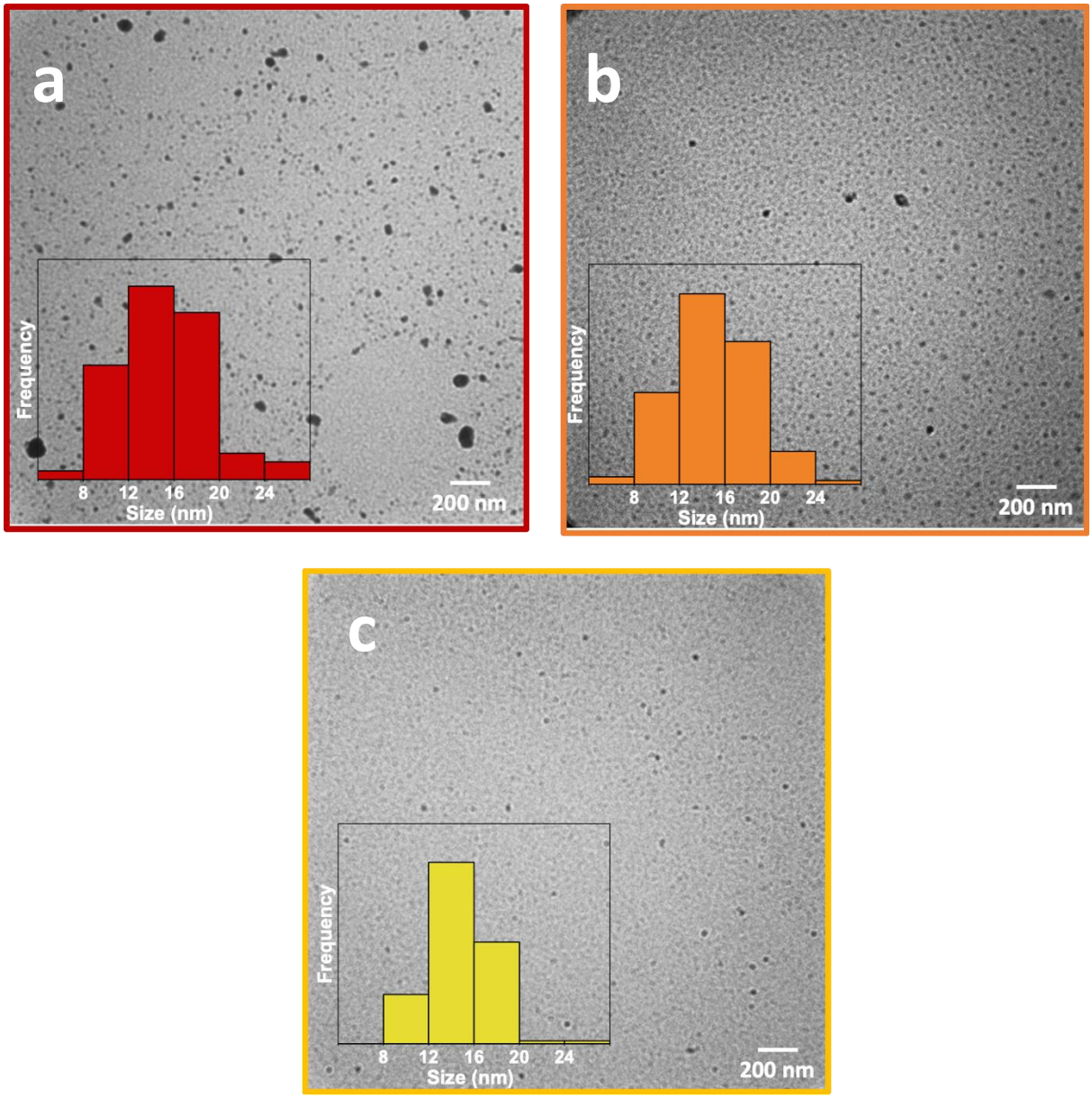

Figure S4. TEM images of L-cysCDs at different precursor ratios. TEM images of cysCDs synthesized at 160 °C and 5 mins with 1 L-CYS : 1 CA ratio(a), 1 L-CYS : 2 CA ratio (b),and 2 L-CYS : 1 CA ratio (c).The cysCDs seem to be stagnant in size as a function of reaction precursor.

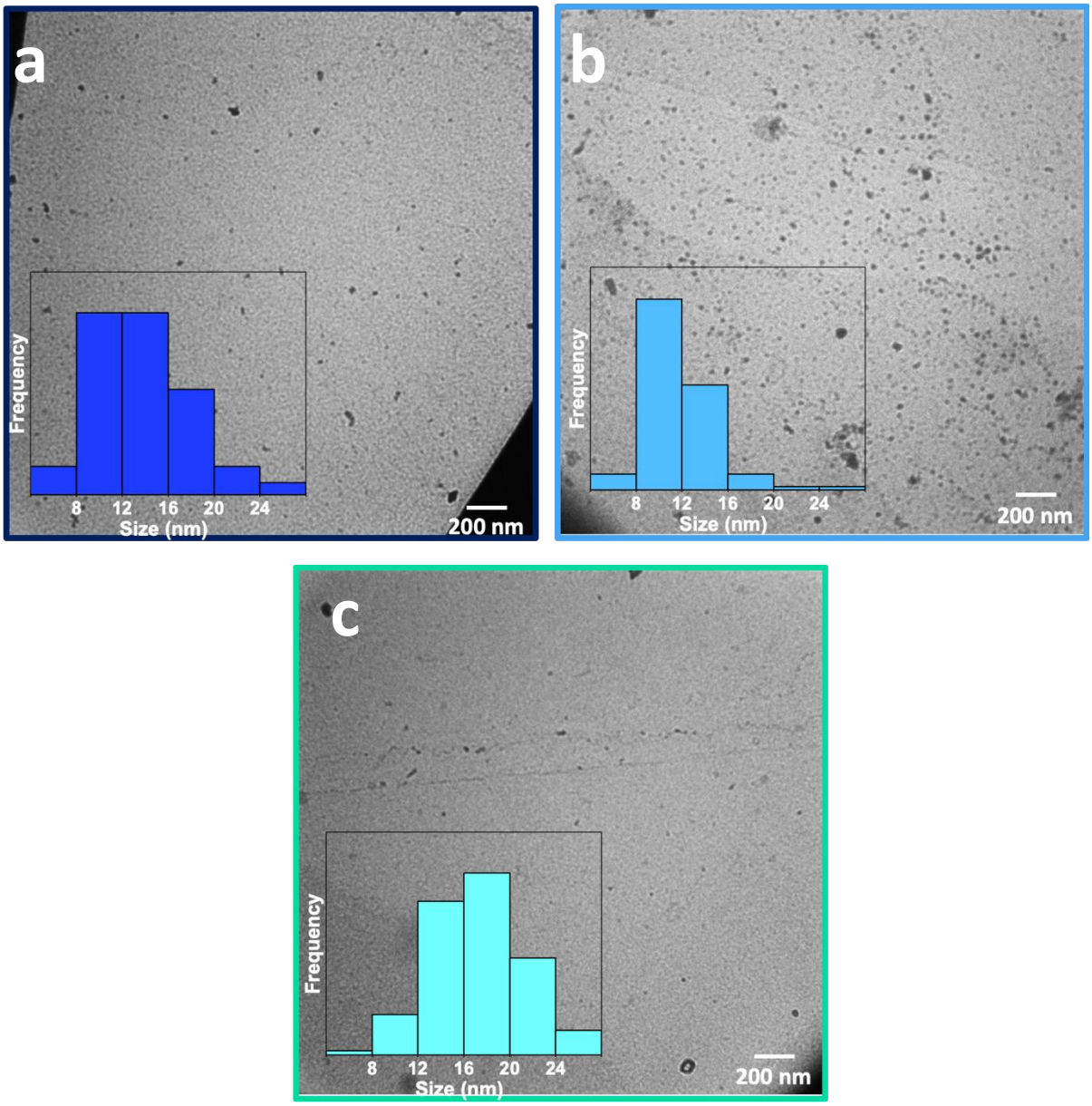

Figure S5. TEM images of D-cysCDs at different precursor ratios. TEM images of cysCDs synthesized at 160 °C and 5 mins with 1 D-CYS : 1 CA ratio(a), 1 D-CYS : 2 CA ratio (b),and 2 D-CYS : 1 CA ratio (c).The cysCDs seem to be stagnant in size as a function of reaction precursor.

