

SYNTHESIS AND CHARACTERIZATION OF CHLORAMPHENICOL SILVER NANOSHELLS FOR TARGETING DIVERSE BACTERIAL INFECTIONS

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ABSTRACT: Nanoparticles offer the possibilities of being used in the treatment of various diseases. Although the synthesis of nanoshells is quite complicated, they exhibit a marked significance in eliciting the required therapeutic activity. The synthesis of silver nanoshells proceeded initially through generation of dielectric core containing chloramphenicol and simultaneous generation of nanoshells by altering the concentrations of Ethanol and Tetraethoxysilane (TEOS). Therefore, 5 varied formulations of chloramphenicol silver nanoshells are generated and the resultant are subjected to various characterization techniques such as drug content, entrapment efficacy, particle size, surface morphology, X-Ray diffraction, HRTEM etc. The results reveal that F1 meets the required criteria and are quite suitable for commercialization and serve as a basis for targeted drug delivery system.

Key Words: Silver nanoshells, chloramphenicol, antibacterial, nanoparticles.

1. INTRODUCTION:

Silver nanoshells are considered as layered nanoparticles that consist of a dielectric core encapsulated by a thin layer of metallic shell. The nanoshells consisting of metals that reveal a strong Plasmon derived optical resonance, and get shifted to longer wave lengths than the corresponding Plasmon resonance of the metal nano spheres. The resonance frequency of metal nanoshells is a highly sensitive function in regard to thickness from core to shell layers. Nanoshells can be tuned to desire structural attributes whose optical resonances range from visible to infra red region. Silver nanoshells range from 1 to 100nm with wide applicability in medical devices including surgical masks and instruments. Although silver nanoshells are not widely used as the gold nano shells, but still they created a tremendous impact on the today's era of medical sciences and especially in the field of novel drug delivery systems. The world today suffers with numerous bacterial infections and demands specific targeting of antibacterial agents that can kill the bacterial cell without affecting the healthier cell. In regard to chloramphenicol, it is a broad spectrum antibiotic isolated from *streptomyces venezuelae*. Chloramphenicol is a lipid soluble drug that exerts its mechanism of action by diffusing through the bacterial cell membrane and reversibly binding to the L16 protein of 50S subunit of bacterial ribosome, where there occurs an inhibition in transfer of amino acids to growing peptide chains. The pharmacokinetic parameters of chloramphenicol reveal that it gets completely absorbed from the gastrointestinal tract and exhibits bioavailability of 80% and when administered through intramuscularly, it exhibits bioavailability of 70%. Chloramphenicol exhibits 50-60% protein binding in adults and 32% protein binding in neonates with a half life of 1.5 to 3.5hrs in patients with impaired renal function and 4.6 to 11.6hrs in patients with severely impaired hepatic function. Therefore, only a small quantity of drug is available in the systemic circulation and might not be sufficient for producing the required therapeutic effect and demands to adjust the dosage regimen. Instead of adjusting the dosage regimen, the drug can be targeted to the specific bacterial cell through metal nanoshells which can deliver the drug molecules to the targeted cell at less risk. Hence, the objectives of investigation lies in fabricating chloramphenicol silver nanoshells that can effectively target the bacterial cell and generates the required therapeutic activity. The silver nanoshells possess a dielectric core whose activation in the presence of UV light can get activated and leads to collapse of the network and directs the drug release. In the above process minute heat energy is required for activation of nanoshells and the supplied heat does not cause the damage of healthier cell. There are several methods for the fabrication of silver nanoshells and found to be quite expensive and are not recommended for commercialization. In the current exploration we set an objective in tracing out a suitable method that can effectively synthesize the silver nanoshells and prove to be commercial with wide applications.

2. Materials and Methods:

2.1 Materials:

The various materials used for the preparation of silver nanoshells include Chloramphenicol, Tetra Ethoxy silane, Ethanol, Ammonia, Stannous chloride, Tin particles, Hydrochloric acid, Triethanolamine, and Silver nitrate.

2.2 Methodology:

2.2.1 Synthesis of Chloramphenicol Silver nanoshells:

The synthesis of Chloramphenicol silver nanoshells proceeds through a two step process which can be detailed as follows:

2.2.1.1 Synthesis of silver core shell:

The synthesis of Chloramphenicol silver nanoshells proceeds through hydrolysis and polycondensation of TEOS in which 0.8gms of chloramphenicol is dissolved in specified concentrations of ethanol and TEOS containing 12ml of ammonia. The mixture is stirred gently at 30°C for 5 hours and the resultant suspension is subjected for ultracentrifugation for complete separation of silica spheres which are washed ultrasonically and dispersed in water.

2.2.1.2 Sensitization of silica core shell:

The sensitization step proceeds through preparation of a solution containing 2.5gms of stannous dichloride, 10ml of Hydrochloric acid, and 0.2gms of tin particles in 140ml of distilled water. The mixture is stirred vigorously under magnetic stirring for 1.5hrs which results in deposition of Sn^{+2} ions on silica spheres. Further, the colloids are subjected to thorough washing with distilled water for complete removal of Sn^{+2} ions.

2.2.1.3 Coating Silicon dioxide cores with silver shells:

The current experimental section involves drop wise addition of 10ml Triethanolamine solution to 2ml of Silver nitrate solution. At the initial stages of addition it leads to the formation of brown coloured (Ag (I) oxide) precipitate which on subsequent additions, it results in colourless complexion. To the current mixture, sensitized silver dioxide particles are incorporated under vigorous stirring and the final volume is adjusted with distilled water. Hence, it leads to complete and uniform deposition of silver seeds on silver particles which on subsequent growth transforms to intense black indicating the formation of silver nanoshells.

