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Appendix A

Table A1. Pre-exponential kinetic factor (A_a) computed from KAS-derived Equation at 10°C/min.

Conversions	A (min^{-1})	A ($nuclei \mu m^{-2} min^{-1}$)
0.05	38820.15566	4.22182E+19
0.1	6.06412E+13	6.59493E+28
0.15	2.43484E+11	2.64797E+26
0.2	9.99855E+12	1.08738E+28
0.25	7.34E+14	7.98E+29
0.3	4.38179E+13	4.77E+28
0.35	9.06E+14	9.85E+29
0.4	7.11E+15	7.73E+30
0.45	4.17E+16	4.54E+31
0.5	2.92E+16	3.17E+31
0.55	1.61E+16	1.75E+31
0.6	1.26E+14	1.37E+29
0.65	5.64E+16	6.13E+31
0.7	1.27E+16	1.38E+31
0.75	8.73E+13	9.49E+28
0.8	2.44E+15	2.65E+30
0.85	2.35E+14	2.55E+29
0.9	2.79471E+14	3.0393E+29
0.95	1.22E+15	1.33E+30

Table A2. Nucleation Rate calculated by Kinetic and Thermodynamic barriers required for Random Nucleation within the Gd₂O₃ matrix concerning Temperature.

T(K)	J_n (nuclei μm^{-2} min^{-1}) 10°C/min	T(K)	J_n (nuclei μm^{-2} min^{-1}) 15°C/min	T(K)	J_n (nuclei μm^{-2} min^{-1}) 20°C/min
562.2	1.735	569.2	3.90	573.17	4.40
572.18	5.19	579.18	11.33	584.22	14.40
579.22	13.0	586.15	27.7	590.18	30.74
585.15	28.4	592.21	60.29	596.29	65.98
591.17	62.79	598.26	131.52	602.2	139.11
597.2	120.92	604.16	245.65	608.26	263.11
603.17	229.29	610.2	462.40	614.19	487.71
612.23	388.94	619.17	763.97	623.24	829.68
624.18	2460.05	631.15	4679.30	636.21	5329.44
640.18	10393.64	647.2	19226.88	653.22	23652.14
663.15	39960.5	671.19	77796.54	677.19	94565.03
685.15	458854.8	694.18	922005.8	700.19	1071619
718.16	3696449	728.23	7516334	735.15	9092955
781.17	2.7×10^8	794.23	5.88×10^8	800.18	6.28×10^8
857.23	3.3×10^{10}	870.24	6.36×10^{10}	881.23	8.22×10^{10}

Table A2.1

Nucleation rate Vs. Temperatures			
	10 °C/min	15 °C/min	20 °C/min
A1	0.49383	0.017077	1.35545
t1	15.775 ± 0.0044	11.378 ± 3.1330	16.628 ± 0.0054

A1 and t1 are the exponential functions constant for the best-fitted plot between nucleation rate and Temperature.

Table A2.2

Nucleation rate Vs. Conversions			
	10 °C/min	15 °C/min	20 °C/min
A1	0.03231	0.11967	0.07705
t1	0.01868 ± 0.135	0.01537 ± 0.407	0.01866 ± 0.136
A2	-0.21021	-0.19791	-0.50477
t2	0.0202 ± 0.161	0.0157 ± 0.432	0.0201 ± 0.162

A1, A2, t1, and t2 are exponential function constants for the best-fitted plot between nucleation rate and conversions.

Table A3. The value of Interfacial energy is calculated by Thermodynamic barriers within the Gd₂O₃ matrix with Temperature.

T(K)	Y (mJ/m ²)- 10 °C/min	T(K)	Y (mJ/m ²)- 15 °C/min	T(K)	Y (mJ/m ²)- 20 °C/min
503.17	49.31	510.17	49.77	513.28	49.89
528.19	44.79	535.22	45.18	540.31	45.37
549.23	50.61	556.19	51.03	560.28	51.37
562.2	51.33	569.2	51.75	573.17	52.08
572.18	51.98	579.18	52.40	584.22	52.79
579.22	52.36	586.15	52.77	590.18	53.10
585.15	52.68	592.21	53.10	596.29	53.43
591.17	53.0	598.26	53.44	602.2	53.76
597.2	53.38	604.16	53.79	608.26	54.12
603.17	53.75	610.2	54.16	614.19	54.45
612.23	54.36	619.17	54.77	623.24	55.10
624.18	54.97	631.15	55.37	636.21	55.76
640.18	55.93	647.2	56.33	653.22	56.77
663.15	57.34	671.19	57.80	677.19	58.24
685.15	58.54	694.18	59.05	700.19	59.49
718.16	60.45	728.23	61.01	735.15	61.50
781.17	63.93	794.23	64.64	800.18	65.07
857.23	67.99	870.24	68.67	881.23	69.37

Table A3.1:

Interfacial Vs Temperature			
	10 °C/min	15 °C/min	20 °C/min
a	18.451 ± 3.997E-15	18.611 ± 2.029E-15	18.742 ± 5.009E-15
b	0.058 ± 6.289E-18	0.058 ± 3.151E-18	0.058 ± 7.709E-18

a and b are linear function constants for the best-fitted plot between interfacial and Temperature.

Table A3.2:

Interfacial Vs Conversions			
	10 °C/min	15 °C/min	20 °C/min
Intercept (I)	51.893 ± 8.437E-15	52.454 ± 1.352E-14	52.895 ± 9.907E-16
b1	-6.603 ± 3.478E-14	-7.330 ± 5.574E-14	-7.914 ± 4.084E-15
b2	18.697 ± 3.270E-14	19.512 ± 5.242E-14	20.266 ± 3.840E-15

I, b1, and b2 are polynomial function constants for the fitted plot between interfacial energy and conversion.

Table A4: Mechanism for $f(\alpha)$ and $I(\alpha)$ functions for some common mechanisms operating in solid-state reactions.