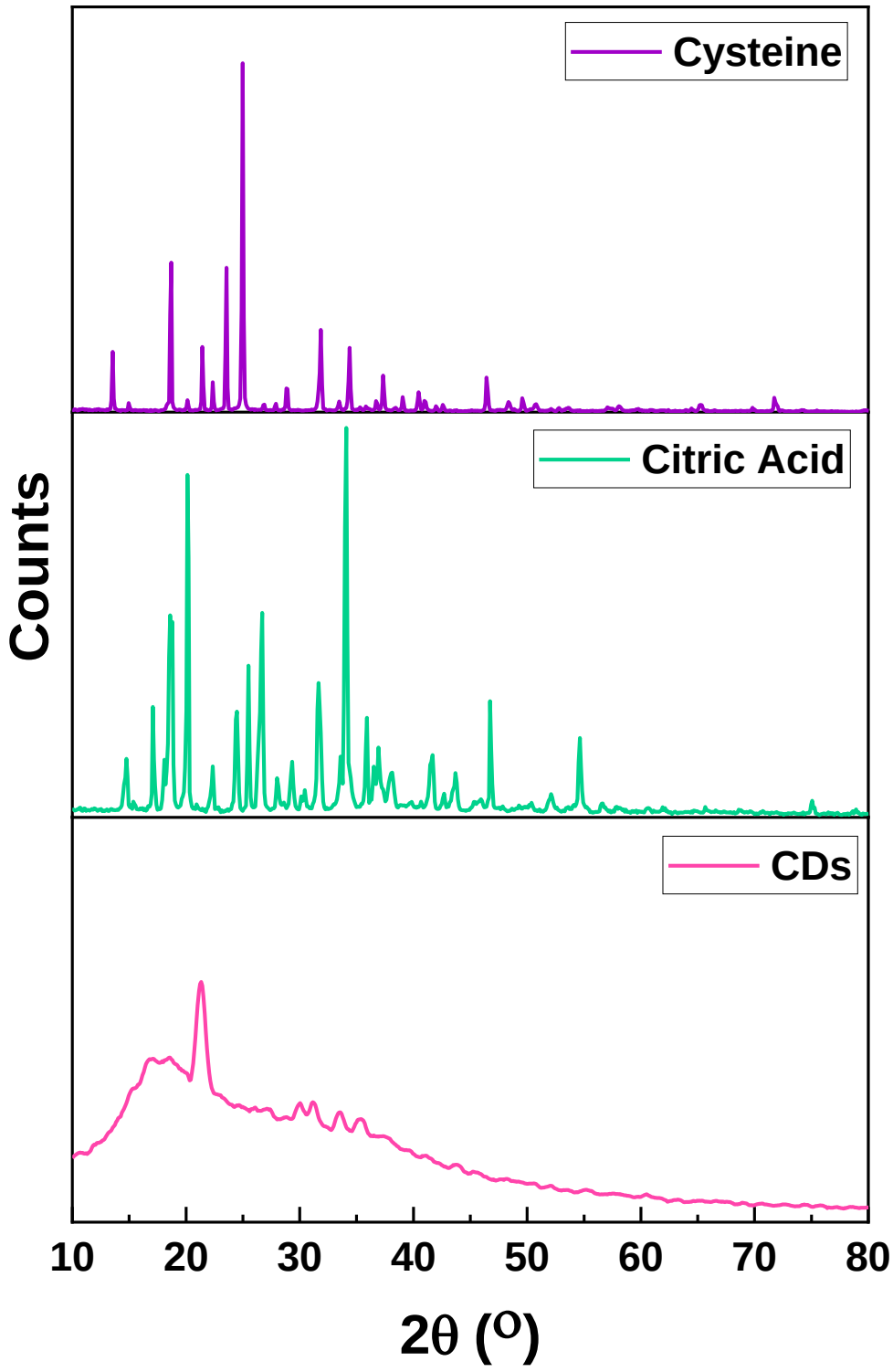

Figure S6. XRD spectra of precursors cysteine and citric acid with L-cysCDs. It is evident that the precursors have decomposed and formed a new system, namely the cysCDs. The apperance of an amorphous halo at 17˚ 2θ is indicative of the (002) plane of graphene. While the sharp reflections at 21°, 30°, 31°, 33° and 35° 2θ on the amorphous halo are indicative of some order semi-crystallization present of the CDs.

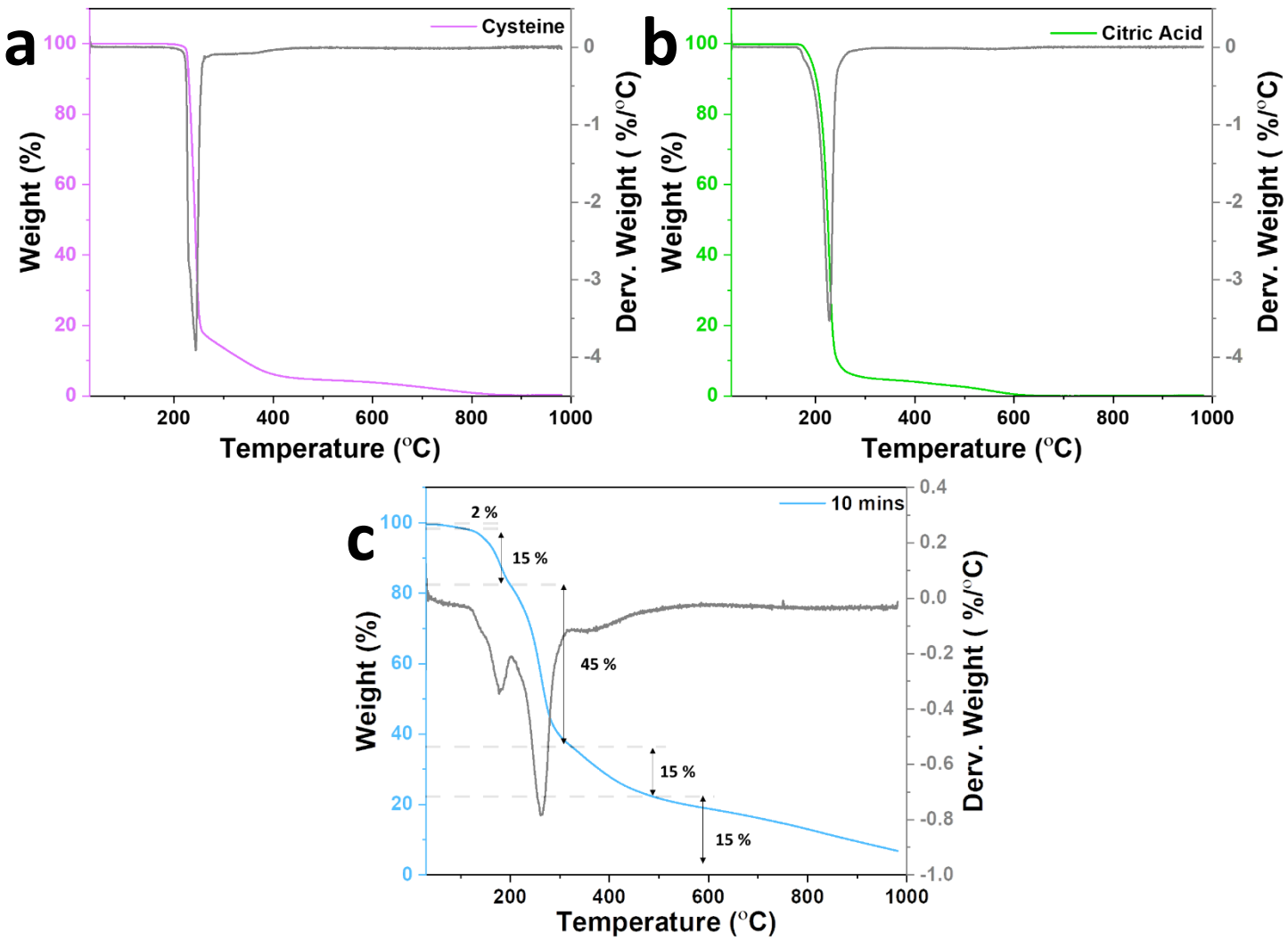

Figure S7. TGA spectra precursors cysteine (a) and citric acid (b) with cysCDs (c). The precursors cysteine (a) and citric acid (b) have a sharp and single weightloss occuring at 200°C; however, the cysCDs (c) has several weight loss steps indicating surface functionalities and an amorphous carbon core decomposition at higher temperature.

