



A Robust Design Criterion for Synthesis, Characterization and Quality Control of Nanoparticles - a Fuzzy Mathematical Approach

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Abstract— *In this paper we present the aspects of statistical quality control for modelling a process of nanoparticles (NPs) preparation, providing feedback on the production and formulation processes in controlled manner. An in process sample preparation and particle sizing system using image processing and Dutta Majumder's generalized shape theory was developed to analyze the particle shape based properties that characterize the synthesis process. The continuous process of improvements will ultimately decrease costs and improve performance of the production system and the product. We have showed that both the nano technology based industry achieves its customers benefit from proper quality control of the process. The particle shape contours were measured using transmission electron microscope to quantify the NPs sphericity. Fuzzy based clustering is used to study the Transmission Electron Microscope image of NPs. To meet the design specification of NPs synthesis under statistical quality control, process capability ratio up to 1.5 gives good results. The results are validated with chemical analysis. This type of characterization helps the researchers in size-based spectral tuning, biological labelling, and toxicity studies and suggests general protocols to address these problems.*

Keywords— *Nano Synthesis, Fuzzy C-Means Clustering, Generalized Shape Theory & Metric, Nanomaterial, Nanoimaging, Process Capability Index.*

I. INTRODUCTION

Nanotechnology[1, 2] may be defined as the development at the atomic, molecular and micro-molecular levels in the length and scale of approximately 1-100 nanometer range, to provide a fundamental understanding of phenomena and materials at the nanoscale and to create and use structures, devices and systems[3] that have novel properties and functions because of their size and shape. The development of nanoparticles (NPs) to desired particle size and shape is an open problem[4]. When developing NPs as catalysts, their size and shape is very important; for a certain volume of material, nanoparticles make the best catalysts when they have a large surface area[5]. Statistical Quality Control (SQC) [2, 3, 6-11] refers to the application of statistical methods to monitor and evaluate systems and to determine whether changing Key Input Variable (KIV) settings is appropriate for the all processes. Nanometer sized particles are of today's interests because of their size-dependent physical properties. The chemical and physical properties of such aggregates, comprising only a few hundred atoms are in a transition region between the bulk and individual atomic or molecular properties. By understanding size related changes in these systems, it is hoped that advanced new materials can be developed together with a raft of new technologies. In contrast to the micro-fabrication technology as in semiconductor and MEMS (Micro Electromechanical Systems) industries nano fabrication utilize " top down " and " bottom up " methods [5, 12]. Structures are self assembled by taking advantage of these and some not yet fully known properties –that are being inspired by 'nature' as biological structures[13] are typically self assembled at the nano scale range using molecular interactions such as Van Der Waals Force, Electrostatic force, Hydrophobic, Hydrogen bond. The preparation of these NPs is a multivariate process, where many factors interplay to affect the final product characteristic. Consequently , in order to obtain nanoparticles having some desired properties , many batches have to be prepared , which is both time and effort consuming , since level of each variables are changed separately at the time , while keeping all the other variables constant. Some classical statistical method combined with some factorial design have been applied to solve such problems , unfortunately they presents some drawbacks related to poor prediction and this gave birth the need for a rigid experimental design in fuzzy mathematical framework (as shown in figure-1). Data can be modeled using [14-16] feed-forward network of the type "Black Box" that can adopts neural training strategy. A Multi Layer Perceptron (MLP) with feed-forward network trained by back propagation is very much useful for the study. Number of optimum hidden neurons may be chosen to 30. Networks trained using the delta rule back propagation of errors (DBP) algorithm. The name "back propagation " refers to a process of propagating the error information backward from the output to the hidden neurons, during which connection weights are modified by the delta learning rules[17]. A gradient descent method [18] is used to minimize the error. The (MSE) is defined as

$$MSE = \left(D_{act} - D_{pred} \right)^2 / N$$

Where D is any measured parameter, like diameter of NPs. Errors are squared to penalize the larger errors and to cancel the effects of the positive and negative values of the differences.

