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Resistance Measurement of blood serum of Bovine,Avian and Caprine

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Abstract

Zinc oxide nanoparticles were synthesized by chemical bath deposition method. The samples of blood serum were characterized by X-ray diffraction (XRD) and transmission electron microscopy (TEM) after mixing it with ZnOsolution . TEM images show various morphological changes of nanostructured ZnO. The average crystallite sizes of ZnO molecule is found to be 0.004nm from XRD. The constituents of nanosized ZnOare found to be of Zn (57.27%),Cl (33.01%), C (8.04%) and O (1.68%) as obtained from EDS. Blood serum of bovine, avian and caprine are characterized by transmission electron microscopy (TEM) and Benesphera Avantor Performance (Biochemistry Analyzer) .The biological parameters- BUN, Creatinine, SGPT and SGOT of the samples are analysed which are found to be in the increasing order.These parameters helps in diagnose of disease related with liver and kidney.

Keywords:

ZnO;
BUN;
Creatinine;
SGPT;
SGOT.

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1. Introduction

From recent studies it is found that most nanoparticles (NPs) show an adverse or toxic effect on blood cells. Studies showthat administration of ZnOnanoparticles to whole blood samples and blood serums of animal and bird cause damage to the blood cells and tissues[1],[2],[3].
Creatinine is a substance that is produced by the body during normal metabolism. Creatinine is an accurate estimation of how well the kidney filtration processes are working. Anything that alters the ability of the kidneys to filter efficiently can cause variations in the level of creatinine in the blood.
The most sensitive and widely used liver enzymes are the aminotransferases. They include aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT). These enzymes are predominantly contained within liver cells and also in the muscle cells to a lesser degree. If the liver is injured or damaged, the liver cells spill these enzymes into the blood, thereby raising the AST and ALT enzyme blood levels and indicating liver disease.
Researchers have investigated that microscopicultrastructural changes occur in mice when ZnO nanoparticles is incorporated in its body [4].In the present study, we measure the four different biological parameters of blood serum of bovine, caprine and avian i.e.SGPT, SGOT, BUN and creatinine . BUN and creatinine levels provide a very accurate estimation of proper functioning of the kidneys. BUN stands for blood urea nitrogen. BUN measures the level of urea in the blood. Urea is eliminated from the body by the

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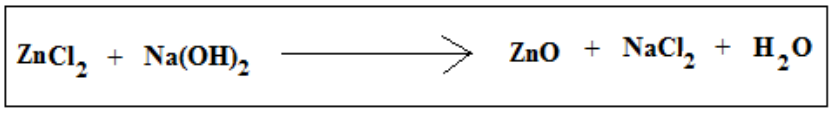
kidneys which is produced when the liver participates in protein metabolism,. Therefore, for the body to maintain a normal level of urea in the blood , both the liver and kidneys must function properly.

2. Research Method

2.1.Synthesis of ZnO nanoparticles : Zinc Chloride (Emsure) is dissolved in 200ml of deionised water in a beaker of 250 ml and placed on the magnetic stirrer and heated up to 70°C and at 450 rpm. Similarly, Polyvinyl alcohol is dissolved in 200 ml of deionised water and placed on the magnetic stirrer and heated up to 70°C respectively for three hours. After this 180ml of Zinc Chloride solution is mixed with 180ml of Sodium hydroxide solution[5] and to this mixture about 10ml of PVA solution is added, the mixture is then placed on the magnetic stirrer and is heated upto 70°C for 1 hour .This solution is then kept inside a black box for overnight and filtered [6].Thus, solution and powder of ZnO nanoparticles are obtained.



2.2. Preparation of slides: Chemically cleaned test slides are taken. Solutions of ZnO is deposited in it.

2.3.Mixing of Blood of animals and birds with ZnO solution: It has been observed that the blood serum and whole blood sample can be mixed with ZnO solution only if pH of both ZnO solution and blood samples are almost same i.e 7.2. As the solution so prepared is highly acidic (pH=4.6), so after mixing it with sample of whole blood and serum respectively, it has been observed that colour of the blood and serum changes immediately, it changes from dark red to light red and colour of serum becomes milky which indicates that haemogolobin of that blood sample decreases as shown in figure1.1 .