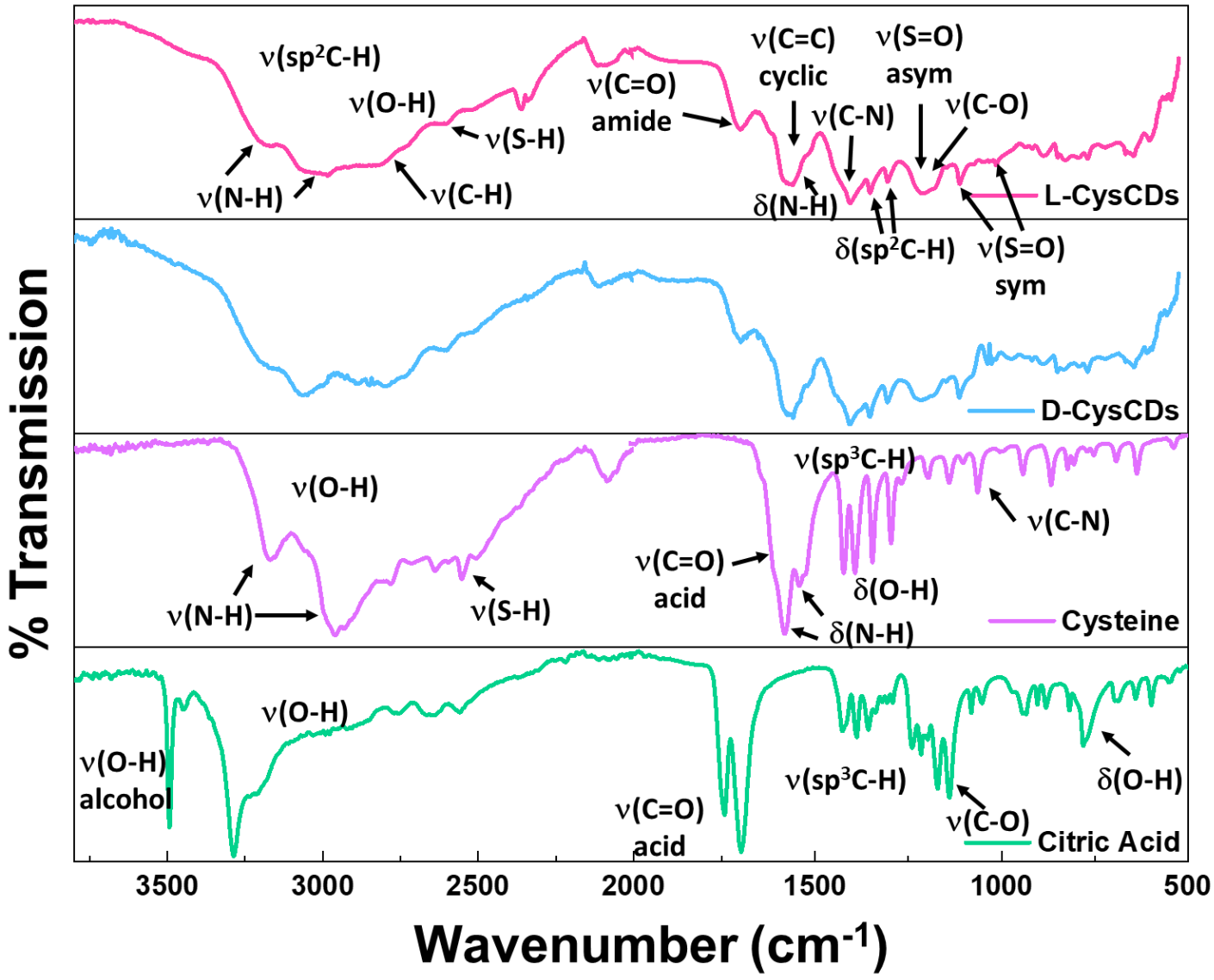

Figure S8. FTIR spectra of L-and D-cysCDs with precursors cysteine and citric acid. It is evident that the precursors have decomposed and formed a new system, namely the cysCDs. There is a transformation in the funtional groups observed in the precursors and the nanoparticles as such as the presence of amide C=O, and S=O, and the changes observed in the fingerprint region of the spectra (highlighted in the L-cysCDs FTIR spectra).

Table S3. The concentration of thiols estimated on the surface of the carbon dot using Ellman's Reagent (DNTB) in 0.1M Tris-HCl buffer at pH 7.5. The absorbance of the samples was taken at 412nm using 950µl of 0.1mM DTNB 50µl 10 $mgmL^{-1}$cysCD sample. A color changed was observed indicating that DNTB reacted with free thiols to produce the colored product, 2-nitro-5-thiobenzoic acid (NTB) and disulfides. The concentration of thiols was calculated using the formula above and the molar coefficient of NTB is 14.15 mM^{-1} cm^{-1} at 412 nm.

$$mM\ free\ thiols = \frac{Absorbance}{Molar\ Coefficent\ of\ DNTB * path\ length} * dilution\ factor$$

		Absorbance at 412 nm (a.u)	For 10 $mgmL^{-1}$ of cysCDs – Thiols (mM)	For 1 $mgmL^{-1}$ of cysCDs – Thiols (mM)
Temperature (All reactions were done at 5 mins)	160 °C	0.132	0.187	0.019
	180 °C	0.066	0.093	0.009
	200 °C	0.054	0.076	0.008
	220 °C	0.054	0.076	0.008
Time (All reactions were done at 160 °C)	5 mins	0.132	0.187	0.019
	10 mins	0.041	0.058	0.006
	15 mins	0.031	0.044	0.004
Ratio (All reactions were done at 160 °C- 5mins)	1 L-CYS : 1 CA	0.041	0.058	0.006
	1 L-L- CYS : 2 CA	0.043	0.061	0.006
	2 L-CYS : 1 CA	0.068	0.096	0.010