3. Mechanism of Action:

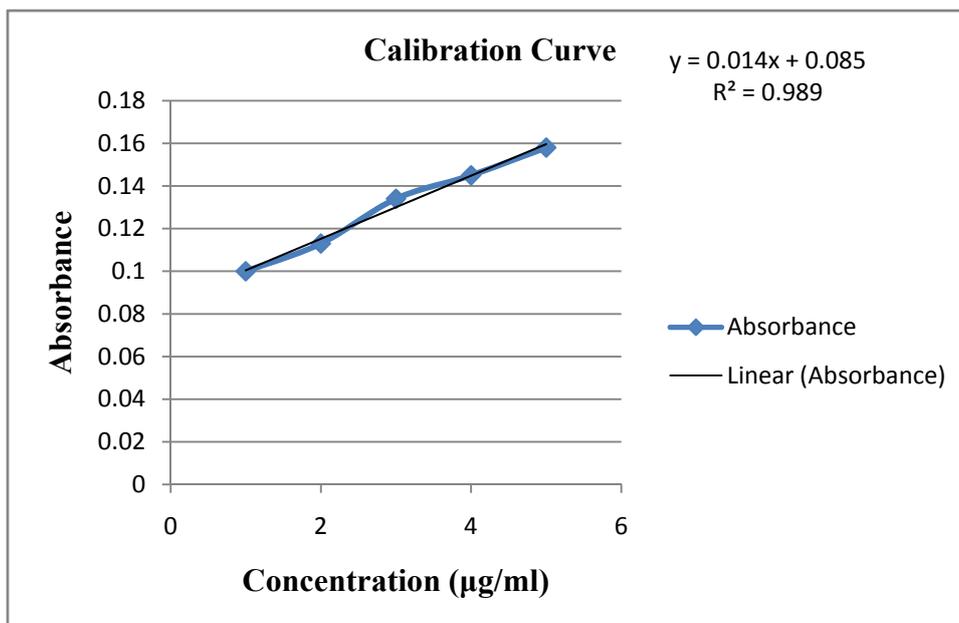
When Nanoshells and polymer matrix are illuminated with resonant wavelength, the nano shells absorb heat and transfer to the local environment which causes the collapse of network and enhances the drug release. In the current investigation, the drug is subjected to core region and encapsulated with silver seeds which on subsequent interactions get transformed to nanoshells. The encapsulation process proceeds through interaction of shell with specific functional groups of drug moieties or through electrostatic stabilization method. When the nano shells get in contact with biological system, it directs the drug release through the above described method. Apart from the above, the silver nano shells are most beneficial in destroying the bacterial cells at het almost half the quantity required to destroy the healthier cells. Therefore, on basis of above theory it can be inferred that silver nano shells are safe and efficacious in biological targeting the drug moieties to intended areas.

4. Preparation of calibration curve:

The calibration curve is generated by dissolving 100mg of chloramphenicol in 100ml of phosphate buffer pH 7.4 which serves as stock solution I. From the above, 10ml of solution is withdrawn and diluted to 100ml with phosphate buffer pH 7.4 which serves as stock solution II. From the resultant, suitable dilutions are generated to beers range i.e. 1 - 6µg/ml whose absorbance is determined spectrophotometrically at 280nm and the corresponding absorbance values (Table 1) are plotted against various concentrations (µg/ml) for generating calibration curve. Further, the calibration curve can be utilized for predicting the slope and intercept values that serves as a basis for characterizing the silver nanoshells.

Table 1: Calibration Curve of Chloramphenicol

Concentration (µg/ml)	Absorbance
1	0.1
2	0.113
3	0.134
4	0.145
5	0.158



5. Results and Discussion

Table2: Formulation table for the Preparation of chloramphenicol

Ingredients	F1	F2	F3	F4	F5
Chloramphenicol (mg)	800	800	800	800	800
Tetraethoxysilane (ml)	0.25	0.5	1	2	3
Ethanol (ml)	1.875	3.75	7.5	15	22.5
Ammonia (ml)	12	12	12	12	12
Tin Particles (gm)	0.2	0.2	0.2	0.2	0.2
Stannous Chloride (gm)	2.5	2.5	2.5	2.5	2.5
Triethanolamine (ml)	10	10	10	10	10
Silver nitrate (gm)	0.2	0.2	0.2	0.2	0.2
Hydrochloride (ml)	10	10	10	10	10

5.1 Drug Content:

The drug content for prepared formulations is traced out by grinding the nanoshells to fine powder and dissolving them in phosphate buffer pH 7.4. The resultant solution is diluted to beers range and analyzed spectrophotometrically at 280nm. The results (Table 3) specify that the % drug content range from 89.52% to 95.2% and among them F1 signifies the highest content containing 95.2% and F5 containing 89.52% as least drug content. The reasons for enhanced drug content might be due to the ratio of TEOS to ethanol in formulations which makes significant quantities of drug molecules to get incorporated in the prepared silver nanoshells and exhibit variations in drug content.

5.2 Entrapment efficacy:

The entrapment efficacy is determined by means of centrifugation method in which the prepared formulations are dissolved in a phosphate buffer pH 7.4 and subjected for centrifugation at 10000 rpm. The supernatant layer is separated and its drug content is analyzed spectrophotometrically at 280nm. Further, the obtained values are used up for generating entrapment efficacy which is depicted in table(3) for reference. The results in view of entrapment efficacy specify F5 as quite superior and optimized which might be due to the elevated concentrations of TEOS and ethanol. Further, as per the theoretical justifications on TEOS properties, it is believed that there exists a direct proportionality relationship between TEOS and particle size which is hypothesized to be a crucial factor for elevated entrapment efficacy of F5.