Mechanism (Nucleation model)	$I(\alpha)$	$g(\alpha)$
A1, sigmoidal rate equation (Avrami equation)	$\frac{[-\ln(1-\alpha)]^{2/3}}{}$	$\frac{(3/2)(1-\alpha)[- \ln(1-\alpha)]^{1/3}}{}$
A2, sigmoidal rate equation (Avrami equation)	$\frac{[-\ln(1-\alpha)]^{1/2}}{}$	$\frac{2(1-\alpha)[- \ln(1-\alpha)]^{1/2}}{}$
A3, sigmoidal rate equation (Avrami equation)	$\frac{[-\ln(1-\alpha)]^{1/3}}{}$	$\frac{3(1-\alpha)[- \ln(1-\alpha)]^{2/3}}{}$
A4, sigmoidal rate equation (Avrami equation)	$\frac{[-\ln(1-\alpha)]^{1/4}}{}$	$\frac{4(1-\alpha)[- \ln(1-\alpha)]^{3/4}}{}$
F1, sigmoidal rate equation (Prout-Tompkins)	$\frac{\ln[\alpha/(1-\alpha)]}{}$	$\frac{\alpha(1-\alpha)}{}$
F2, sigmoidal rate equation (Contracting area)	$\frac{1-(1-\alpha)^{1/2}}{}$	$\frac{2(1-\alpha)^{1/2}}{}$
F3, sigmoidal rate equation (Contracting volume)	$\frac{1-(1-\alpha)^{1/3}}{}$	$\frac{3(1-\alpha)^{2/3}}{}$
F4, sigmoidal rate equation (Random nucleation (1))	$\frac{1/(1-\alpha)}{}$	$\frac{(1-\alpha)^2}{}$
F4, sigmoidal rate equation (Random nucleation (2))	$\frac{1/(1-\alpha)^2}{}$	$\frac{(1-\alpha)^3/2}{}$
R1, Reaction rate models (First order)	$\frac{-\ln(1-\alpha)}{}$	$\frac{1-\alpha}{}$
R1, Reaction rate models (Second order)	$\frac{(1-\alpha)^{-1}-1}{}$	$\frac{(1-\alpha)^2}{}$
R1, Reaction rate models (Third order)	$\frac{[(1-\alpha)^{-2}-1]/2}{}$	$\frac{(1-\alpha)^3}{}$
R1, Reaction rate models (Fourth order)	$\frac{2[(1-\alpha)^{-1/2}-1]}{}$	$\frac{(1-\alpha)^{3/2}}{}$
D1, Diffusion models (1 dimensional)	$\frac{\alpha^2}{}$	$\frac{1/2\alpha}{}$
D2, Diffusion models (2 dimensional-Valensi)	$\frac{(1-\alpha)\ln(1-\alpha)+\alpha}{}$	$\frac{[-\ln(1-\alpha)]^{-1}}{}$

D3, Diffusion models (3 dimensional-Jander))	$\frac{[1-(1-\alpha)^{1/3}]^2}{1}$	$\frac{(3/2)(1-\alpha)^{2/3}}{[1-(1-\alpha)^{1/3}]}$
D4, Diffusion models (4 dimensional-Ginstling))	$\frac{1-2\alpha/3-(1-\alpha)^{2/3}}{1}$	$\frac{(3/2)/[(1-\alpha)^{-1/3}-1]}{1}$
P2, Power Law	$\frac{\alpha^{3/2}}{1}$	$\frac{(2/3)\alpha^{-1/2}}{1}$
P3, Power Law	$\frac{\alpha^{1/2}}{1}$	$\frac{2\alpha^{1/2}}{1}$
P4, Power Law	$\frac{\alpha^{1/3}}{1}$	$\frac{3\alpha^{2/3}}{1}$
P5, Power Law	$\frac{\alpha^{1/4}}{1}$	$\frac{4\alpha^{3/4}}{1}$

Section A1:

The size range of ultra-small gadolinium oxide nanoclusters during nucleation is being calculated at all three heating rates using equation 1, and according to Temperature, the sizes are listed below in Table a5:

Table A5. Variation in size of prepared Gd₂O₃ with the Temperature

Heating rate @ 10°C/min		Heating rate @ 15°C/min		Heating rate @ 20°C/min	
Temperature (K)	Size (nm)	Temperature (K)	Size (nm)	Temperature (K)	Size (nm)
503.17	1.15	510.17	1.14	513.28	1.14
528.19	0.99	535.22	0.99	540.31	0.98
549.23	1.08	556.19	1.08	560.28	1.07
562.2	1.07	569.2	1.07	573.17	1.06
572.18	1.06	579.18	1.06	584.22	1.06
579.22	1.06	586.15	1.06	590.18	1.05
585.15	1.06	592.21	1.05	596.29	1.05
591.17	1.05	598.26	1.05	602.2	1.05
597.2	1.05	604.16	1.04	608.26	1.04
603.17	1.04	610.2	1.04	614.19	1.04
612.23	1.04	619.17	1.04	623.24	1.04
624.18	1.03	631.15	1.03	636.21	1.03
640.18	1.02	647.2	1.02	653.22	1.02
663.15	1.01	671.19	1.01	677.19	1.01
685.15	1.00	694.18	1.00	700.19	1.00
718.16	0.99	728.23	0.98	735.15	0.98
781.17	0.96	794.23	0.95	800.18	0.95
857.23	0.93	870.24	0.92	881.23	0.92

Our study includes the random nucleation process, ascertained by experimental $z(\alpha)$ master plots. During the starting phase of such nucleation, the first nuclei appear randomly, and during the formation of such nuclei, the steadiness in the nucleation is only possible when the formed nuclei size (r) is equal to the standard critical size of nucleation (r^*), as shown by the Equation below -

$$r^* = \frac{2\beta_a \gamma v}{3\beta_v k_B T \ln(S)} \quad (1)$$

Where, β_a is 4π , β_v It is $4\pi/3$, γ is abbreviated as surface free energy per unit area, v is the specific volume of the nanomaterial, T is the specific Temperature, and S is supersaturation. At a specific temperature, the first nucleus appears when the radius of the nuclei formed is equivalent to the standard critical size of nucleation, as described in Eq.(1), and the value of supersaturation (S) reaches 2.718. Our study includes the formation of first nuclei by the R3 mechanism, ascertained by $z(\alpha)$ master plots, as a starting point of nucleation (when actual nucleation starts). Thereby, this study includes the value of supersaturation (S) to be 2.718. Consequently, by putting the value of Boltzmann constant, interfacial energy, specific volume, specific Temperature, supersaturation value, and other constants like β_a and β_v , the size range of ultra-small gadolinium oxide nanoclusters at various temperature increments (ranging from 503.17K to 857.23 K) during the event of nucleation has been calculated by using Eq. (1) and is summarized in Table A5.