Fig.1.1. From Left side : Blood sample of bovine (cattle) ; ZnO mixed with blood sample (turns light red in colour) ; ZnO mixed with blood serum (turns milky)

As pH of blood is 7.2 , sodium hydroxide solution is again added drop by drop using micropipette of range 2-20 microlitre to the Zinc oxide solution to make pH value of that solution nearly equal to the pH value of blood[7]. pH is recorded after every dropwise addition of sodium hydroxide solution to zinc chloride solution which is found as 7.5. After this 0.5ml of Zinc oxide solution having pH greater than 7.5 is added to 1ml of blood of cattle and is kept for overnight. No haemolysis is observed immediately . Again 0.5ml of Zinc oxide solution is added to the same proportion of blood serum of the same cattle and is kept for one night. No haemolysis is observed. But in the next morning again haemolysis is observed in both blood and blood serum. Due to the occurrence of haemolysis , pH of ZnO solution has been made exactly equal to the pH value of blood sample(i.e.7.2).20µl of NaOH solution is added to 70µl prepared ZnO solution dropwise and after addition it has been observed that pH of ZnO solution becomes 7.2. Again 20µl of the ZnO solution (pH=7.2) is added to1ml of blood of cattle as well to blood serum and after addition no haemolysis is observed as shown in figure 1.2. Hence, the sample are used for manual test to observe whether any changes have taken place.

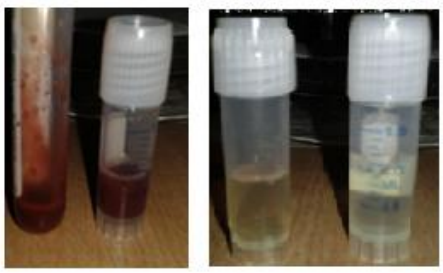


Fig.1.2. From left side : Blood sample of bovine (cattle) ; ZnO mixed with blood sample (No haemolysis seen) ; Blood serum of bovine (cattle) ; ZnO mixed with blood serum (No haemolysis seen)

It has been found that RBC (red blood cells) counts shrinks and WBC (white blood cells) are vanished. Again a new set of experiment is performed with the same ZnO solution. ZnO solution (pH=7.2) of 2µl is added to the 1.5ml of the same blood serum of bovine .The four biological parameters are tested using biochemistry analyzer as shown in the table1.1. Besides this again different amount of ZnO solution is

added to different amount of whole blood of the same bovine and the comparison and the observation is shown in table 1.2.

Table1.1 Showing 2µl is added to the 1.5ml of the blood serum of bovine

Sl.No	Biological Parameters	Blood Serum	ZnO Solution added to serum	Unit
1.	Creatinine	1.5	1.5	mg/dl
2.	SGPT/ALT	20.9	24.0	U/L
3.	SGOT/AST	39.0	48.6	U/L
4.	BUN	49.3	54.1	mg/dl

Table1.2.Table showing ZnO solution is added to whole blood of the same bovine

Sl.No	ZnO Solution added to whole blood	Observation
1	0.5ml ZnO is added to 0.5ml blood	RBC shrinks,no WBC seen,causing cell death
2	0.02ml ZnO is added to 0.5ml blood	RBC shrinks,no WBC seen,causing cell death
3.	0.001ml ZnO is added to 2ml of blood	RBC's shrink in lesser proportion ,WBC's exist

In the next set of experiment 1µl ZnO is mixed with 1ml of blood serum of 5 different blood serums of bovine, caprine and avian. The five different samples of blood serum of the three different species were then undergone clinical test for testing four biological parameters-Bun, creatinine,SGPT and SGOT. Also the above mentioned five samples of each three species were then mixed with ZnO and then again clinical test has been performed . It has been found that there is an increase in the values of the parameters after mixing all the 15 serums (5 serum of each species)with ZnO. Table 1.3 , 1.4 and 1.5 shows the comparison chart of biological parameters (after mixing with ZnO) of three species-bovine,avian and caprine respectively . Table1.6 shows the normal range of biological parameters of bovine,avian and caprine.