Table 3: Drug content, Entrapment efficacy, particle size & Zeta potential from F1 - F5

Formulation Code	Drug Content (%)	Entrapment Efficacy (%)	Particle size (nm)	Zeta Potential (mV)	%CRD
F1	95.2	82.3	50	+48	94.73
F2	91.36	85.4	51	+52	89.23
F3	90.89	88.6	100	+59	84.15
F4	92.16	92.3	200	+57	79.48
F5	89.52	94.1	0.5	+61	78.80

5.3 Particle size:

The particle size is determined using optical microscopy technique in which the size of nanoshells increased proportionately with concentrations of tetraethoxysilane. The results (Table 3) specify the particle size between 50nm to 0.5 μ m among which F5 points out as the highest particle size and F1 exhibits lowest particle size. The decreased particle size enhances the surface area of the particles and encourages the drug dissolution and pharmacokinetics parameters of silver nanoshells.

5.4 Zeta potential:

The zeta potential for the prepared formulations is assessed using MarvenZetasizer Nano-ZS (Malvern Instruments, Malvern, UK). The surface charge of nanoparticles is recommended for the assessing the physical stability of formulation (Table3). It is believed that surface charge of nanoparticles is quite useful in assessing the stability characteristics of nanoshells. The results display a positive charge on the surface of nanoshells which prevents them from agglomeration and directs them towards the negative charge of bacterial cells. Hence the system acts as a targeted drug delivery system and generates an antibacterial activity.

5.5 In-vitro Release Studies:

The in-vitro drug release studies are performed using USP dissolution apparatus type II which reveal that the formulations exhibit a slow and sustain release (Tables 4). Among all the prepared formulations F4 exhibits 79.48 % drug release at 12hrs, while F1 produces 94.73% drug release. In view of the drug release studies it can be inferred that the formulation producing least drug release in due course of time should be considered as highly optimized formulation and the results point out F4 as optimized formulation. Tracing out the theoretical background behind such release, it might be purely due to the concentration of excipients used in the formulation and more precisely due to the concentrations of TEOS, and ethanol which show a profound impact on drug release. The formulation data reveals that a proportionate increase in the concentrations of above excipients generated nanoshells of versatile sizes. The particles of decreased size exhibit an enhanced surface area which encourages them to undergo enhanced dissolution and vice versa. In the prepared formulations, F5 exhibits decreased *in-vitro* drug release of 78.80% and F1 exhibits elevated drug release of 94.73% which makes sense to consider F1 as optimized as it has undergone a maximum drug release in due course of time.

Table 4: Percentage CRD from F1 to F5

Time (hrs)	% Cumulative Drug release				
	F1	F2	F3	F4	F5
0.5	10.58	6.30	7.50	5.14	6.70
1	18.34	13.76	12.60	11.06	13.80
2	25.88	22.24	21.60	17.48	25.40
3	30.83	33.30	33.45	29.95	34.70
4	45.11	42.17	42.60	35.22	42.70
5	56.93	55.80	53.10	44.23	50.4
6	63.45	68.66	61.05	55.54	59.3
7	76.16	76.37	70.35	63.38	66.3
8	88.88	80.36	79.35	71.10	70.40
12	94.73	89.23	84.15	79.48	78.80

5.6 Drug release Kinetics:

The *in-vitro* drug release data is subjected to various kinetic parameters for assessing the type of drug release and kinetic profile from the dosage form. The results reveal a linear relationship and elevated R^2 values which confirms zero order kinetics. Further, the Higuchi values range from 0.948 to 0.973 which confirms that the drug follows diffusion mechanism. Apart from these, the KorsmeyerPeppas data specify the “n” values from 0.728 to 0.898 which confirms non-fickian diffusion (anomalous drug transport).

Table 5: Drug Release Kinetics

Formulation Code	Kinetic Parameters				
	Zero Order	First Order	Higuchi	Korsmeyer Peppas	
	Regression Coefficient	Regression Coefficient	Regression Coefficient	Regression Coefficient	“n” Value
F1	0.940	0.600	0.948	0.980	0.728
F2	0.925	0.636	0.949	0.985	0.875
F3	0.927	0.628	0.955	0.989	0.826
F4	0.948	0.667	0.949	0.989	0.898
F5	0.919	0.597	0.973	0.984	0.798