The criterion for the formation of a stable crystal nucleus is met only when the size of the formed nucleus (r) is equal to the standard critical size of nucleation (r^*), according to the Eq. (1)

Section A2:

A confidence interval (CI) is an estimate's uncertainty level, a range of numbers. For investigating the real difference or experimental errors in activation energy between two conversion points, we need to compute the confidence interval between two conversion points. The steps are listed below:

1. We collected the thermogravimetric data at 10 °C, 15 °C, and 20 °C.
2. Now we computed the activation energy using non-isothermal models at each conversion point.
3. Then we calculated the standard deviation for computing the standard error for activation energy at each conversion point.
4. Now we computed the standard errors for the difference in activation energy using the formula listed below:

$$SE = (SE1^2 + SE2^2)^{1/2}$$

Where SE1 and SE2 stand for standard errors of the activation energy at each conversion point, which represent the amount of uncertainty or variation in computed activation energy.

5. Finally, we have calculated the confidence interval for the difference in activation energy using the formula listed below:

$$95\% \text{ CI} = (\text{diffe} \pm t\text{-value} * \text{SE})$$

Where diffe is the difference in activation energy.

t-value is a critical value for t distribution for CI, which fixed in our case is 2.77, and for computing t value, the degree of freedom (dfr) is calculated using the formula listed below:

$$\text{dfr} = n_1 + n_2 - 2 \quad \text{Where } n_1 \text{ and } n_2 \text{ are sample size.}$$

6. Further, if the calculated value of the confidence interval does not contain zero, then the change in activation energy is statistically significant.
7. Hence, from Table A6, it is clear that the calculated value of the confidence interval is not zero; then, we conclude that the change in activation energy between two conversion points is not in the range of experimental error, which further shows that the change is statistically significant

Table A6. Calculation of confidence interval at each conversion points.

Conversion (α)	E_a (3 TGA runs) *			E_a (Initial)*	Standard Deviation (SD)*	Standard Error (SE)*	95 % CI (Confidence interval)	CI (Zero/Non-Zero)	Statically Significant
	Exp 1	Exp2	Exp3						
0.05	67.41	67.55	67.44	67.41	0.073	0.042		Non-Zero	YES
0.1	134.6	136.58	136.47	134.6	1.11	0.64	(65.04, 68.97)	Non-Zero	YES
0.15	127.08	123.21	124.83	127.08	1.94	1.12	(11.10, 3.93)	Non-Zero	YES
0.2	150.10	146.55	146.55	150.10	2.04	1.18	(18.49, 27.54)	Non-Zero	YES
0.25	158.29	157.12	157.18	158.29	0.65	0.38	(4.73, 11.64)	Non-Zero	YES
0.30	150.63	147.27	148.06	150.63	1.75	1.01	(10.66, 4.65)	Non-Zero	YES
0.35	168.6	168.5	168.45	168.6	0.07	0.04	(15.15, 20.78)	Non-Zero	YES
0.40	169.21	169.56	169.43	169.21	0.17	0.10	(5.65, 13.92)	Non-Zero	YES
0.45	174.13	173.25	176.67	174.13	1.77	1.02	(2.05, 7.78)	Non-Zero	YES
0.5	177.9	176.67	177.32	177.9	0.61	0.35	(0.75, 6.78)	Non-Zero	YES
0.55	181.82	181.15	184.89	181.82	1.99	1.15	(0.57, 7.26)	Non-Zero	YES
0.60	187.97	187.68	190.24	187.97	1.40	0.80	(2.24, 10.05)	Non-Zero	YES
0.65	180.04	182.38	179.47	180.04	1.54	0.89	(11.27, 4.58)	Non-Zero	YES
0.70	174.79	174.90	175.11	174.79	0.16	0.09	(7.73, 2.76)	Non-Zero	YES
0.75	173.64	174.60	174.1	173.64	0.48	0.27	(5.43, 10.5)	Non-Zero	YES
0.80	172.12	173.20	172.50	172.12	0.54	0.31	(2.68, 0.35)	Non-Zero	YES
0.85	167.07	167.62	168.08	167.07	0.50	0.29	(6.24, 3.85)	Non-Zero	YES
0.90	171.83	176.40	175.43	171.83	2.40	1.39	(0.81, 8.70)	Non-Zero	YES
0.95	167.89	168.40	168.10	167.89	0.25	0.14	(7.82, 0.05)	Non-Zero	YES

*All the values of activation energy, standard deviations and standard errors are in **kJ/mol**.