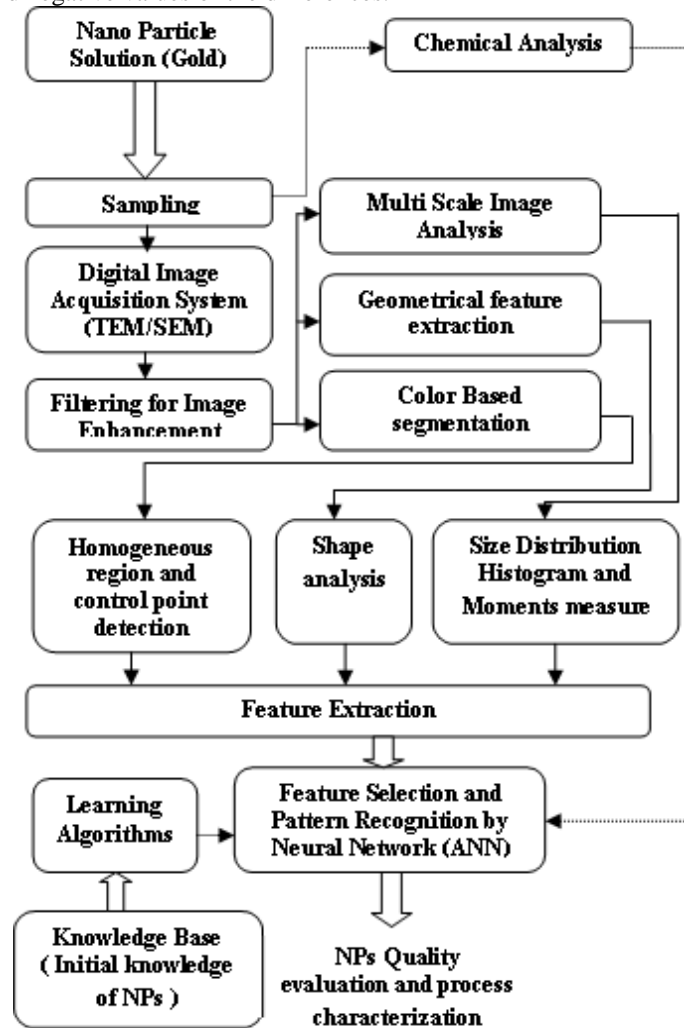


Fig.1. In process quality control of nano particles

II. MATERIALS AND METHODS

Thiol , aspartic acid , Citrate protected gold nanoparticles[19] of different size and shape were synthesized in the laboratory[20]. Transmission Electron Microscopy (TEM) and spectroscopy study for Imaging and particle size distribution of Gold NPs were performed to observe the surface topology of the particles. Fuzzy based process characterization model are used for synthesis of the gold NPs.

A. Synthesis of Gold NPs

• Synthesis of thiol protected gold nanoparticles

Aqueous solution of HAuCl₄ is mixed with solution of ToABr in toluene. Vigorous stirring causes transfer of HAuCl₄ into the organic layer . Dodecanethiol is added to the organic phase followed by addition of aqueous solution of NaBH₄ is slowly added with vigorous stirring. Aqueous solution of sodium borohydride is slowly added with vigorous stirring. A deep brown coloured solution appears at the interface. Excess ethanol is added to the separated brown coloured solution and kept overnight which causes precipitation of GNP . The ethanolic solution is filtered with nylon filter and the precipitate is re-dispersed in toluene

• Synthesis of aspartic acid protected gold nanoparticles

90 ml of 10-4M aqueous solution of chloroauric acid is prepared. The solution is heated up to boiling condition. 10 ml of 10-2M aspartic acid solution is added to the boiling solution. Aspartic acid acts as the reducing agent. The reduction process is continued under constant stirring. Heating is stopped. The reduction of the metal ions is evident with appearance of red color.