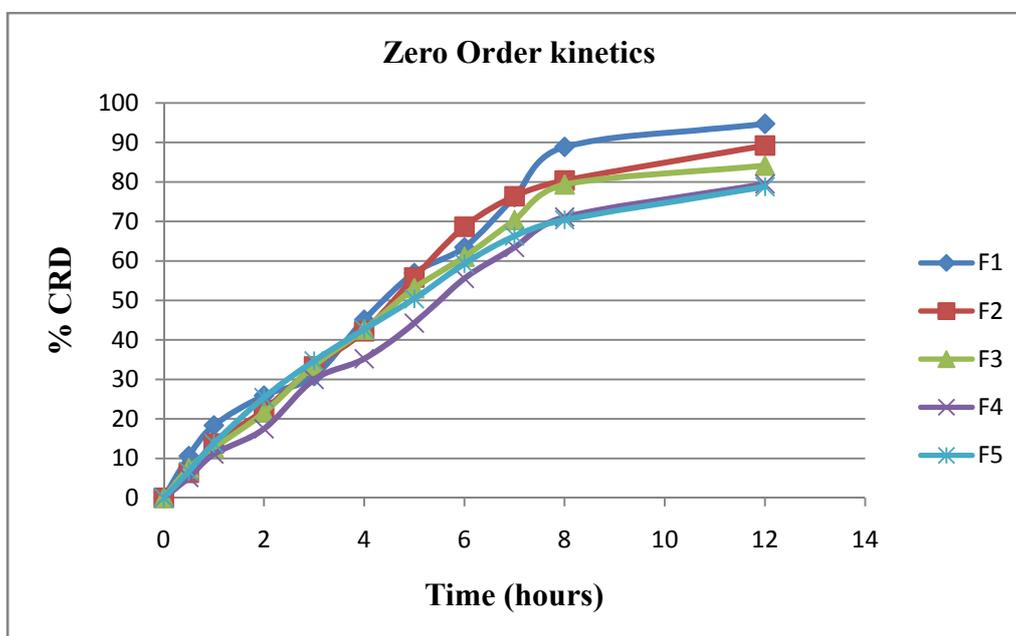


Figure 1: Zero order Kinetics from F1 to F5

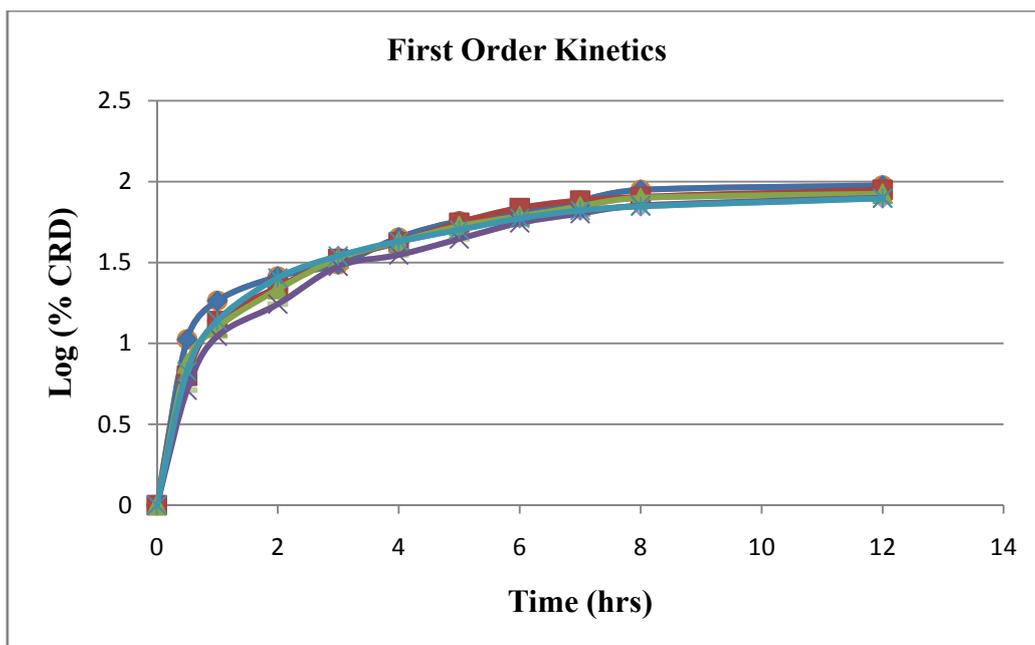


Figure 2: First Order Kinetics form F1 to F5

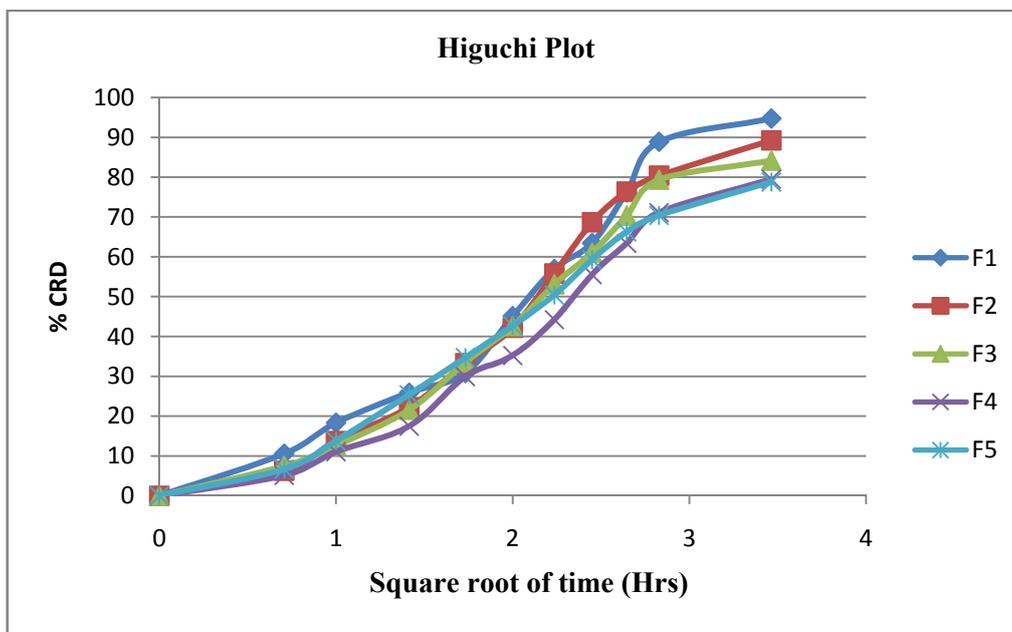


Figure 3: Higuchi plot from F1 to F5

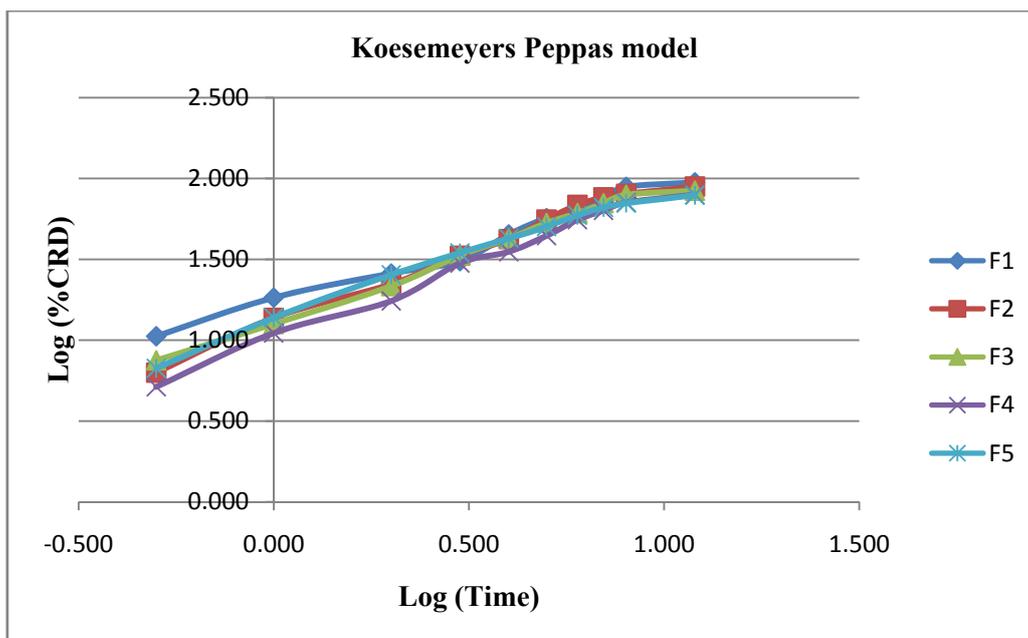


Figure 4: KorsemeierPeppas Plot from F1 to F5

5.7 FTIR Studies:

FTIR studies for pure drug chloramphenicol and their combination with various excipients are carried out prior to the formulation of silver nanoshells. The IR spectra of chloramphenicol and their specific combination with excipients are generated by mixing them with potassium bromide with nearly 100times of their weight and converting it in the form of a pellet. The pellet thus produced is utilized for