Appendix B

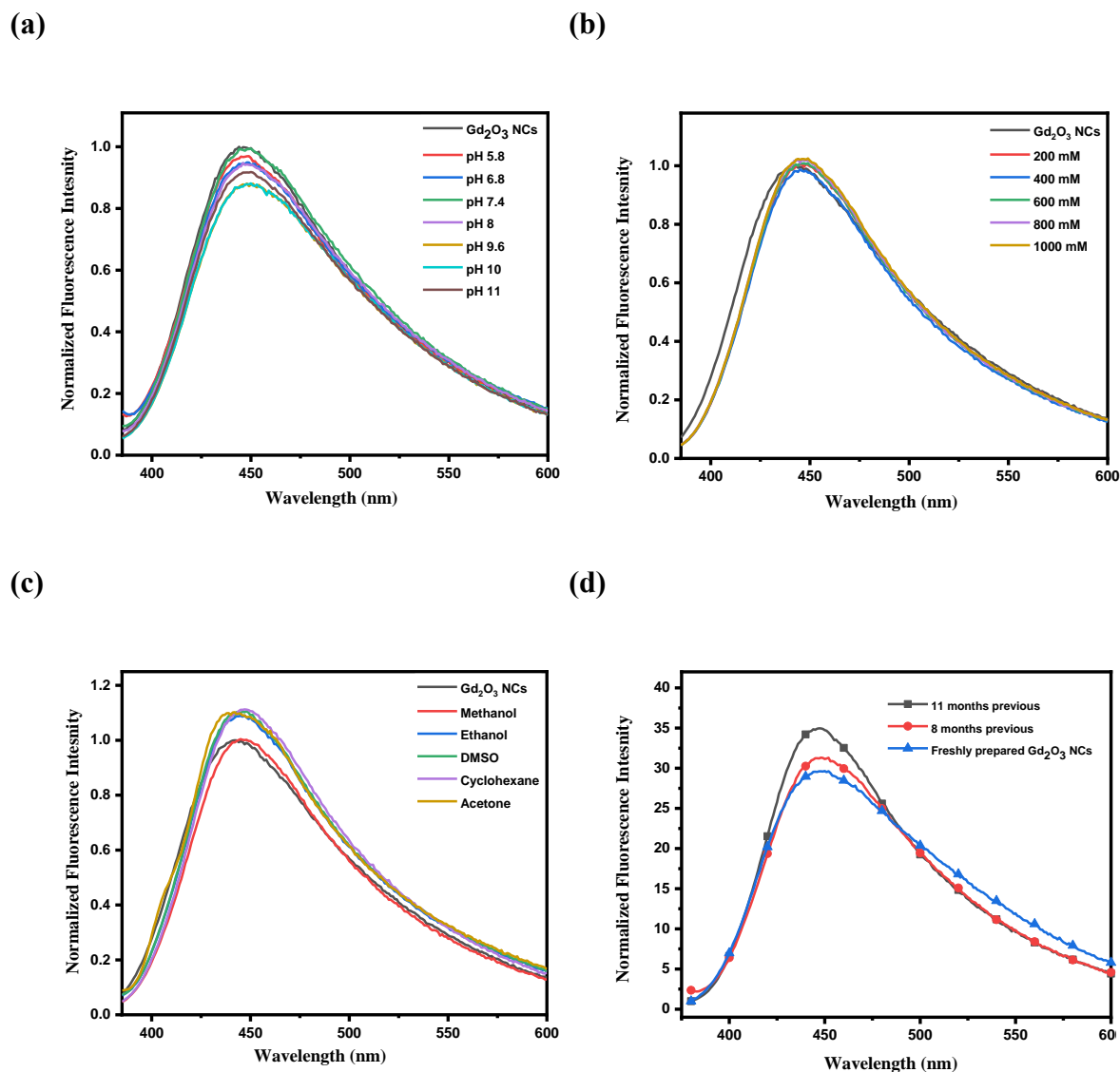
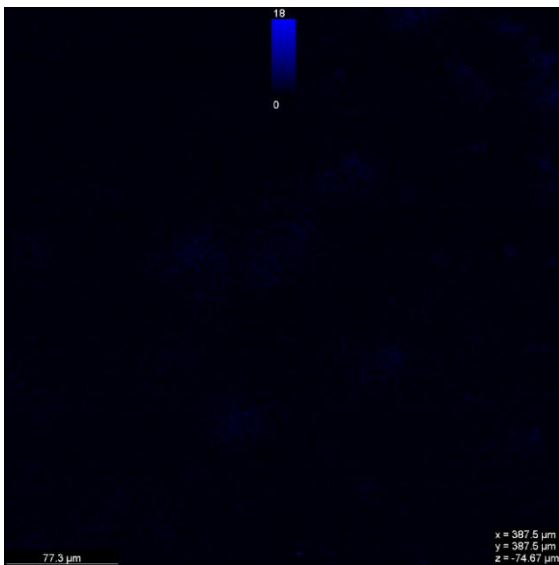
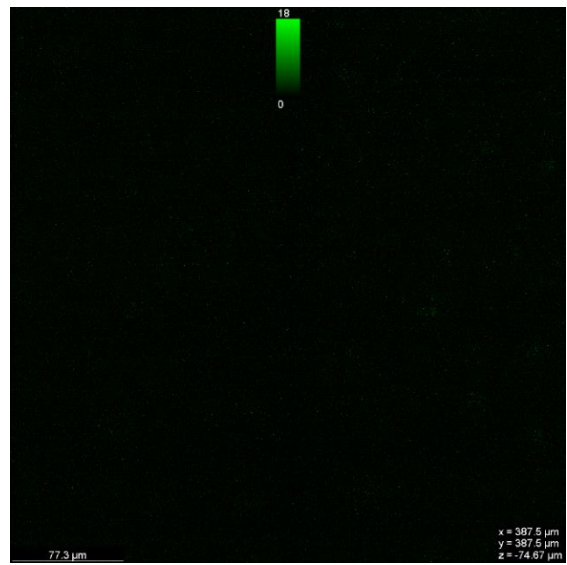


Figure B1: (a) pH. dependent normalized fluorescence emission spectra at different pH. (5.8-11) at λ_{EX} 365 nm, (b) Normalized fluorescence emission spectra for Gd_2O_3 NCs after the addition of various concentrations of freshly prepared NaCl solution at λ_{EX} 365, (c) Normalized fluorescence emission spectra of freshly prepared Gd_2O_3 NCs with different solvent at λ_{EX} 365, (d) Normalized fluorescence emission spectra for freshly prepared Gd_2O_3 NCs, previous 8 months and nearly 11 months old at λ_{EX} 365.

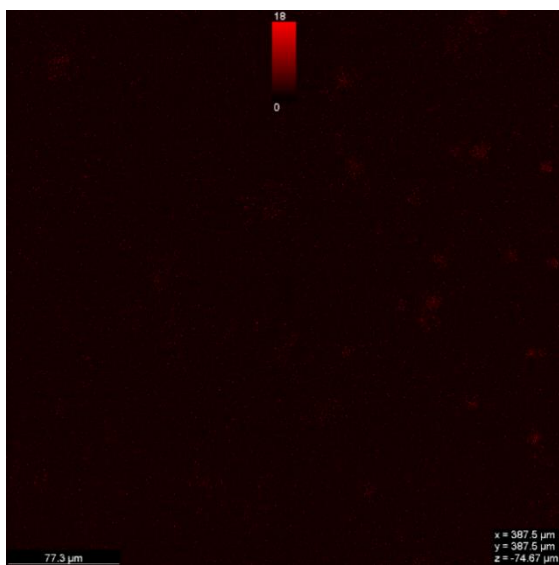
(a)



(b)



(c)



(d)

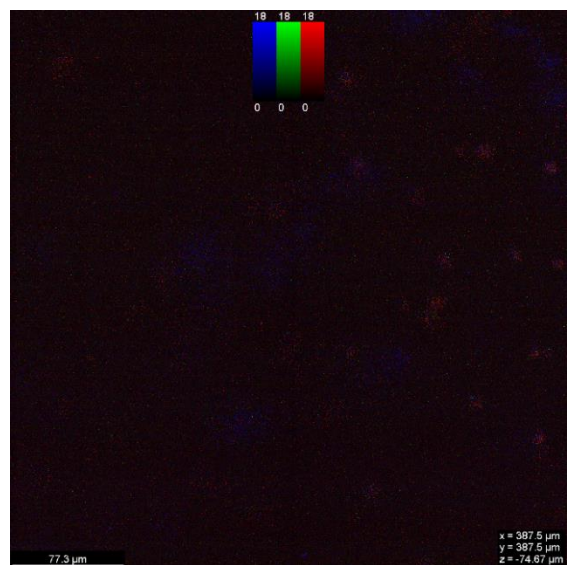


Figure B2: Multifluorescent confocal image of U 87-MG cells treated with Chitosan polymer.