• Synthesis of Citrate capped gold nanoparticles

50 ml of 1mM aqueous solution of chloroauric acid is prepared . The solution is heated up to boiling under reflux condition. 5 ml of 1% tri sodium citrate dihydrate solution is added to the boiling solution. Sodium citrate here reduces the gold chloride. Stirring was continued until the color of the solution gradually changed from faint yellowish to clear to grey to purple to deep purple, and finally wine-red. Negatively charged citrate ions were absorbed onto the GNPs, introducing the surface charge that repels the particles and prevents them from aggregation.

• *Synthesis of Bovine Serum Albumin (BSA) capped gold nanoparticles*

80ml of 1% aqueous chloroaurate solution and 20 ml of 1% tri sodium citrate dihydrate solution is prepared separately. Both the solutions are warmed at 60°C and mixed under continuous stirring condition. Heated up to 95°C and cooled on ice immediately after appearance of wine red coloration. Under continuous stirring mode, 1ml of 2.4 microgram/ml BSA solution is mixed with ice cold colloidal gold solution. After 5 minutes phosphate buffer saline (PBS) is added and centrifuged at 10,000 rpm for 30 minute at 4°C. The pellet of BSA capped GNP is kept re-suspended in PBS solution.

B. Imaging of Gold NPs

Gold NPs, synthesized in our laboratory, were measured by field emission scanning electron microscope (FE-SEM, FEI Quanta 200F). AFM (AFM-STM, Ntegra Ts-150) study was performed to see the surface topology of these NPs. Both of these study was performed in Indian Institute of Technology (IIT), roorkee,India. Transmission Electron microscope microscopy (TEM) , FE-SEM and EDAX study of the custom made gold NPs were done by MK Implex , Canada. The z average of all the samples were measured by Dynamic light Scattering (DLS,Malvern). In this paper only TEM images of gold NPs and their different SPR spectra study including PH stability analysis are used to model the characterization.

C. NPs Image Data Clustering Using Fuzzy C-Means

TEM Image data of Gold NPs were classified using fuzzy C-Means clustering[21] algorithm to identify the particle from its background and to study details inside the image. The fuzzy-C Means based clustering process plays an important role in tracing the Gold NPs more flexible and robust to deal with noisy and uncertain data [22]. In this section, we bring up this algorithm as an iterative optimization procedure to be used for classification. Let $X = \{x_1, x_2, x_3, \dots, x_n\}$ be a set of samples to be divided (clustered) into c classes. Here we consider color as a feature for classification in RGB color space. We consider the red, green, blue (RGB) pixel values of a nano particle TEM image as a data set (X). The criterion function used for the clustering process is

$$J(V) = \sum_{k=1}^n \sum_{x_k \in C_1} |x_k - v_i|^2 \tag{1}$$

Where v_i , is the sample mean or the center of samples of cluster i , and $V = \{v_1, \dots, v_c\}$. To improve the similarity of the samples in each cluster, we can minimize this criterion function so that all samples are more compactly distributed around their cluster centers.

In summary, the c-means clustering procedure consists of the following steps:

S-1: Determine the number of clusters c .

S-2: Partition the input samples into c clusters based on an approximation. If no rule of approximation exists, the Samples can be partitioned randomly.

S-3: Compute the Cluster Centers

S-4: Assign each input sample to the class of the closet cluster center.

Repeat steps 3 and 4 until no change in J can be made.

For cluster validity. We consider three types of measures : partition coefficient, partition entropy and compactness and separation validity function.

D. Shape and size analysis of gold nano particles

The perception of shape has been used for pattern recognition, computer vision, shape analysis[23], and image registration. Here we proposed a generalized method of shape analysis and shape based similarity measures, shape distance and shape metric to measure the NPs shape.