developing the spectrum which on interpretation generates the information on incompatibilities of chloramphenicol with its excipients. Further, the FTIR studies developed on the basis of above procedure reveal that the characteristic peaks of chloramphenicol were present at their respective wavelengths and there is a complete absence of incompatibilities with the excipients used in the formulation. Hence, from the results, it can be concluded that chloramphenicol is compatible with the specified excipients and the developed dosage form meets the stability, safety, and efficacy attributes which is a feasibility factor in patient compliance.

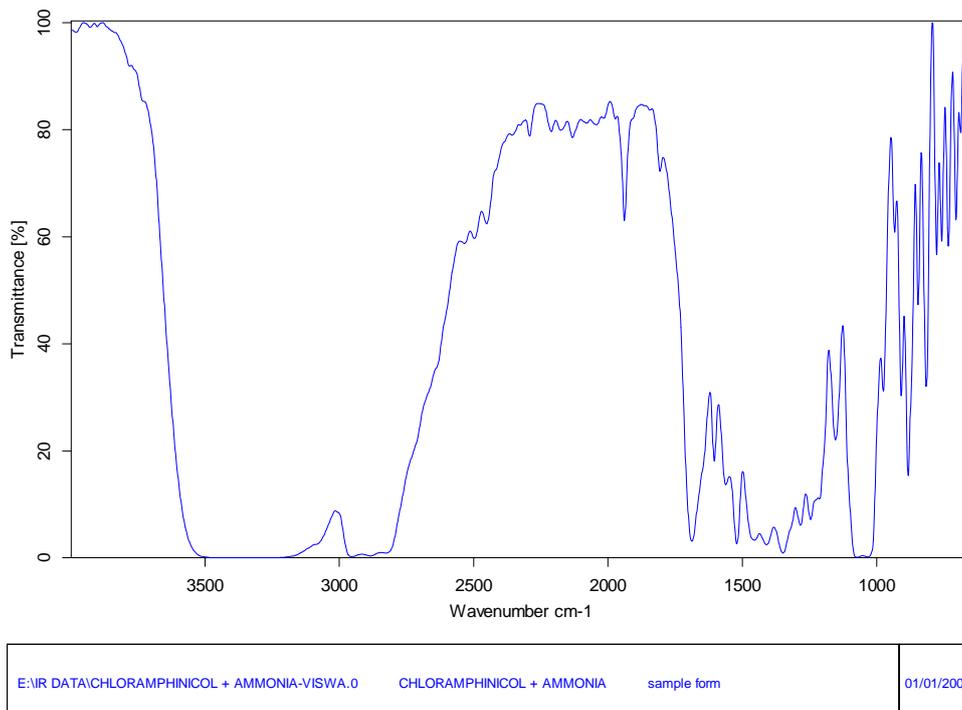
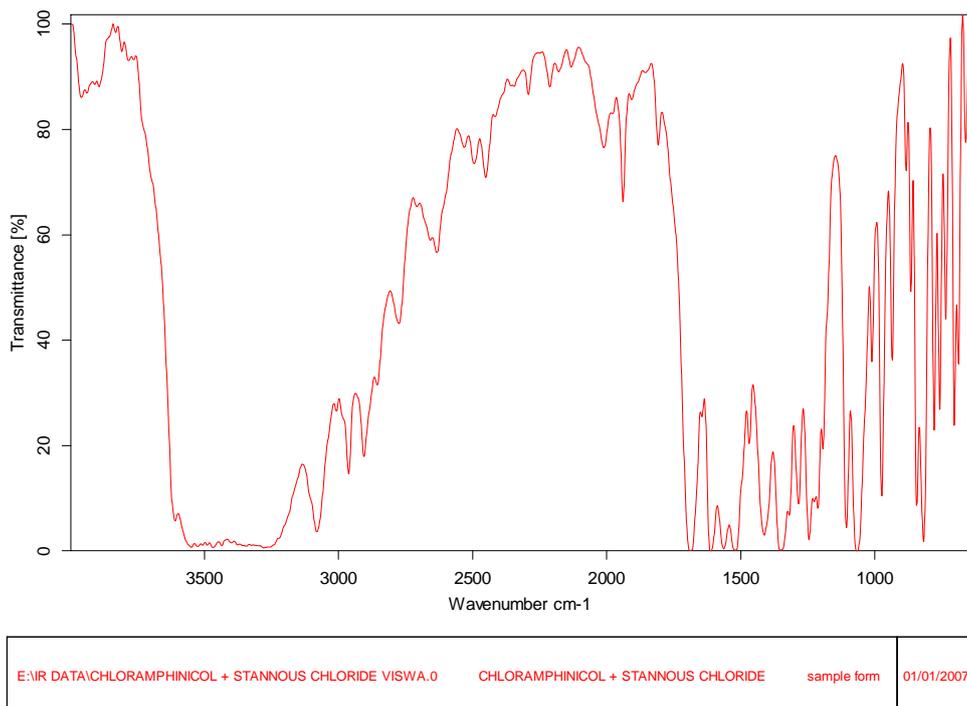
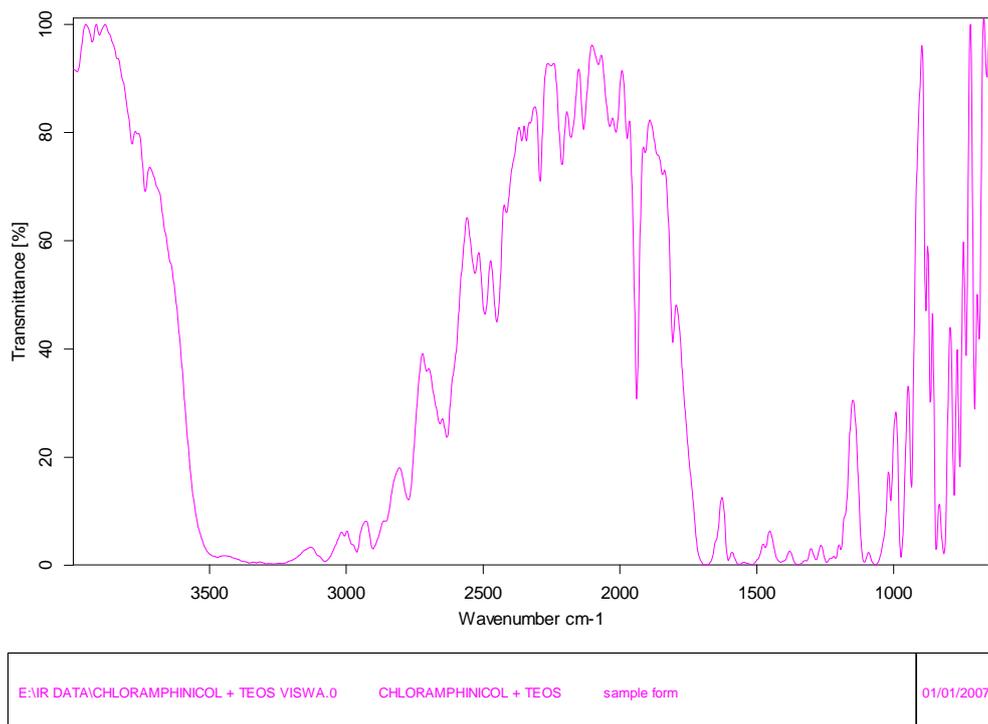