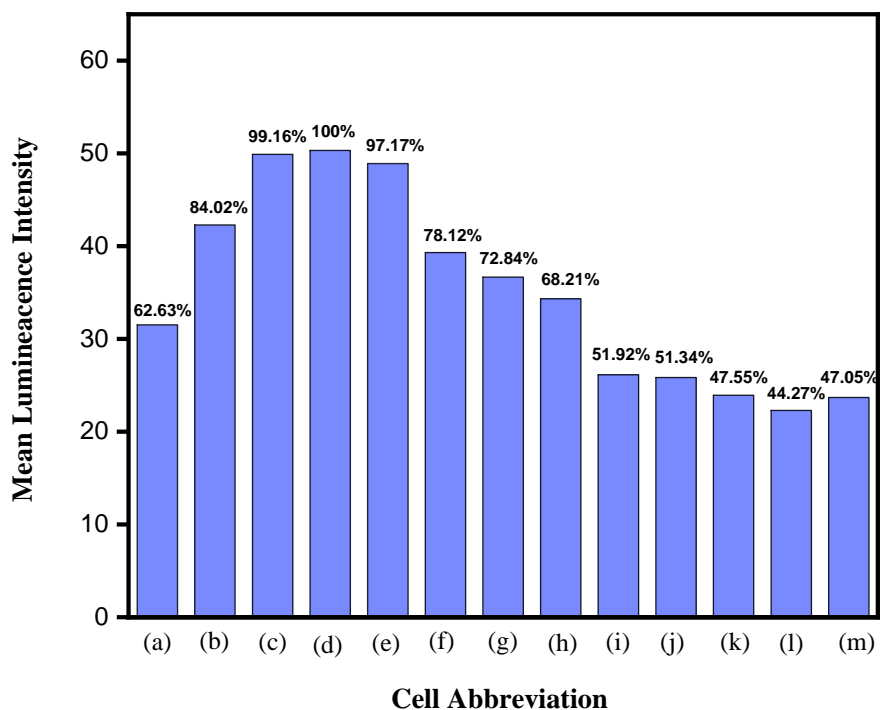


Figure B3: Mean Luminescence Intensity with Cell depth penetration in U87-MG cell line at λ_{Ex} 366 nm. Where (a) = 0 μm , (b) = 2 μm , (c) = 4 μm , (d) = 6 μm , (e) = 8 μm , (f) = 10 μm , (g) = 12 μm , (h) = 14 μm , (i) = 16 μm , (j) = 18 μm , (k) = 20 μm , (l) = 22 μm , (m) = 24 μm .

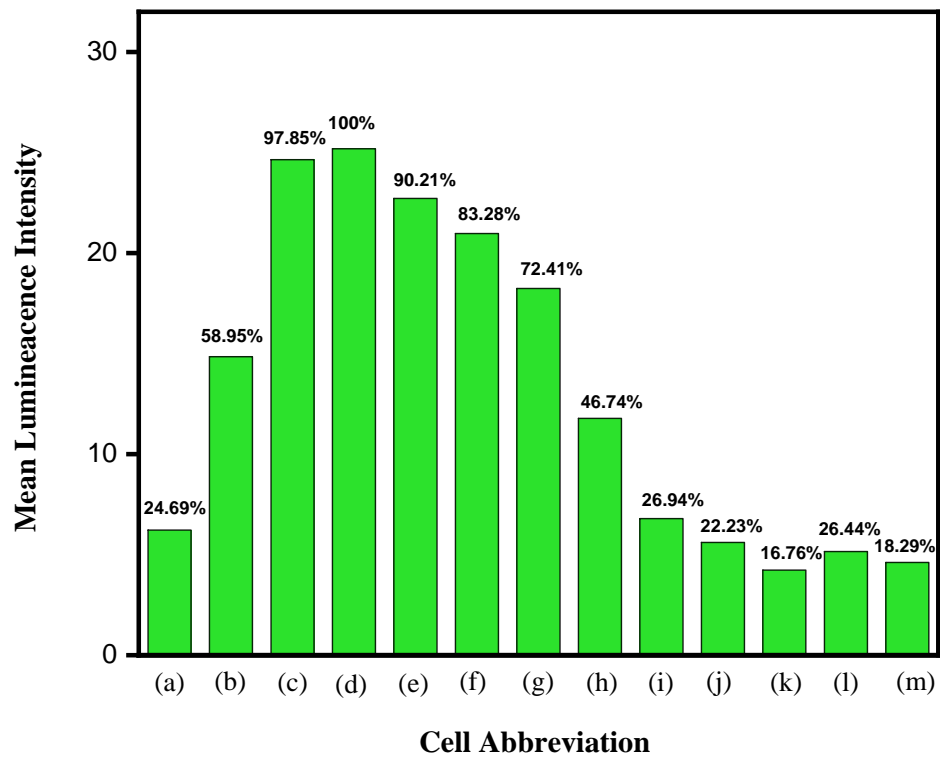


Figure B4: Mean Luminescence Intensity with Cell depth penetration in U87-MG cell line at λ_{Ex} 472 nm. Where (a) = 0 μm , (b) = 2 μm , (c) = 4 μm , (d) = 6 μm , (e) = 8 μm , (f) = 10 μm , (g) = 12 μm , (h) = 14 μm , (i) = 16 μm , (j) = 18 μm , (k) = 20 μm , (l) = 22 μm , (m) = 24 μm .