The shape of an object can be defined as a subset X in R^2 if (a) X is closed and bounded , (b) Interior of X is non-empty and connected and (c) Closure property holds on interior of X . This representation of shape remains invariant with respect to translation, rotation and scaling. Moreover another object Y in R^2 is of same shape to object $X \in R^2$ if it preserves translation, rotation and scaling invariance[24]. In term of set these three transformations can be represented as Translation : $Y = \{(x + a),(y + b): x, y \in X\}$

$$\tag{2}$$

Rotation : $Y = \{P1(\alpha).P2(\beta)X\}$ where $P1$ & $P2$ are rotation around x and y axes .

$$\tag{3}$$

Scaling : $Y = \{(kx, ky): x, y \in X\}$

$$\tag{4}$$

Distance d_1 between shape X and Y in F is defined as follows: $d_1(X,Y)=m_2((X-Y)\cup(Y-X))$ \tag{5}

where m_2 is Lebesgue measure in R^2 and d_1 satisfies following rules: (i) $d_1(X,Y) \geq 0$, (ii) $d_1(X,Y) = 0$ if and only if $X = Y$ (iii) $d_1(X,Y) = d_1(Y,X)$ and (iv) $d_1(X,Y) + d_1(Y,Z) \geq d_1(X,Z)$

We consider, two nano particles are of same shape[25, 26] if and only if one of the images is translation, Scaling and rotation[23] of other.

To extract the feature of the boundary of the Region of Interest (ROI) it is helpful to represent the closed contour with a

set of direction. The direction code may be taken among “n” selected points on the contour, which has same distance between any two consecutive points. The direction d makes an angle 45° with direction i, where real number $d \in 1$ to 8 and $i = (1,2,..8)$. Let $d_m = (d_{ij})$, $j = 1$ to n where $m = A, B$ are the contour starting from each reference point A and B and are denoted by d_A and d_B respectively. If d_2 is a rotation of d_1 then $d_2 = d_1 + \gamma$ for any real number γ . For all j we can write $d_2 = d_1 + \gamma \forall j$ and the distance function D, in terms of the direction code between the contour of interest and the model is defined as:

$$D(d_1 d_2) = \sum_{j=1}^n \min((d_{1j} d_{2j}), 8 - (d_{1j} d_{2j})) \quad (6)$$

The normalized value of D is D/n and the shape similarity measure between the two shapes is given by $\mu = 1 - D/n$, smaller value of D indicates higher degree of similarity[27].

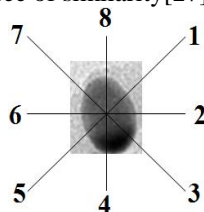


Fig. 2. Chain code representation

E. Statistical process control

Process capability[28] is the long term performance level of the process after it has been brought under statistical control. In case of NPs preparation process, it is the ability of the combination of people, machine, methods, material and measurements to produce a product that will consistently meet the design requirements or customer expectation (size and shape). Here in different stage we have performed the process capability study, that uses control charts to detect and eliminate the unnatural causes of variation until a state of statistical control is reached. Process capability indices measure the degree to which the process produces output that meets the customer specification.

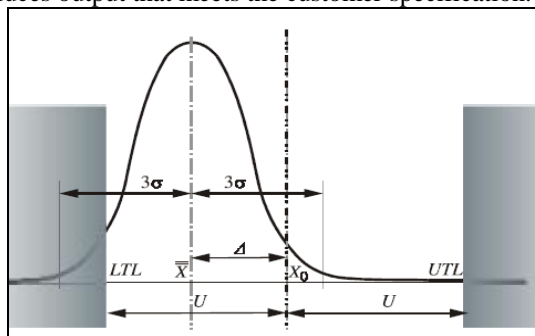


Fig. 3. Graphical details of process capability (UTL = Upper Specification Limit, LTL = Lower Specification Limit, X_0 = Nominal value of the Process, s = Standard Deviation of the Process)