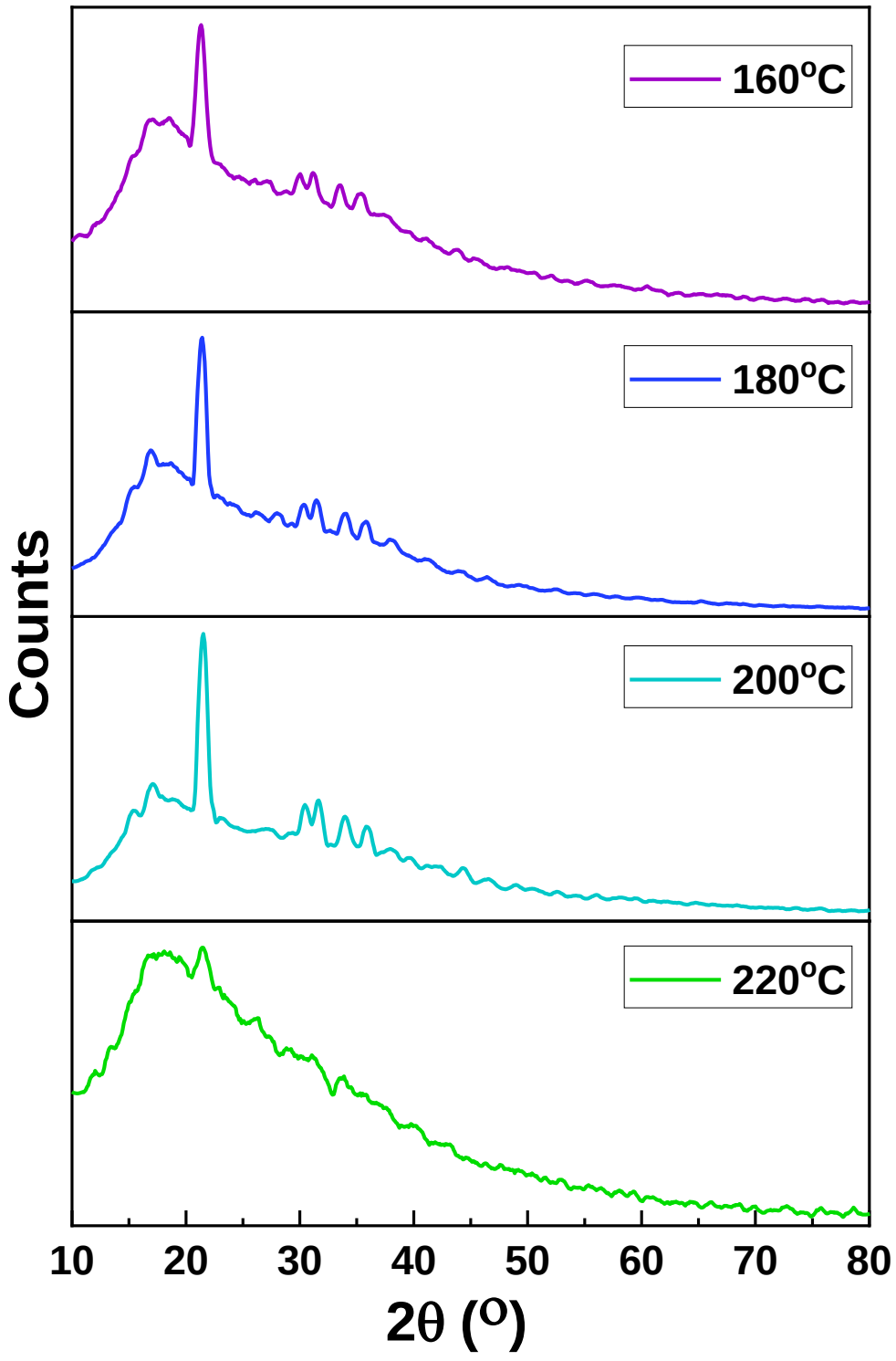

Figure S9. XRD spectra of L- cysCDs at different synthesis temperatures. The apperance of amphrous halo at 17˚ 2θ is indicative of the (002) plane of graphene and the sharp reflections at 21°, 30°, 31°, 33° and 35° 2θ indicate a semi-crystalline state present in the cysCDS. However, as synthesis temperature is increased the cysCDs become more disordered.

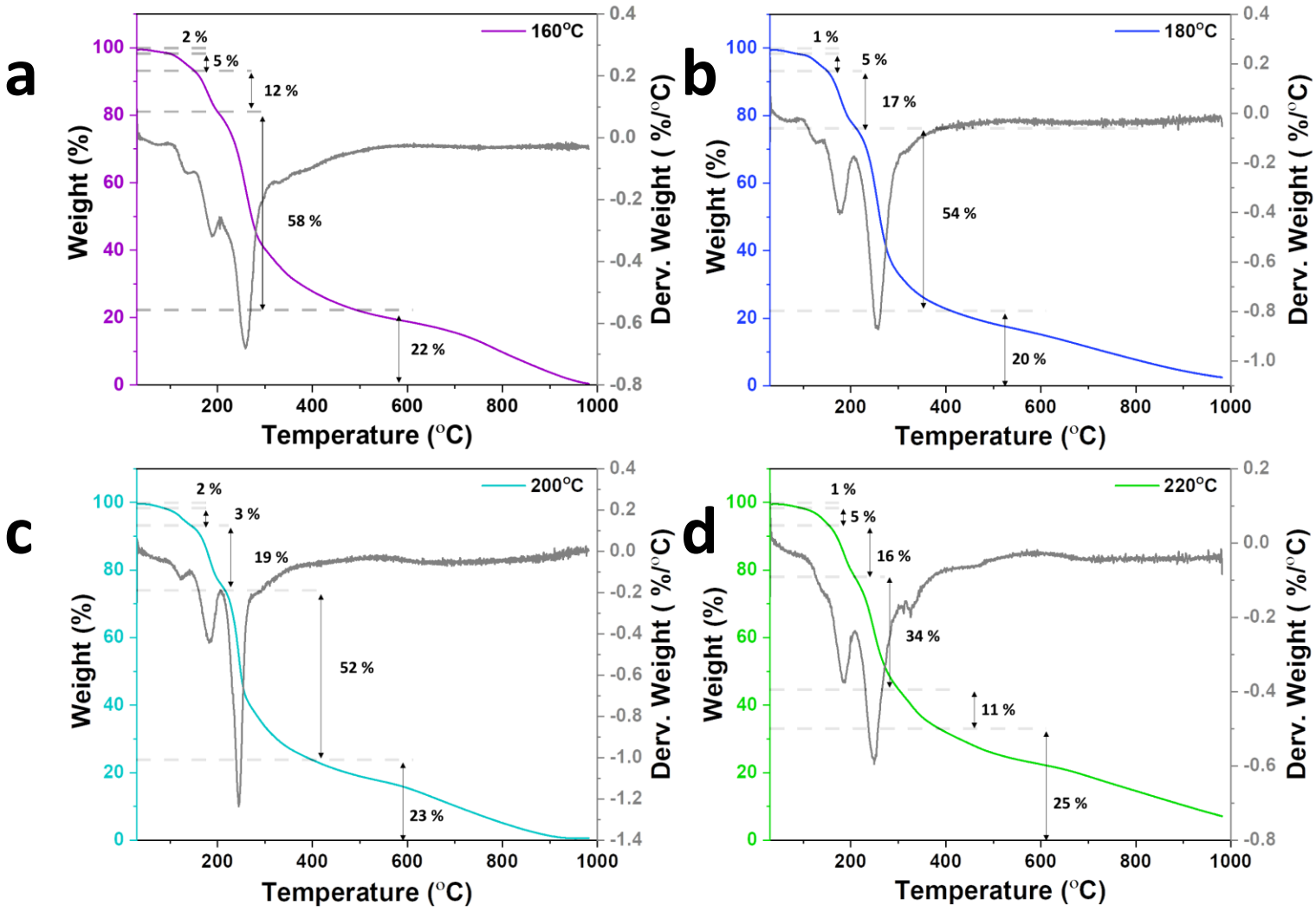

Figure S10. TGA spectra of L- cysCDs at various synthesis temperatures- 160°C (a), 180°C (b), 200°C (c), 220°C (d). The weight loss profile of the cysCDs changes indicating changes in the decomposition profile likely ascribed to changes in surface functionality. The 2% weight loss from 30-100°C and 5% loss from 100-150 °C is due to residual moisture and solvent adsorbed on the surface. The large weight loss of 58 % is between 200-500 °C is as due to decomposition of the functional groups on the surface containing oxygen, nitrogen and sulfur. The loss occurring past 500 °C is due to the decomposition of the carbon core of the dot.