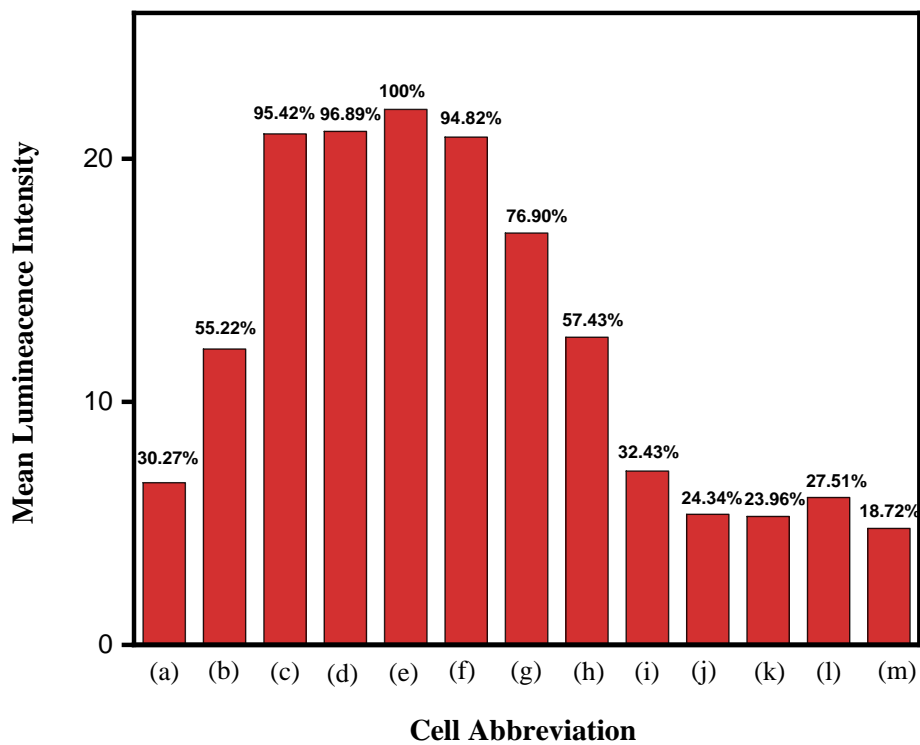


Figure B5: Mean Luminescence Intensity with Cell depth penetration in U87-MG cell line at λ_{EX} 472 nm. Where (a) = 0 μm , (b) = 2 μm , (c) = 4 μm , (d) = 6 μm , (e) = 8 μm , (f) = 10 μm , (g) = 12 μm , (h) = 14 μm , (i) = 16 μm , (j) = 18 μm , (k) = 20 μm , (l) = 22 μm , (m) = 24 μm .

Appendix C

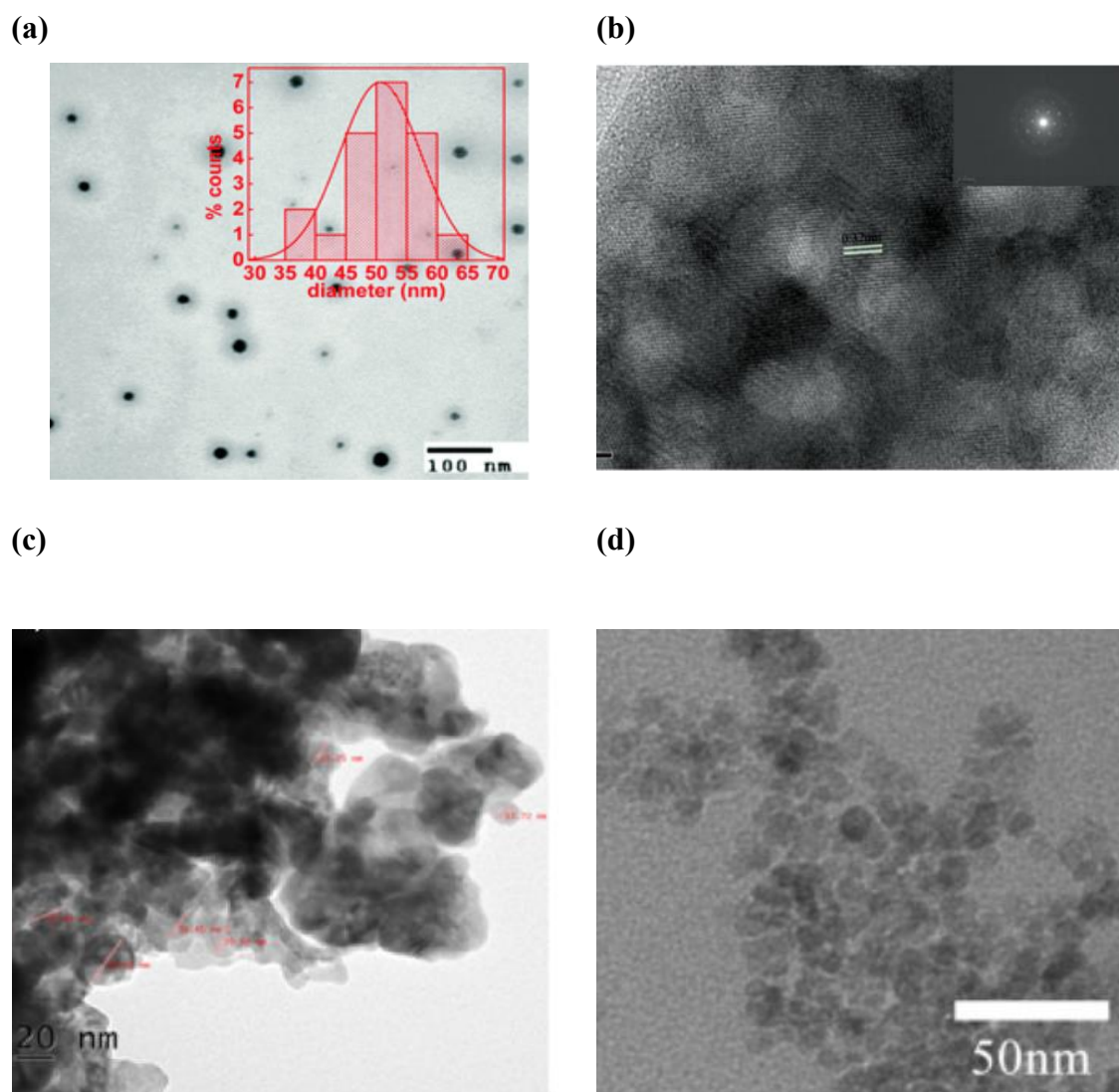


Figure C1: Representative image for TEM for various Gd_2O_3 nanomaterials from table 2.1 (a) Ref.217, (b) Ref. 219, (c) Ref.220, (d) Ref. 221.