Table1.3:Comparison of biological parameters of Bovine

Sample Nos.	SGPT(U/L)		SGOT(U/L)		BUN(mg/dl)		CREATININE(mg/dl)	
	Serum	Serum+ZnO	Serum	Serum+ZnO	Serum	Serum+ZnO	Serum	Serum+ZnO
B1	46.2	51.3	37.4	40.2	64.3	73.6	1.8	2.1
B2	31.5	33.9	40.6	48.8	41.3	45.7	1	1.3
B3	32.3	36.8	54.6	59.2	67.3	71.4	2.1	2.5
B4	71.2	77.3	45.6	49.2	71.3	78.3	1.7	1.9
B5	17.4	21.3	26.3	33.5	44.6	48.2	1.1	1.4

Table1.4:Comparison of biological parameters of Avian

Sample Nos.	SGPT(U/L)		SGOT(U/L)		BUN(mg/dl)		CREATININE(mg/dl)	
	Serum	Serum+ZnO	Serum	Serum+ZnO	Serum	Serum+ZnO	Serum	Serum +ZnO
A1	19.1	20.3	161.7	172.4	1.2	1.4	0.4	0.5
A2	10.1	12.3	144.2	156.4	0.9	1.3	0.2	0.5
A3	14.2	15.9	366.4	371.6	1	1.2	0.3	0.4
A4	12.3	14.2	240.7	247.2	1.2	1.4	0.2	0.4
A5	10.1	13.1	272.4	278.9	1.1	1.6	0.1	0.3

Table1.5:Comparison of biological parameters of Caprine

Sample Nos.	SGPT(U/L)		SGOT(U/L)		BUN(mg/dl)		CREATININE(mg/dl)	
	Serum	Serum+ZnO	Serum	Serum+ZnO	Serum	Serum+ZnO	Serum	Serum+ZnO
C1	32.7	35.2	43.6	47.9	39.2	42.8	0.6	0.9
C2	63.1	67.9	71.2	74.6	59.6	63.2	1.7	2
C3	18.6	21.3	71.6	74.6	39.6	43.4	0.8	1.5
C4	11.1	15.4	65.3	68.3	30.5	34.1	0.5	0.7
C5	13.4	16.9	80.9	83.6	37.3	41.2	0.8	0.9

Table1.6:Normal range of biological parameters of Bovine,Avian and Caprine

Normal Range	Bovine	Avian	Caprine
SGPT(U/L)	8-57	20	15-52
SGOT(U/L)	9-49	131-486	66-230
BUN(mg/dl)	18.8-55.4	1.1	18.8-55.4
Creatinine(mg/dl)	0.5-1.6	0.1-0.4	0.5-1.6

2.4. Measurement of resistance of blood serum of bovine , avian and caprine

The resistance of 15 samples of blood serums (5 blood serum of each species) and same 15 samples mixed with ZnO are measured using multimeter and also using Arduino so as to check how resistance changes after mixing serum with ZnO.Figure1.3 below shows the circuit diagram to measure the resistance of blood serum using multimeter and Arduino. Table 1.7 and 1.8 shows the values of resistances. It has been observed that resistance increases when serum is mixed with ZnO when measured in both the cases.i.e using multimeter and using Arduino.

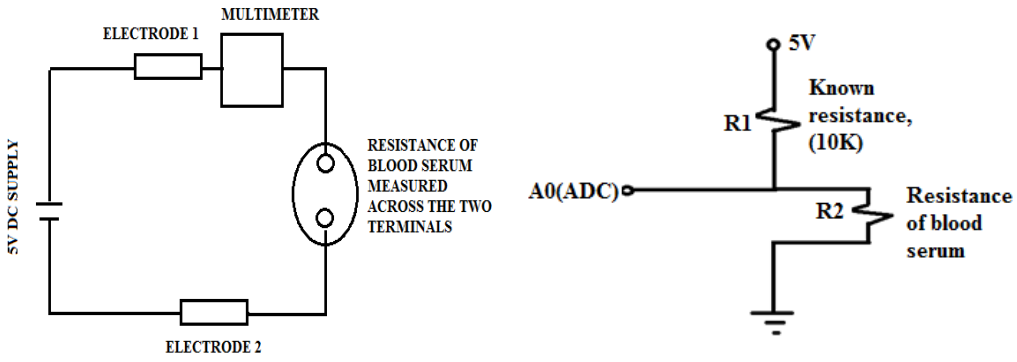


Figure 1.3.Left:Circuit diagram for measuring the resistance of blood serum using multimeter; Right:Circuit diagram for measuring the resistance of blood serum using Arduino