Figure 5: FTIR Spectrum of Chloramphenicol and Ammonia



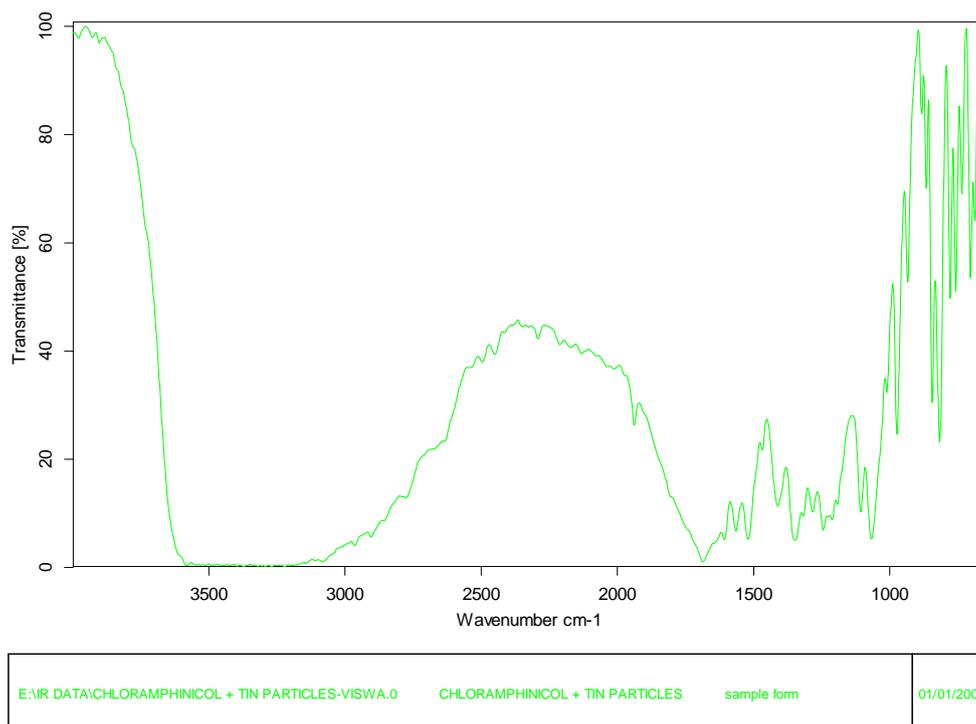
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Figure 6: FTIR Spectrum of Chloramphenicol and Stannous chloride