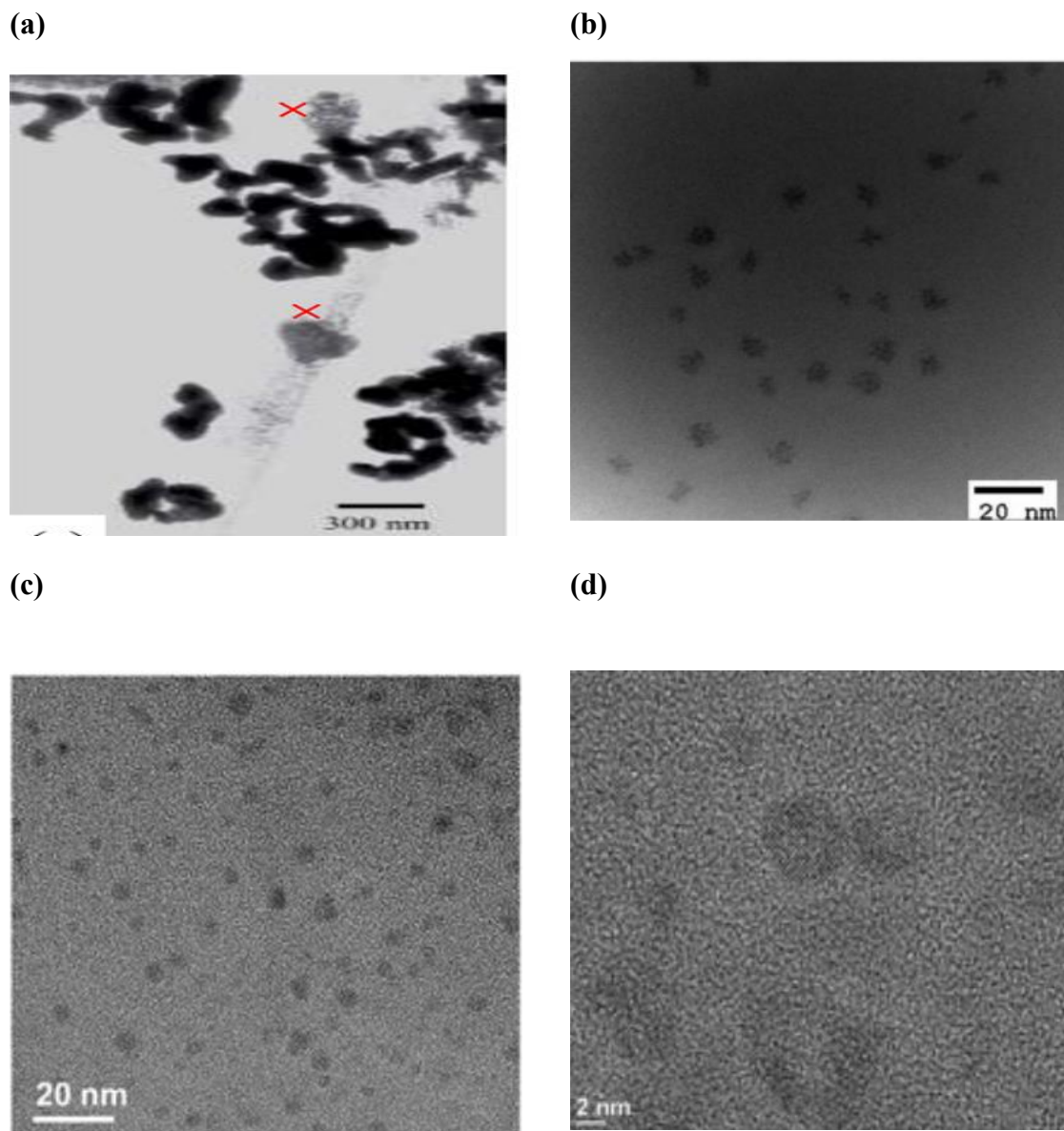


Figure C2: Representative image for TEM for various Gd₂O₃ nanomaterial from table 2.1 (a) Ref.223, (b) Ref. 224, (c) Ref.225, (d) Ref. 229.

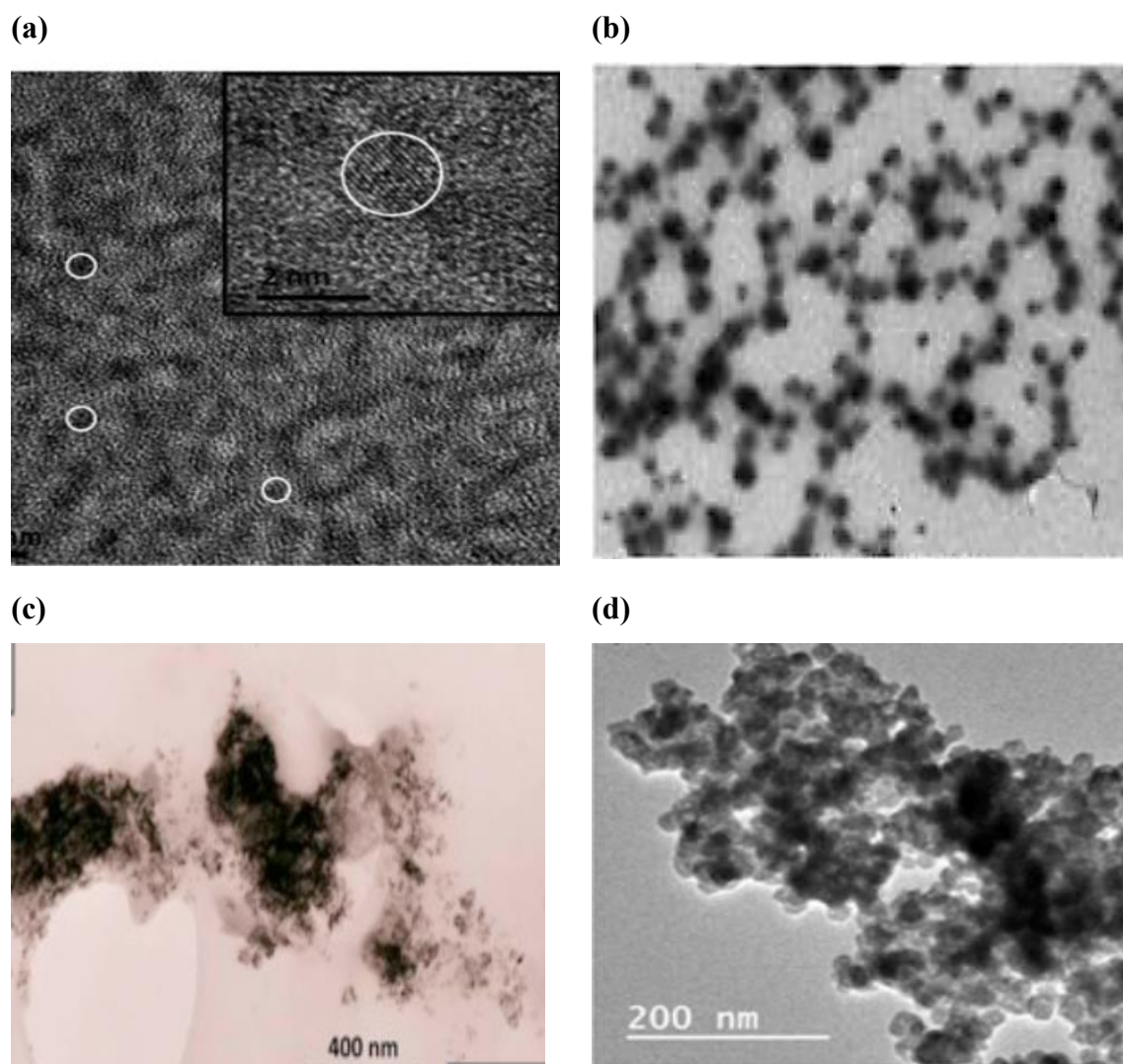


Figure C3: Representative image for TEM for various Gd_2O_3 nanomaterial from table 2.1 (a) Ref.231, (b) Ref. 232, (c) Ref.233, (d) Ref. 234.