Table I Definition of different process capability ratio

Index	Estimated Equation	Usage
C_p	$(UTL - LTL) / 6s$	Tolerance interval T is defined as a difference between the upper tolerance limit UTL and the lower tolerance limits LTL. That means $T = UTL - LTL$. s is the process standard deviation.
C_{pk}	$(U - \Delta - U_{KE}) / 3s$ Where $\Delta = X_0 - X_m$	X_0 -Check standard nominal value. X_m -process average mean. U_{KE} - Check standard uncertainty.
C_{pm}	$(UTL - LTL) / 6k\Omega$ Where $\Omega = (s_2 + (x_m - x_0)^2)^{1/2}$	Ω is the standard deviation around the check standard nominal value X_0 . $K = 3$ to 10

III. EXPERIMENTAL RESULTS AND DISCUSSION

We have synthesized gold nanoparticles [5, 29] confinement at 2nm, 5nm, 10nm and 20nm. Diameter of the NPs synthesized in the laboratory, were measured by field emission scanning electron microscope (FE-SEM, FEI Quanta 200F). AFM (AFM-STM, Ntegra Ts-150) study was performed to see the surface topology and confinement. SPR spectra study including PH stability analysis is used to study the properties of quantum confinement. Typically its diameter (D) and the level of confinement, is characterized by the Bohr radius of an excitation (RB). Strong confinement (The size of the Quantum Dot) occurs when $D < 2RB$ and for weak confinement $D > 2RB$. The size of strong confinement (in case of Quantum Dot) can be characterized by the Fermi wavelength inside the host materials, which is typically between 10 nm and 1 μ m.

A. Imaging and characterization of gold nanoparticles

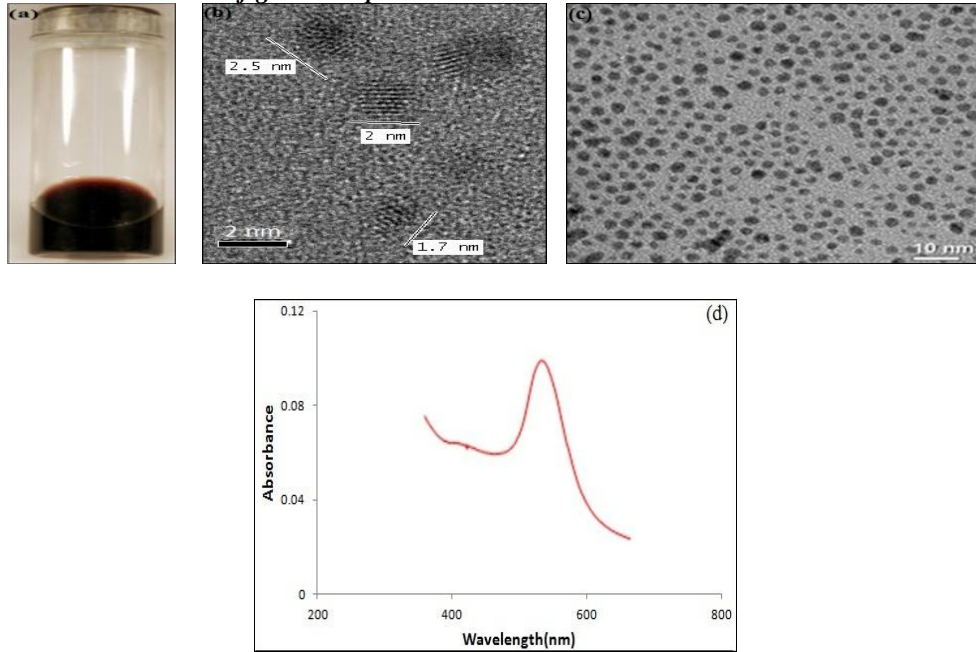


Fig. 4. (a) Interface colour of thiol capped gold nanoparticle (b) TEM image of thiol capped GNP 2nm (c) TEM image of thiol capped GNP 3- 5nm (d) Surface Plasmon peak of thiol capped gold nanoparticle