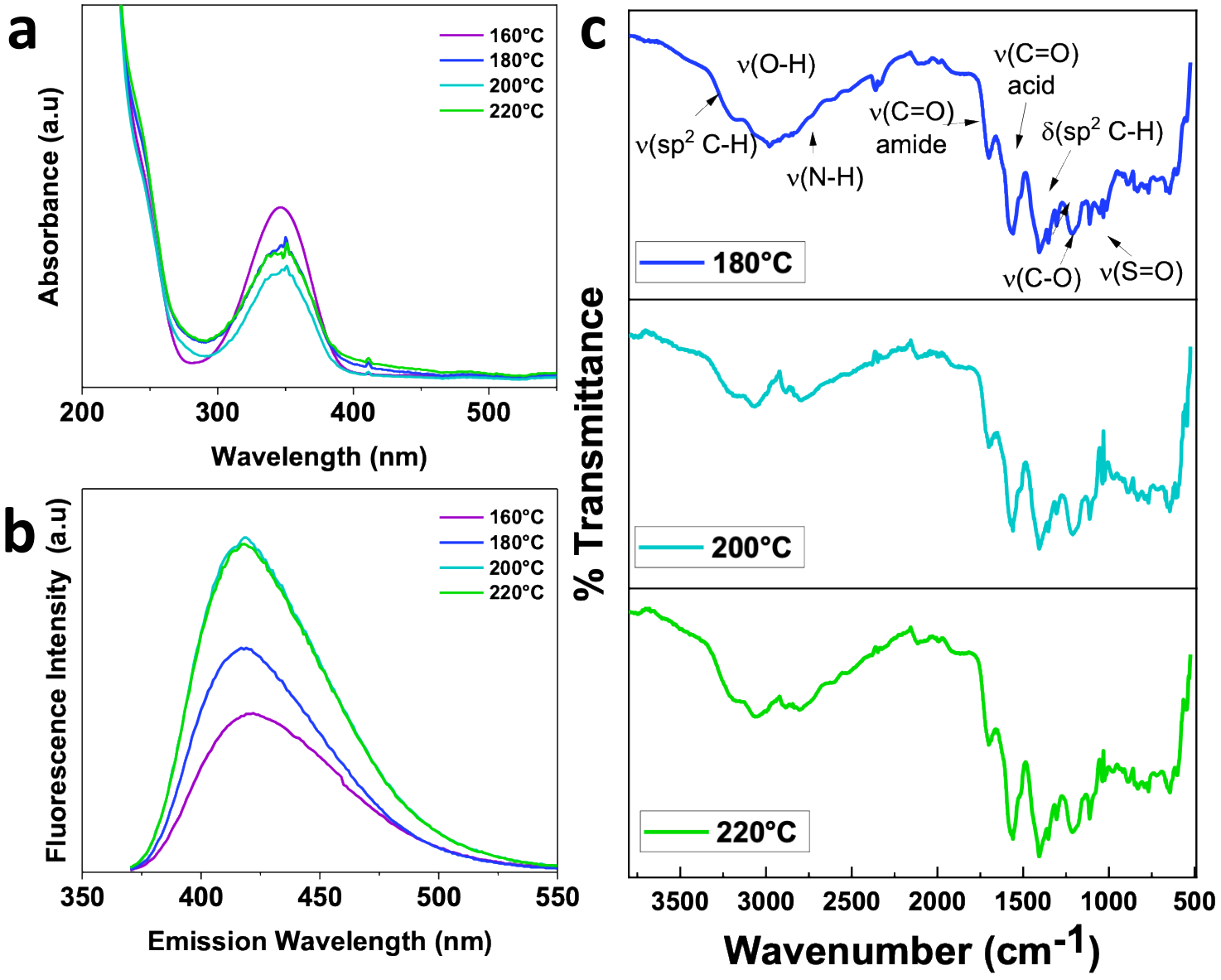

Figure S11. The optical and physical properties of cysCDs synthesized at different reaction temperature at 5 minutes reaction time and 1:1 ratio of L-cysteine to citric acid. (a) The cysCDs synthesized at various temperatures exhibit absorbance bands at ~250 nm and 350 nm corresponding to π → π* and n → π* transitions, respectively, (b) the fluorescence intensity of the cysCDs increase with an increasing reaction temperature at an excitation wavelength of 350 nm, (c) the FTIR analysis of the CDs shows that there is no significant change in the functional groups present on the surface of the CDs.

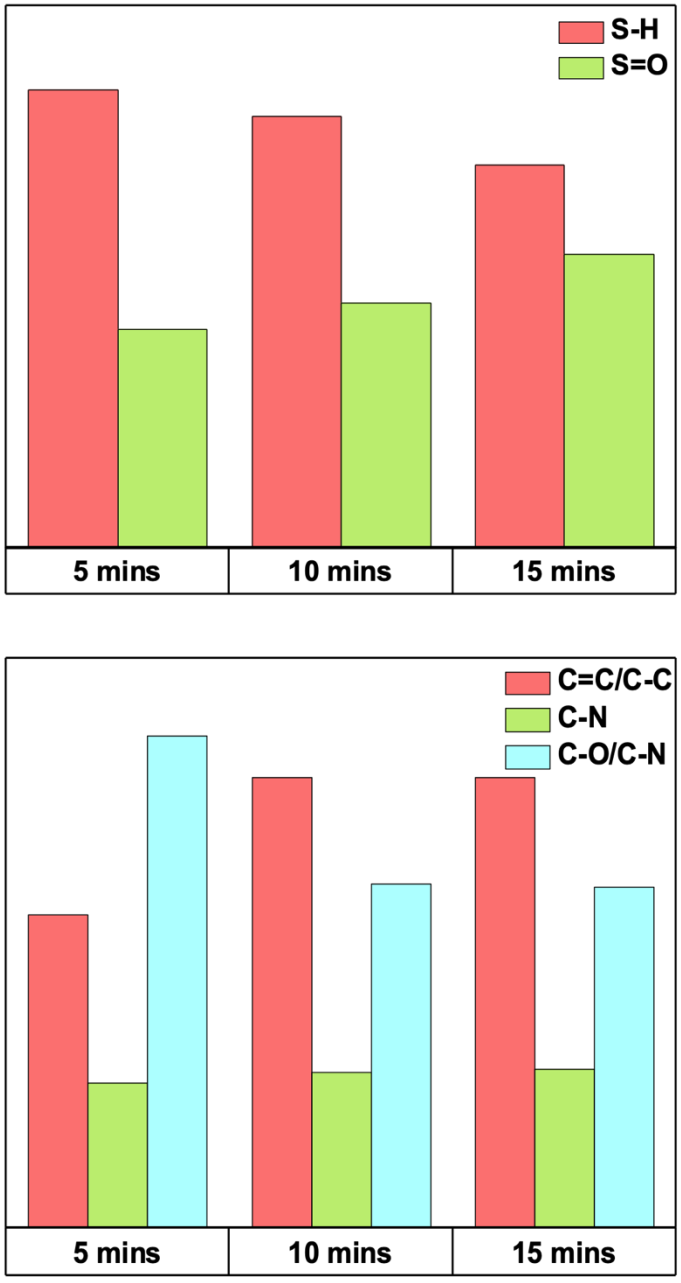

Figure S12. The XPS spectra of L- cysCDs at different synthesis time at 160°C. (a) A decrease in S-H and an increase in S=O functionalities in cysCDs is observed as an increase in reaction time (b) similarly, an increase in the C=C/C-C is observed as an increase in synthesis time of the cysCDs further supporting the formation of a hybridized core at the expense of functional groups on the surface of the CDs.