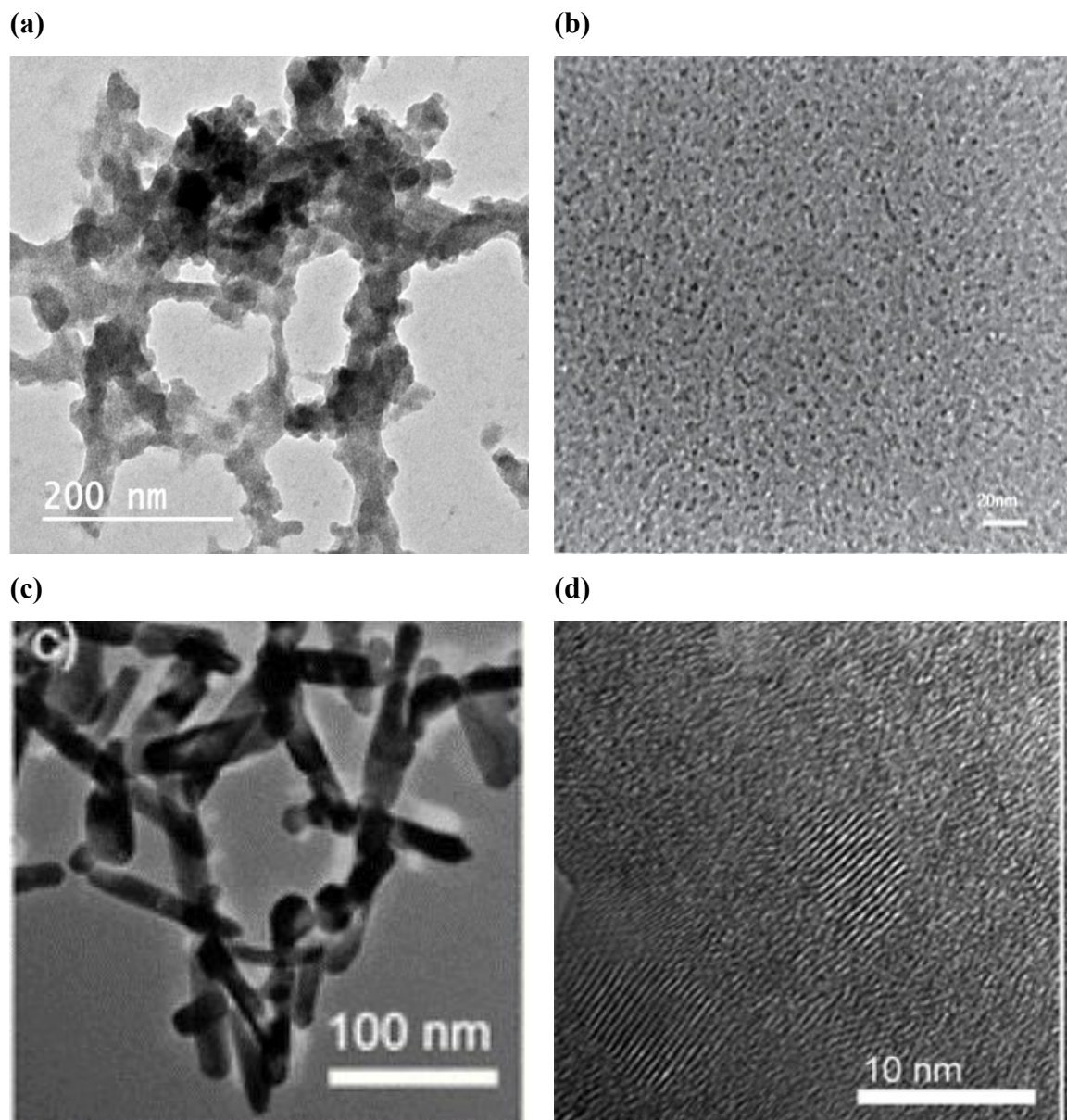


Figure C4: Representative image for TEM for various Gd_2O_3 nanomaterial from table 2.1
(a) Ref.235, (b) Ref. 237, (c) Ref.239, (d) Ref. 206.

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synergistic combination therapies against clinically relevant pathogens and
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11. Prachi Srivastava, **Vivek Kumar Verma**, Deepa Dehri, Dr. A K Agrwal, Dr. Manoj
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synthesis of metallic magnesium nanoparticles and their potential application in-vitro brain cell imaging and In vivo imaging system (IVIS).” Colloids and Surfaces B: Biointerfaces. IF 5.9 (Submitted).



Regd. No. 2123/GO/Re/S/21/CPCSEA

Date: 03 May, 2022

IAEC Approval Number: **IIT(BHU)/IAEC/2022/077**

CERTIFICATE

This is to certify that the project proposal entitled **“Design and development of nanoparticle and fluorescence Nano-systems for possible application in health care”** submitted by **Mr. Vivek Kumar Verma** under supervision of **Dr. Manoj Kumar** has been approved/recommended by the IAEC of *Indian Institute of Technology, Banaras Hindu University, Varanasi* in its meeting dated **03/05/2022** and has been sanctioned **36 Male/Female Wistar Rats (200-250 gm), 18 Male/Female Swiss Albino Mice (20-25 gm)** under this proposal for a duration of **Twelve (12) months.**

Prof. Sushant Kumar
Shrivastava
Name & Signature

Chairman

Dr. Vinod Tiwari
Name & Signature

Member Secretary

Dr. Shesh Narayan Mishra
Name & Signature

Main Nominee of CPCSEA

Note: The CPCSEA Guideline should be followed strictly while handling the animals