B. Shape tracing and size distribution of Gold nanoparticles

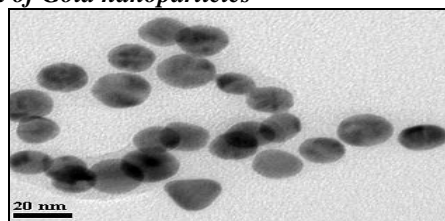


Fig.5. Original TEM Image of Gold nanoparticles 20nm

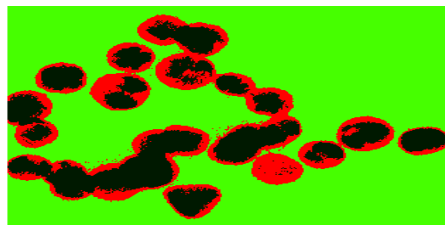


Fig.6. Segmented TEM Image of Gold nanoparticles 20 nm in 3 classes



Fig.7. Trace of Gold nanoparticles 20nm for its shape measurement in TEM image

TABLE II PIXEL VALUE WITH MEMBERSHIP AFTER CLUSTERING INTO 3 CLASSES, WITH CLUSTER CENTRE

R	G	B	Initial assignment			Afte 9th iterations		
			μ_1	μ_2	μ_3	μ_1	μ_2	μ_3
111	111	111	0.37	0.1	0.53	0.02	0.72	0.26
111	111	111	0.12	0.74	0.14	0.02	0.72	0.26
127	127	127	0.86	0.1	0.04	0	0.99	0
131	131	131	0.61	0.1	0.29	0	1	0
145	145	145	0.36	0.1	0.54	0.04	0.92	0.05
160	160	160	0.11	0.74	0.15	0.19	0.71	0.1
183	183	183	0.85	0.1	0.05	0.66	0.27	0.07
212	212	212	0.6	0.1	0.3	0.99	0	0
218	218	218	0.35	0.1	0.55	1	0	0
206	206	206	0.1	0.74	0.16	0.97	0.02	0.01
207	207	207	0.84	0.1	0.06	0.98	0.02	0.01
215	215	215	0.59	0.1	0.31	1	0	0

Cluster Centre			
Color	V1	V2	V3
R	87.3	46.5	11.9
G	13.3	83.9	38.9
B	52.8	12.8	91.3

TABLE III VARIATION OF PROCESS CAPABILITY INDEX WITH PROCESS AVERAGE MEAN DIAMETER OF NANOPARTICLE

d_{avm}	c_p	c_{pk}	c_{pm}
20.01	1.667	2.500	0.61
0			9
20.00	1.667	2.417	0.67
9			7
20.00	1.667	2.333	0.74
8			5
20.00	1.667	2.250	0.82
7			7
20.00	1.667	2.167	0.92
6			5
20.00	1.667	2.083	1.04
5			1
20.00	1.667	2.000	1.17
4			9
20.00	1.667	1.917	1.33
3			3
20.00	1.667	1.833	1.49
2			1
20.00	1.667	1.750	1.61
1			7
20.00	1.667	1.667	1.66
0			7
19.99	1.667	1.583	1.61
9			7
19.99	1.667	1.500	1.49
8			1
19.99	1.667	1.417	1.33
7			3
19.99	1.667	1.333	1.17

6			9
19.99	1.667	1.250	1.04
5			1
19.99	1.667	1.167	0.92
4			5
19.99	1.667	1.083	0.82
3			7
19.99	1.667	1.000	0.74
2			5
19.99	1.667	0.917	0.67
1			7
19.99	1.667	0.833	0.61
0			9

Standard nominal value(x_0)=20nm; process average value is d_{avm} for 21 nos of batch production; process uncertainty=.02