Table1.6 Measurement of Resistance of 5 different samples of bovine,avian and caprine before and after mixing with ZnO using multimeter

BOVINE			AVIAN			CAPRINE		
Sample Nos.	Resistance (k)		Sample Nos.	Resistance (k)		Sample Nos.	Resistance (k)	
	Serum	Serum+ZnO		Serum	Serum+ZnO		Serum	Serum+ZnO
B1	7.24	10.35	A1	9.98	12	C1	11.35	15
B2	10.46	15	A2	11	17	C2	5	8.4
B3	12.07	13.44	A3	13.3	15.38	C3	14	15.23
B4	1.9	11.6	A4	13	19	C4	13.5	16.5
B5	17	18	A5	17.22	19.89	C5	7.19	9.1

Table1.7 Measurement of Resistance of 5 different samples of bovine,avian and caprine before and after mixing with ZnO using Arduino

BOVINE			AVIAN			CAPRINE		
Sample Nos.	Resistance (Ω)		Sample Nos.	Resistance (Ω)		Sample Nos.	Resistance(Ω)	
	Serum	Serum+ZnO		Serum	Serum+ZnO		Serum	Serum+ZnO
B1	27741.9	30497.76	A1	21926.8	25088.7	C1	29106.5	30256.33
B2	29106.5	30023.35	A2	34163	35136.13	C2	32514	33050
B3	25700	26204.2	A3	28810	29559.47	C3	33231	34547.3
B4	26204.2	27088.7	A4	30820.3	32164.2	C4	27947.8	28320.98
B5	28663.8	29106.5	A5	26723	30497.63	C5	28518.4	29256.33

It has been observed that resistance increases after addition of ZnO to 5 different samples of blood serum of three species.

3.Results

3.1.TEM and EDS Study of ZnO

With the help of TEM(JEOL-100CX),TEM images of ZnO solution is obtained .The shape of ZnO nanoparticle is observed to be hexagonal as shown in figure1.4(f).

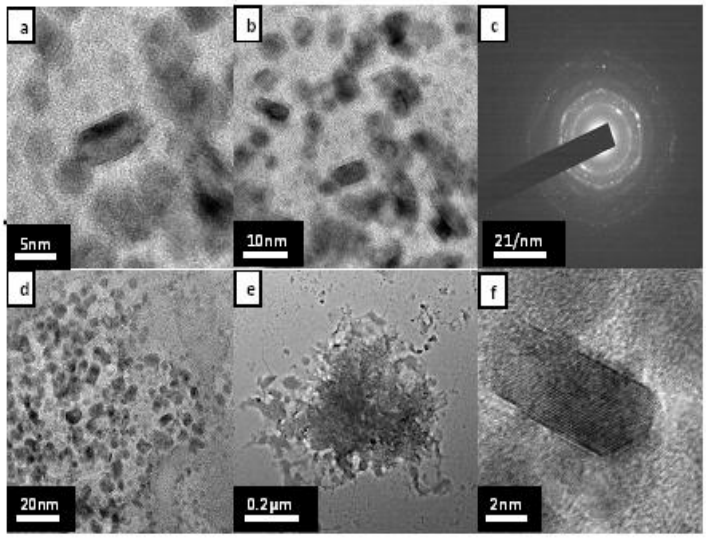


Fig.1.4 TEM images of ZnO at different magnifications

EDS was used for compositional analysis of the prepared ZnO solution[8],[9].Percentage of elements present is shown in table1.8.