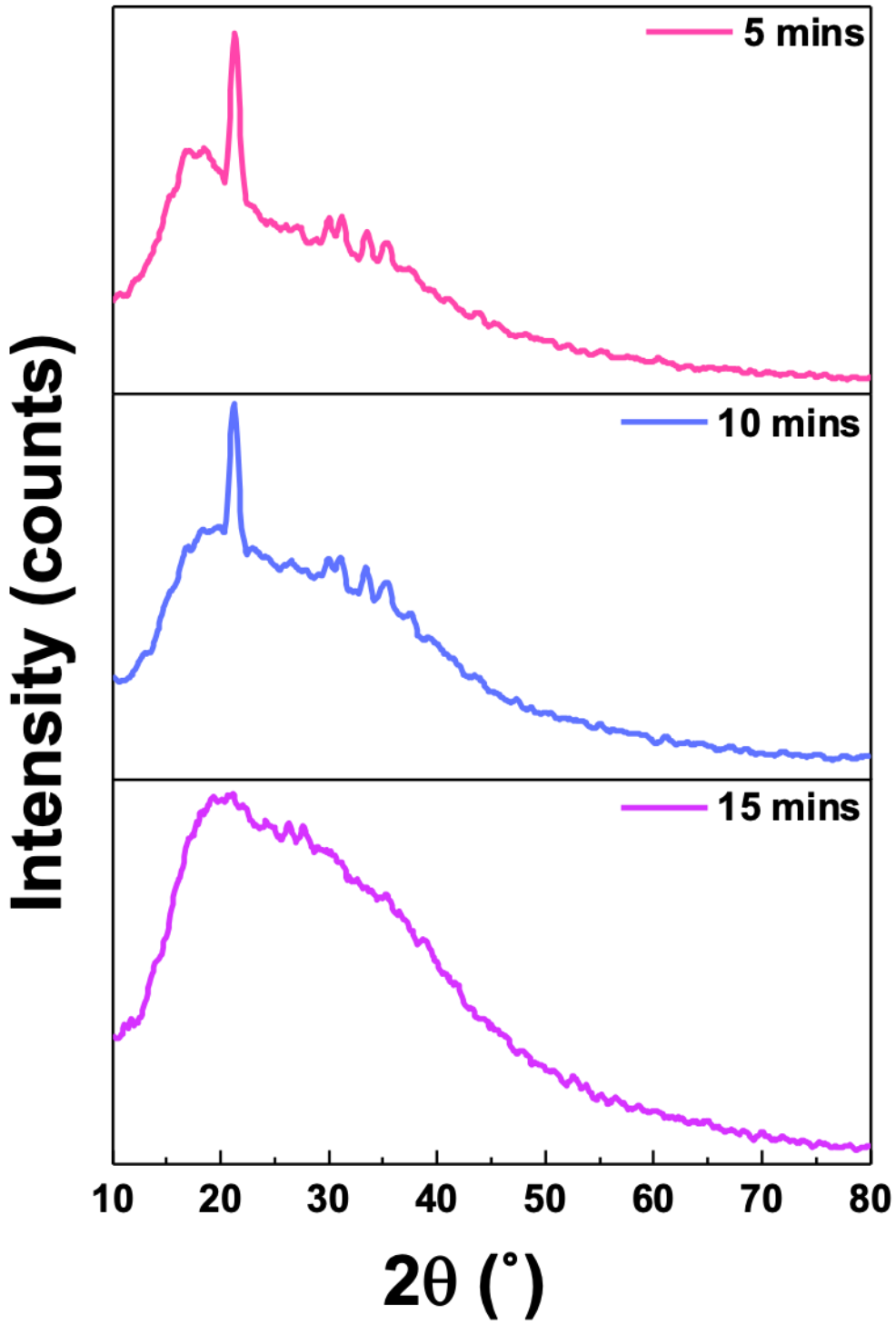

Figure S13. XRD spectra of L-cysCDs prepared at different synthesis times at an optimized reaction temperature of 160°C. The apperance of amphrous halo at 17˚ 2θ is indicative of the (002) plane of graphene and the sharp reflections at 21°, 30°, 31°, 33° and 35° 2θ indicate semi crystalline states present on the CDs. As reaction time is increased at 160°C, the CDs become increasingly amphrous.

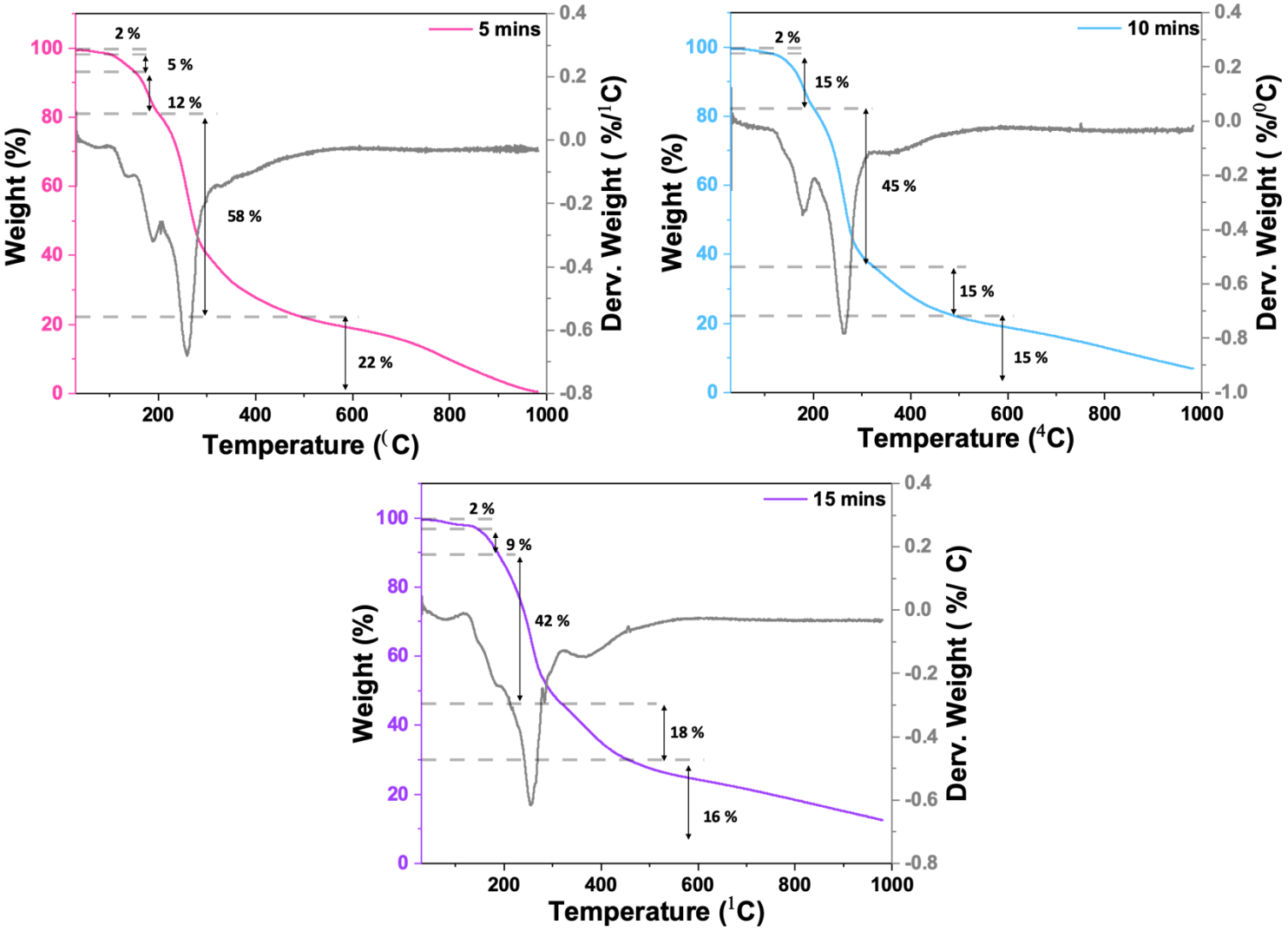

Figure S14. TGA spectra of L- cysCDs prepared at different synthesis times at an optimized reaction temperature of 160°C- 5 mins (a), 10 mins (b), 15 mins (c). The weight loss profile of the cysCDs changes indicating changes in the surface composition through variation in the weight loss profiles observed at the different reaction times. The weight loss at 30-100°C and 100-150 °C is due to residual moisture and solvent adsorbed on the surface of the cysCDS. The large weight loss between 200-500 °C is as due to decomposition of the functional groups on the surface containing oxygen, nitrogen and sulfur. The loss occurring past 500 °C is due to the carbon core of the nanodot.