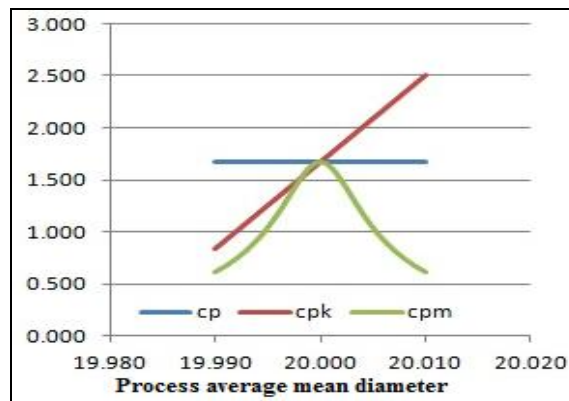


Fig.8. Variation of process capability indexes with process average mean (targeted value 20nm for gold nanoparticles)

Table IV Parts per million (ppm) rejection level vs c_{pk} value, where s is the standard deviation

Cpk	S	ppm
0.1	0.3	764177.28
0.2	0.6	548506.12
0.3	0.9	368120.18
0.4	1.2	230139.46
0.5	1.5	133614.45
0.6	1.8	71860.53
0.7	2.1	35728.71
0.8	2.4	16395.05
0.9	2.7	6934.04
1	3	2699.93
1.1	3.3	966.96
1.2	3.6	318.29
1.3	3.9	96.23
1.4	4.2	26.7
1.5	4.5	6.8
1.6	4.8	1.5887
1.7	5.1	0.34
1.8	5.4	0.0668
1.9	5.7	0.012
2	6	0.002

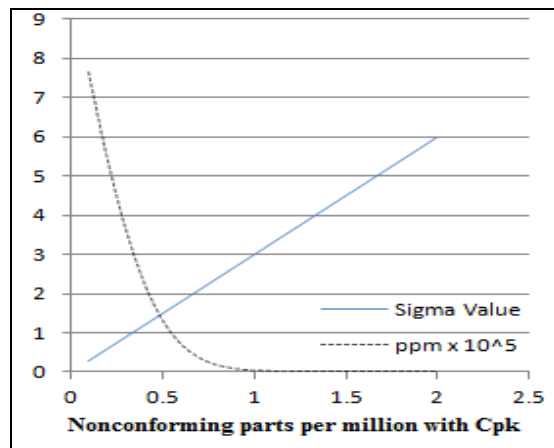


Fig.9. PPM Rejection with Cpk Value (ppm rejection is 7 when Cpk is 1.5 with Standard deviation 4.5)

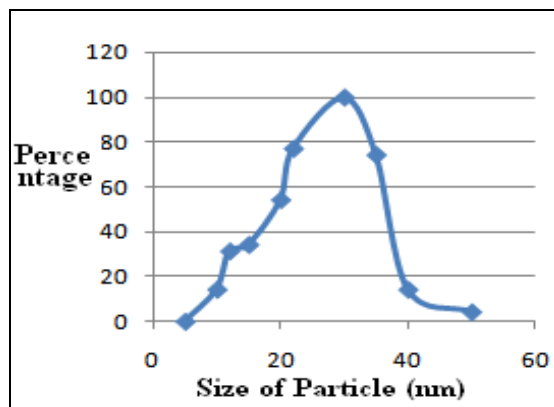


Fig.10. Size distribution of Gold nanoparticles 20 nm with an average size of 28.33nm.

IV. CONCLUSION

In this paper we presented a novel and simple method for measurement of the size, shape and other spectral response of gold NPs [29-31]. Our shape based characterization techniques gives good result for synthesis process modeling[32]. These values may be used as the input of an artificial neural network (ANN) to in process (real time) tuning of the synthesis process of gold NPs. Pre processing of training data have played an important role in modeling applications by neuro-fuzzy computing[33]. Chemical routes for the Polymer based nano-composites preparation offer the advantage of (a) a cluster or atomic level control, and (b) an efficient scale up for processing and production. The size and shape of the nanoparticles are measured using fuzzy based clustering and DDM's generalized shape theory and gives better results. The flexibility offered by the choice of different chemical agents as control parameters for prescription of process capability index [11, 34-36] of real time process control in a neuro-fuzzy environment gives best platform to control the process and synthesis.

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