Table1.8. Composition of ZnO solution found from EDS

Element	Line	k factor	Absorption corm	Weight%	Weight%sigma	Atomic%
C	K_SERIES	1.706	1.0000	8.04	0.70	25.94
O	K_SERIES	1.353	1.0000	1.68	0.34	4.06
Cl	K_SERIES	1.155	1.0000	33.01	1.26	36.06
Zn	K_SERIES	3.907	1.0000	57.27	1.49	33.94
Totals				**100.00**		

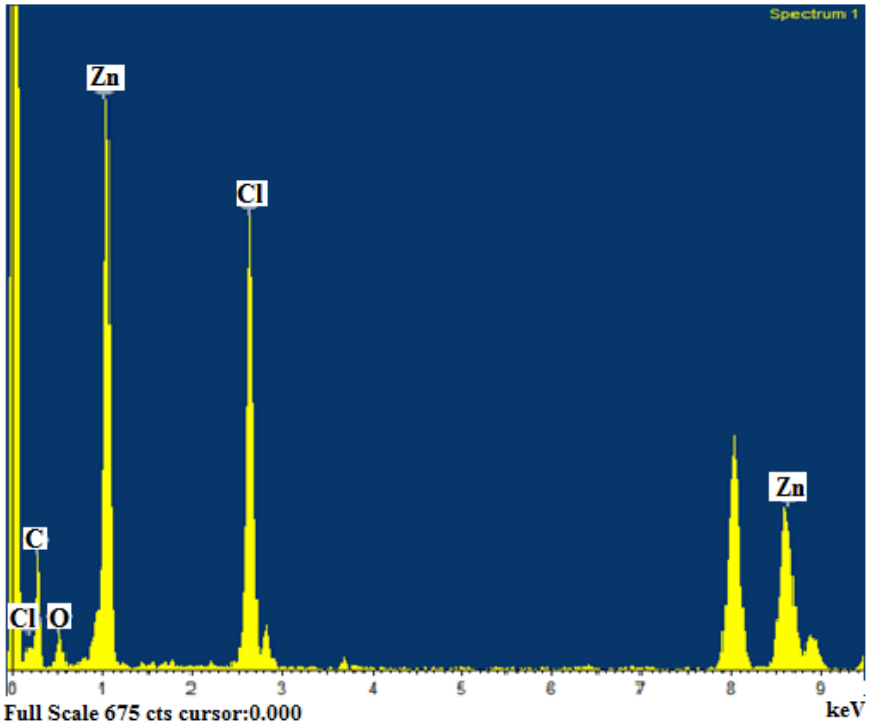


Fig.1.5.EDS of ZnO solution

EDS test is performed by putting the prepared ZnO solution on carbon coated grid. The results shows that solution contains Carbon of 8.04% (due to the presence of PVA and carbon coated grid), Chlorine of 33.01% and Zn of 57.27% (due to Zinc chloride) , Oxygen of 1.68% (because of PVA and NaOH).

3.2.1.TEM Study of Blood serum of Bovine(cattle)

1ml blood serum of bovine is used for TEM study.The images are observed using TEM(JEOL-100CX) as shown in the figure below.