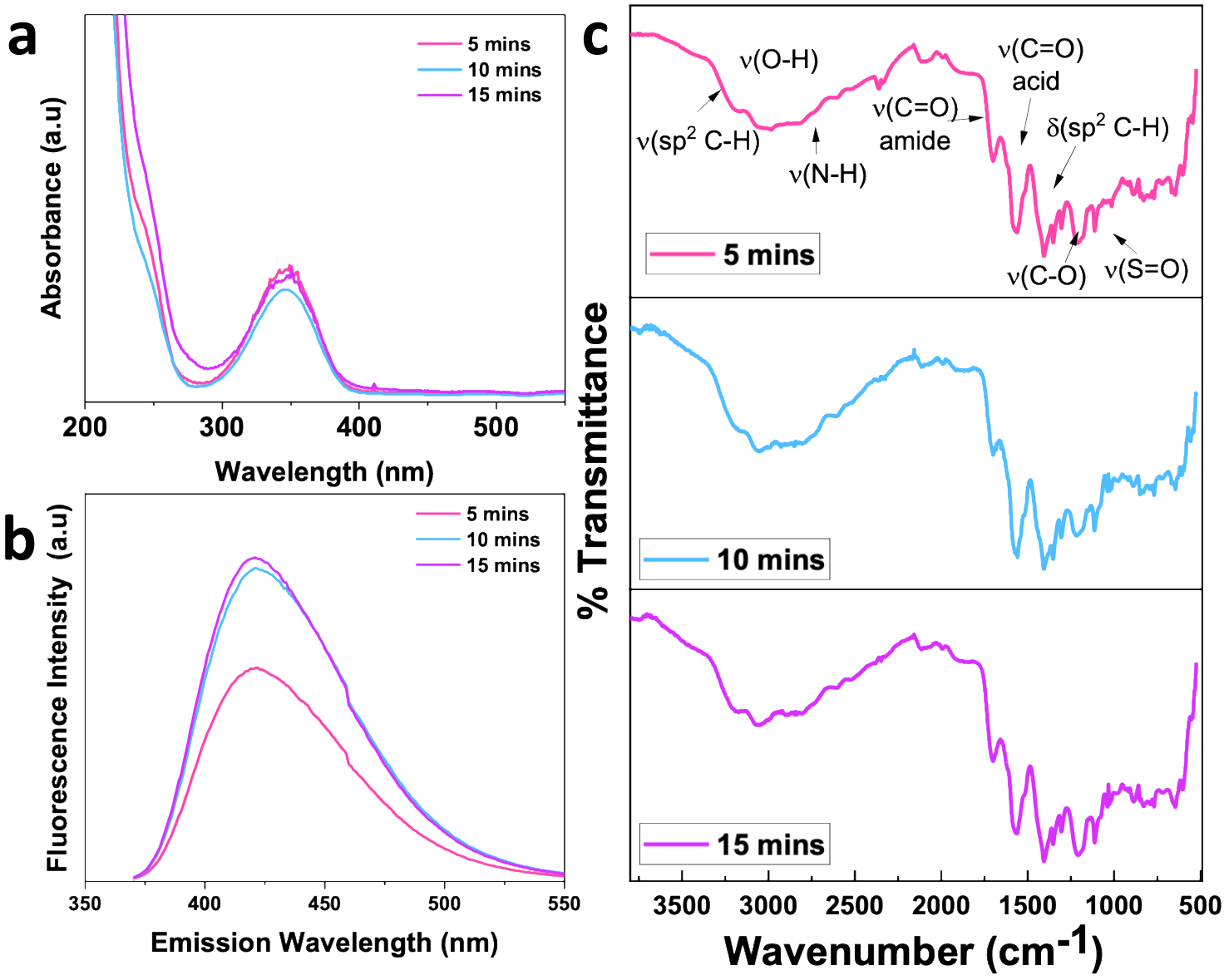

Figure S15. The optical and physical properties of cysCDs prepared at different synthesis times at an optimized reaction temperature of 160°C and 1:1 ratio of L-cysteine to citric acid. (a) The cysCDs synthesized at various reaction times have absorbance bands at ~250 nm and 350 nm corresponding to π → π* and n → π* transitions, (b) the fluorescence intensity of the cysCDs increase with an increasing reaction time at excitation wavelength of 350 nm, (c) the FTIR analysis of the CDs shows that there is no significant change in the functional groups present on the surface of the CDs.

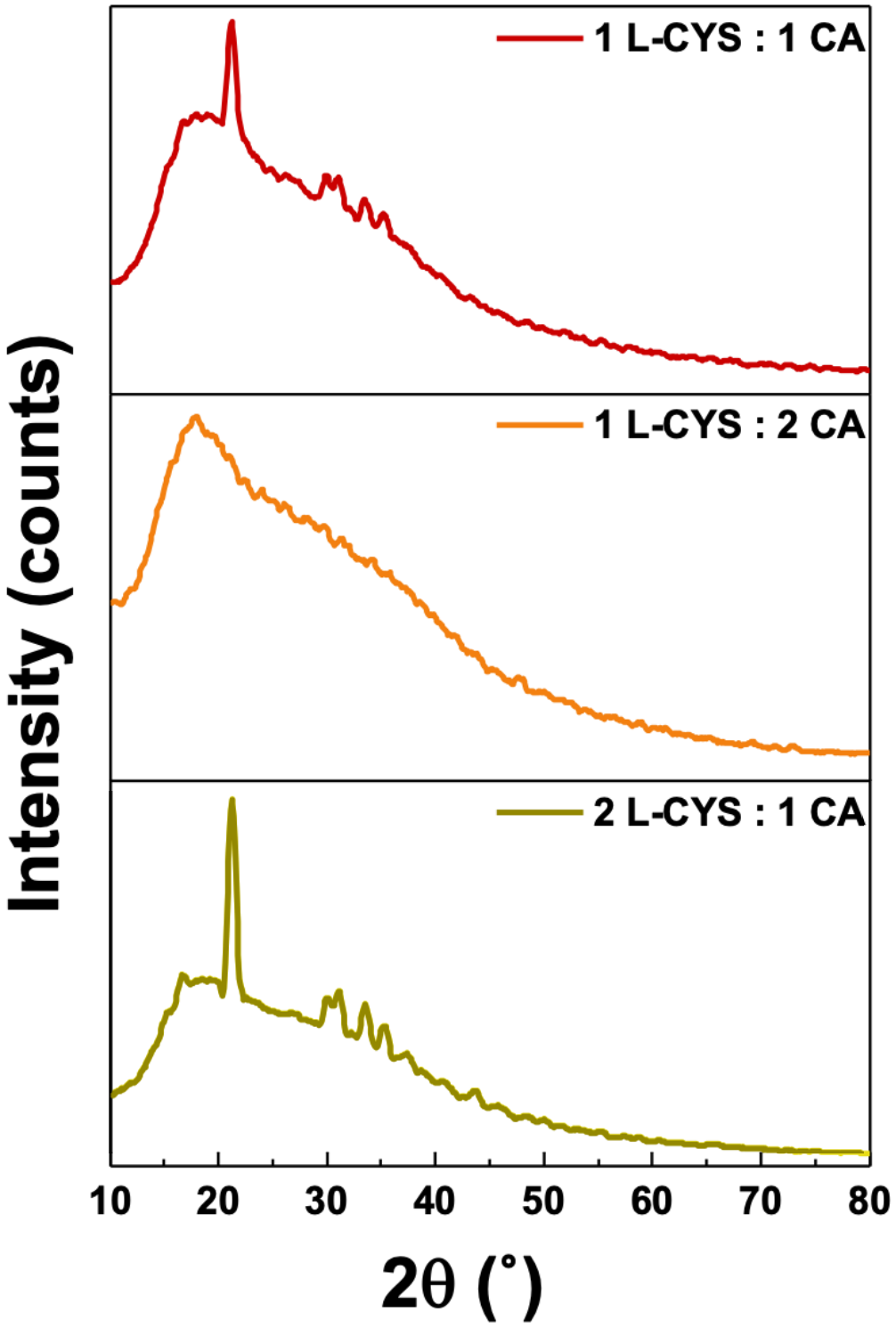

Figure S16. XRD spectra of L- cysCDs using different precursor ratios at a reaction tempreature of 160 °C and a 5 min reaction time. The apperance of amphrous halo at 17˚ 2θ is indicative of the (002) plane of graphene and the sharp reflections at 21°, 30°, 31°, 33° and 35° 2θ are indicative ordered structures in the nanoparticle. The increase in cysteine precursor increases the crystallinity in the nanoparticle.