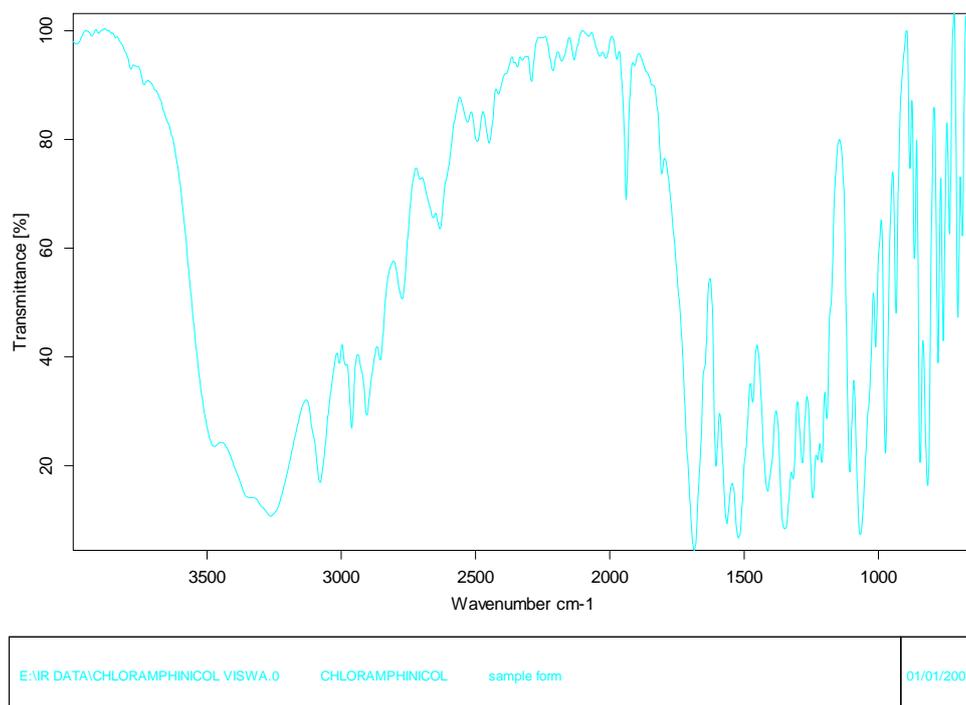
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Figure 7: FTIR Spectrum of Chloramphenicol and Tetraethoxysilane



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Figure 8: FTIR Spectrum of Chloramphenicol and Tin particles



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Figure 9: FTIR Spectrum of Pure Chloramphenicol

5.8 Powdered X-Ray Diffraction Studies:

XRD study is a common method not only recommended for the determination of nanoshell composition, but also for the estimation of their size using Bragg's law. As per the analytical procedure, X-rays are mounted on powdered sample which get scattered and interfered constructively at a particular angle 2θ . The scattered rays from the "reflecting planes" and the corresponding diffracted X-rays are detected, processed, and counted. The orientation and interplanar spacing between these planes are represented by three integers h, k, l indices which

cut a, b, c axis of a unit cell in l second. Further, the relationship between diffraction peaks to d- spacing allows estimation of mineral composition which can be represented by Bragg's law as follows:

$$(n\lambda) = (2d\sin\theta)$$

As described above, the XRD spectrum for powdered silver nanoshells (Table 11) are predicted between “2 θ ” and “Lin counts” in which the counts signify the number of reflections due to crystal planes. Simultaneously, the collaborative information on d-spacing's and intensity are used for identifying the type of material by comparing them with standard patterns mentioned in international powder diffraction files compiled by the joint committee of powder diffraction standards (JCPDS) which is currently referred as International Centre for Diffraction Data (ICDD). Apart from these, for converting the value of $1/d^2$ to an integer we consider to multiply it by means of z value and currently, we consider “z” value as 0.064 which results λ value as 1.54 Å. The corresponding peaks generated at 38.159 Å, 44.321 Å, 64.448 Å, 77.381 Å are assigned as (100), (111), (200), (220), (311) reflections which signify face centered cubic (FCC) particles respectively.

Table 11: chloramphenicol XRD data and their corresponding Miller Indices

S.No	2 θ values	θ values	“d”	$1/d^2$	$(1/d^2)/z$	h	k	L
1	22.501	11.250	3.948	0.064	1	1	0	0
2	38.164	19.082	2.356	0.180	3	1	1	1
3	44.317	22.158	2.042	0.239	4	2	0	0
4	64.450	32.225	1.444	0.479	8	2	2	0
5	77.383	38.691	1.232	0.659	10	3	1	0