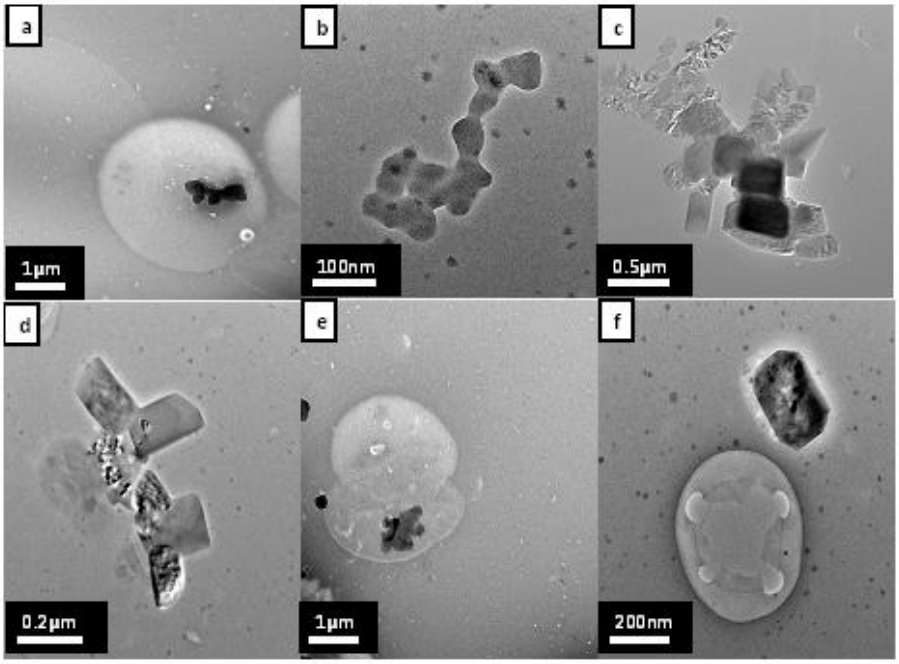


Fig.1.6 TEM images of Blood serum of Bovine

3.2.2.TEM Study of Blood serum of Bovine(cattle) mixed withZnO

1ml blood serum of bovine is mixed with 1 µl of ZnO using micropipette .TEM images are observed using TEM(JEOL-100CX) as shown in the figure below. The irregular black spots of serum proteins are observed.

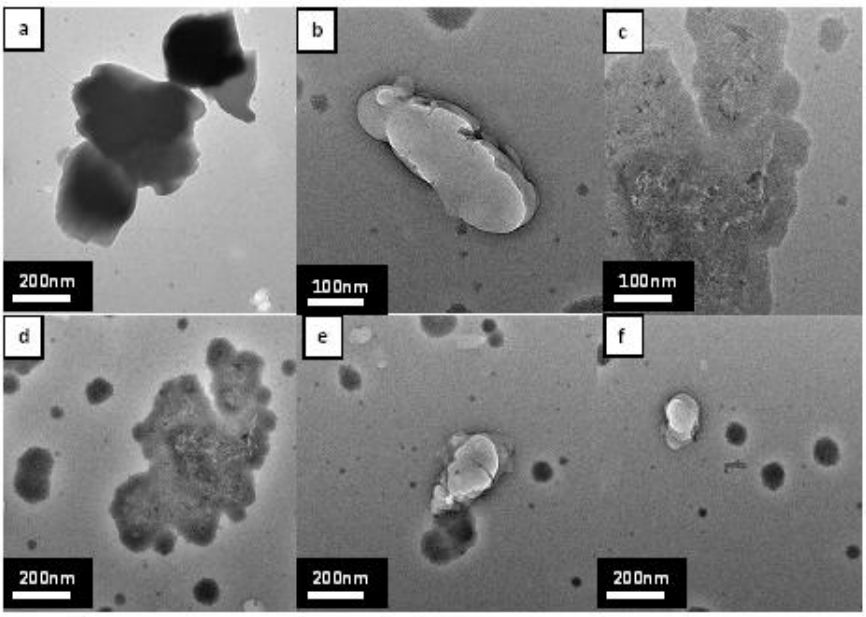


Fig.1.7. TEM images of Blood serum of Bovine mixed with ZnO

3.2.1.TEM Study of Blood serum of Avian(poultry)

1ml blood serum of avianis used for TEM study. The images are observed using TEM(JEOL-100CX) as shown in the figure below.