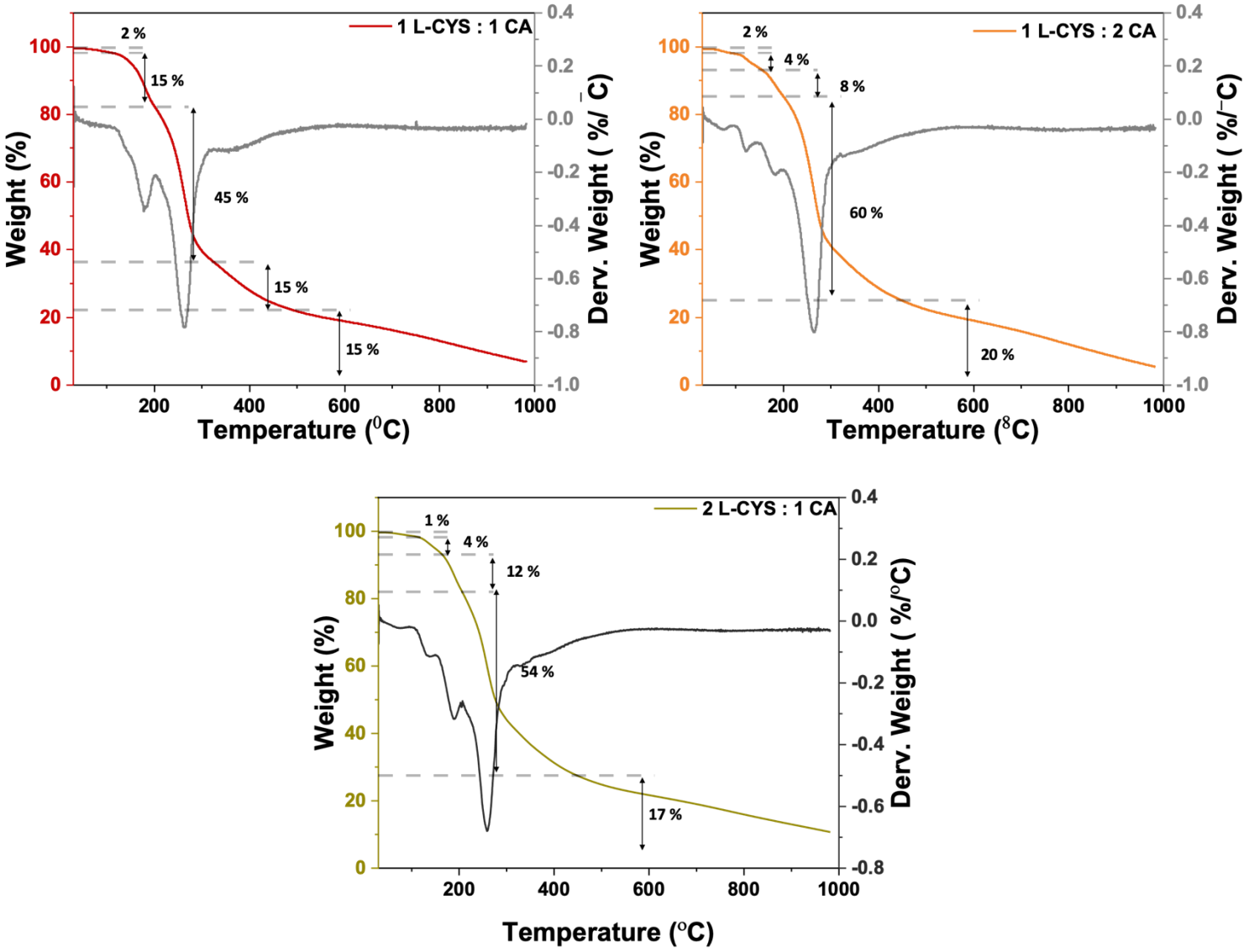

Figure S17. TGA spectra of L- cysCDs using different precursor ratios at a reaction tempreature of 160 °C and a 10 min reaction time- 1:1 L-cysteine to citric acid (a), 1:2 L-cysteine to citric acid (b), 2:1 L-cysteine to citric acid (c). The weight loss profile of the cysCDs changes indicating changes in the surface functionalities as concentration of precursors are changed. The weight loss at 30-100°C and 100-150 °C is due to residual moisture and solvent adsorbed on the surface of the cysCDS. The large weight loss between 200-500 °C is as due to decomposition of the functional groups on the surface containing oxygen, nitrogen and sulfur. The loss occurring past 500 °C is due to the carbon core of the nanodot.

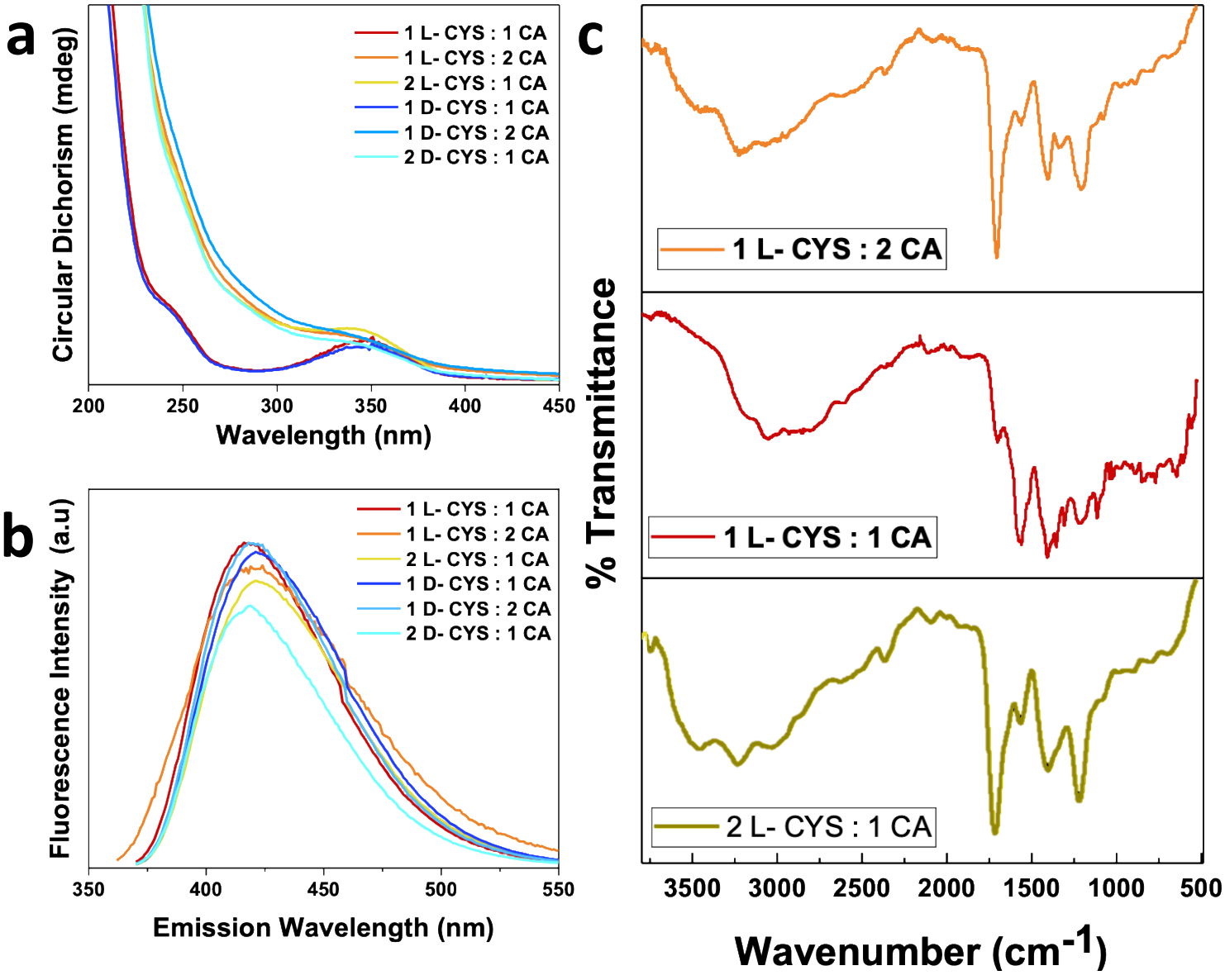

Figure S18. The optical and physical properties of cysCDs using different precursor ratios at a reaction tempreature of 160 °C and a 5 min reaction time. (a) The cysCDs has absorbance bands at ~250 nm and 350 nm corresponding to π → π* and n → π* transitions similar to the other cysCDs, (b) the fluorescence intensity of the cysCDs remain same at different ratios when excited at a wavelength of 350 nm, (c) the FTIR analysis of the CDs shows that there is no significant change in the functional groups present on the surface of the CDs.