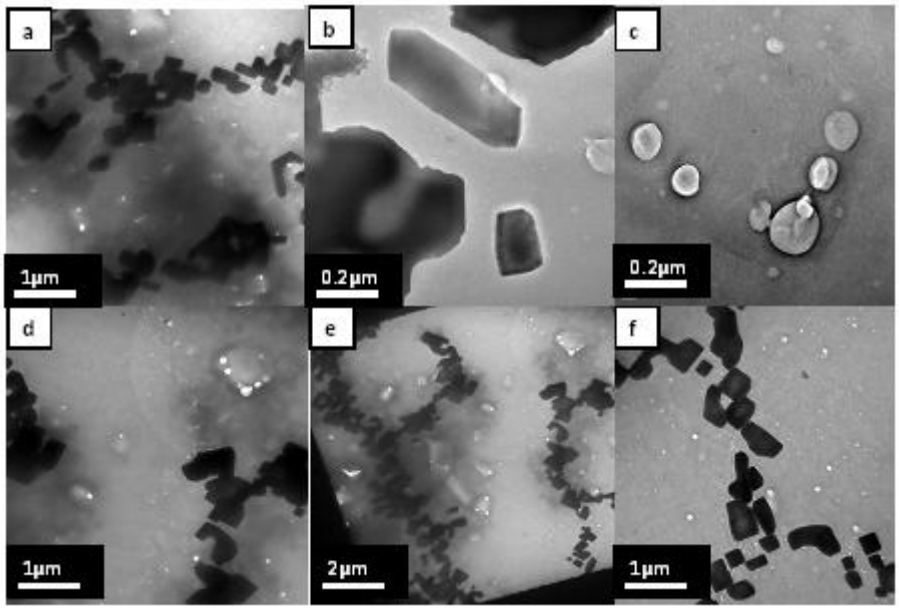


Fig.1.8. TEM images of Blood serum of Avian

3.2.2.TEM Study of Blood serum of Avian(poultry) with ZnO

1ml blood serum of avian is mixed with 1 µl of ZnO using micropipette .TEM images are observed using TEM(JEOL-100CX) as shown in the figure below.

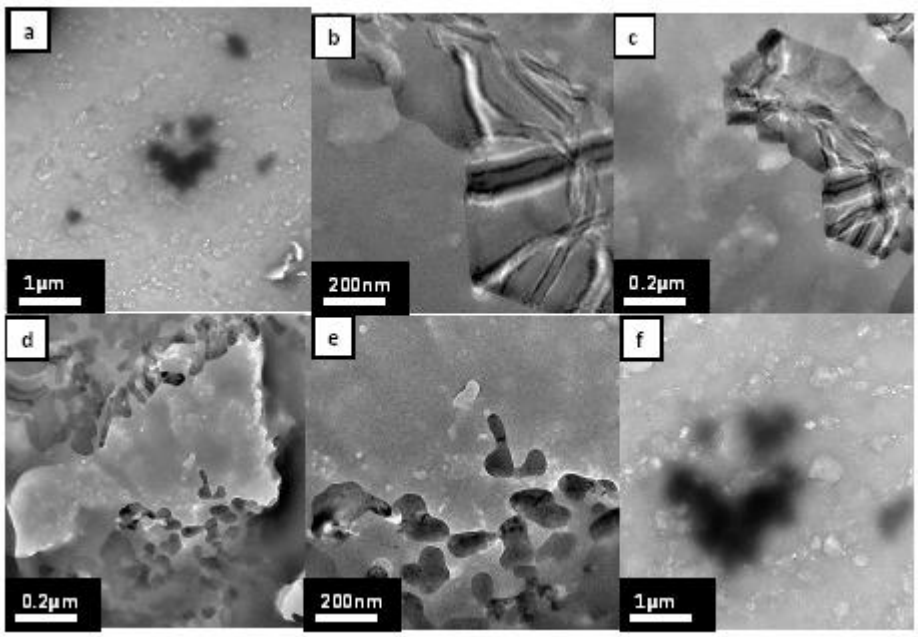


Fig.1.9. TEM images of Blood serum of Avian mixed with ZnO

3.3.1.TEM Study of Blood serum of Caprine(goat)

1ml blood serum of caprineis used for TEM study. The images are observed using TEM(JEOL-100CX) as shown in the figure below.

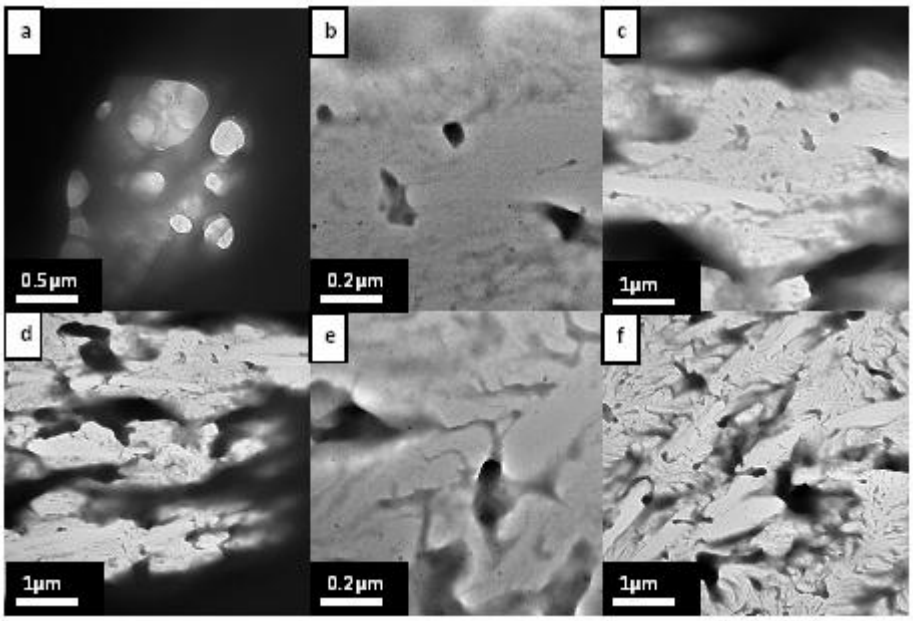


Fig.1.10. TEM images of Blood serum of Caprine

3.3.2.TEM Study of Blood serum of Caprine(goat) with ZnO

1ml blood serum of avian is mixed with 1 µl of ZnO using micropipette .TEM images are observed using TEM(JEOL-100CX) as shown in the figure below.

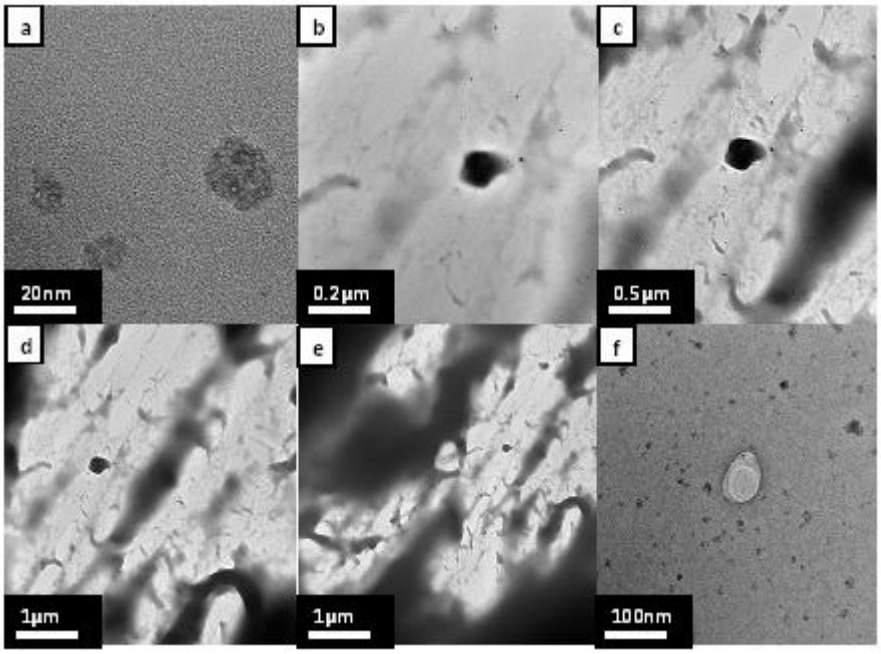


Fig.1.9. TEM images of Blood serum of Caprine mixed with ZnO

4. Conclusion

From XRD and TEM study, it is found thatZnO particles have nanosized structure[10].Thesenanosized ZnO mixed with blood serum has a hexagonal structure as observed in figure 1.4(f) .The crystallite size found from XRD is 0.004 nm. Percentage of Zn and O in the formation of ZnO is 57.27% and 1.68% as obtained from EDS .In TEMstudy, the irregular black spots of about 30-40nm are serum proteins. The average size of serum albumin is 36 nm. Bubble like structure arethe aggregated lipoproteins. It has been observed that mixing of 1µl ZnO with 1ml blood serum cause an increase in order of the biological parameters viz. BUN,creatinine, SGPT and SGOT and electrical parameter(resistance) which will help to analyse the variation of biological parameters of blood serum, after the incorporation of ZnO nanoparticle in it.

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