CLP-Ag nano shell

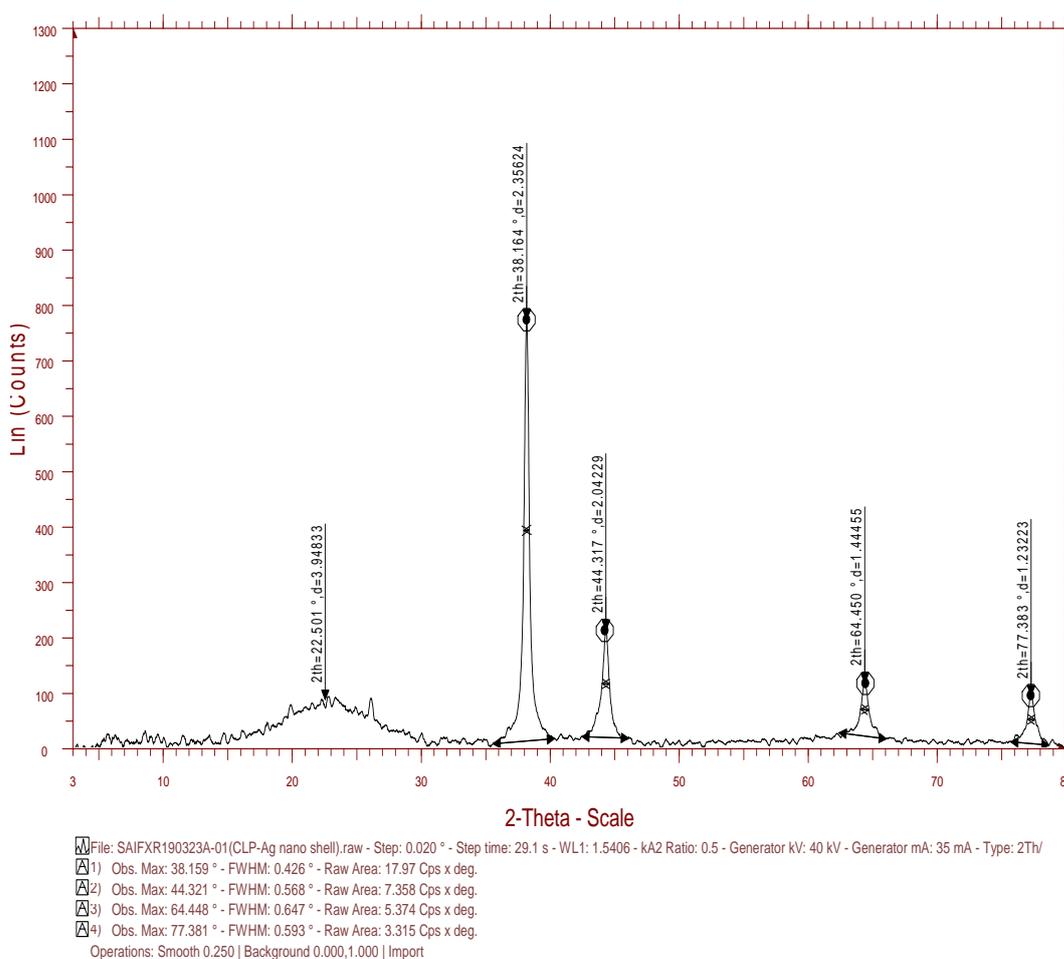
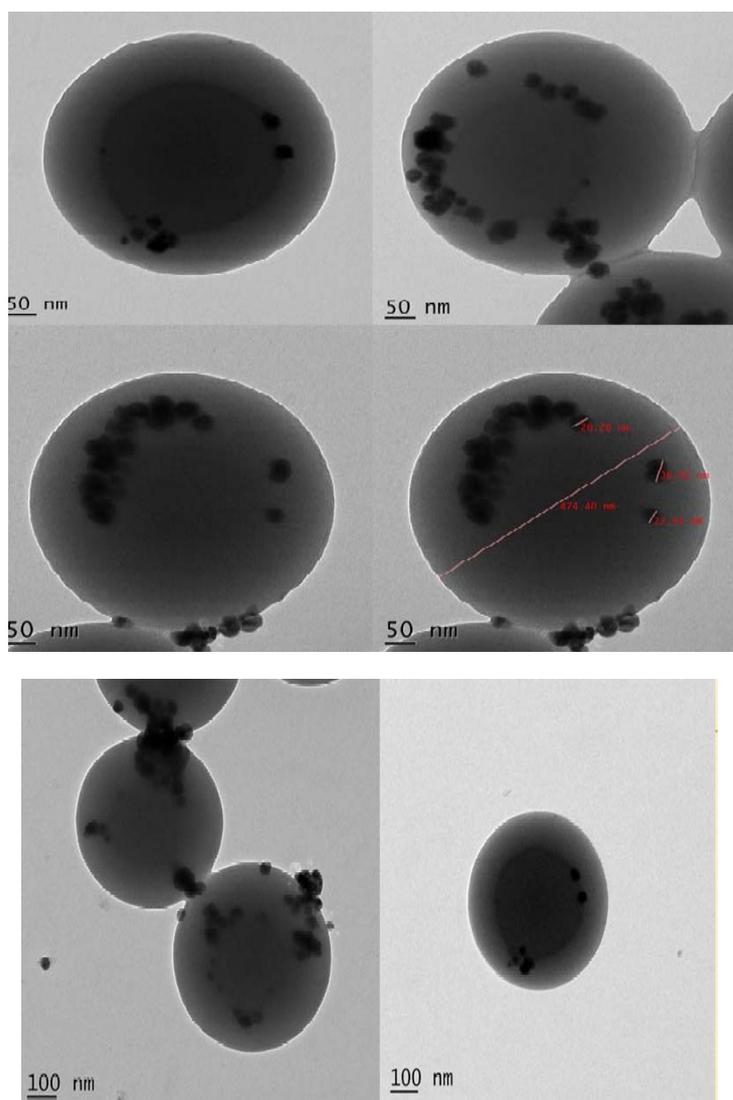


Figure 10: XRD Spectrum of Chloramphenicol silver nanoshells (F1)

5.9 High Resolution Transmittance Electron Microscopy (HR TEM):

HR TEM is a specialized imaging mode in Transmittance Electron Microscopy (TEM) that allows a direct imaging of atomic structure of the sample. HR TEM utilizes both transmitted and scattered beams to generate interference images that are extensively recommended in analyzing the structural attributes and lattice imperfections on atomic resolution scale. It is highly specialized in tracing out information on point defects, dislocations, stacking faults, precipitate grain boundaries and surface electrons using a positive electrode potential. The instrument consists of gun that generates a source of electrons which passes through “condenser lenses” and gets transformed into a monochromatic beam. The resultant strikes the target specimen and focused through “objective lens” that gets converted to an image. The resultant is fed through “intermediate and projector lenses” which gets enlarged depending upon the required magnification.



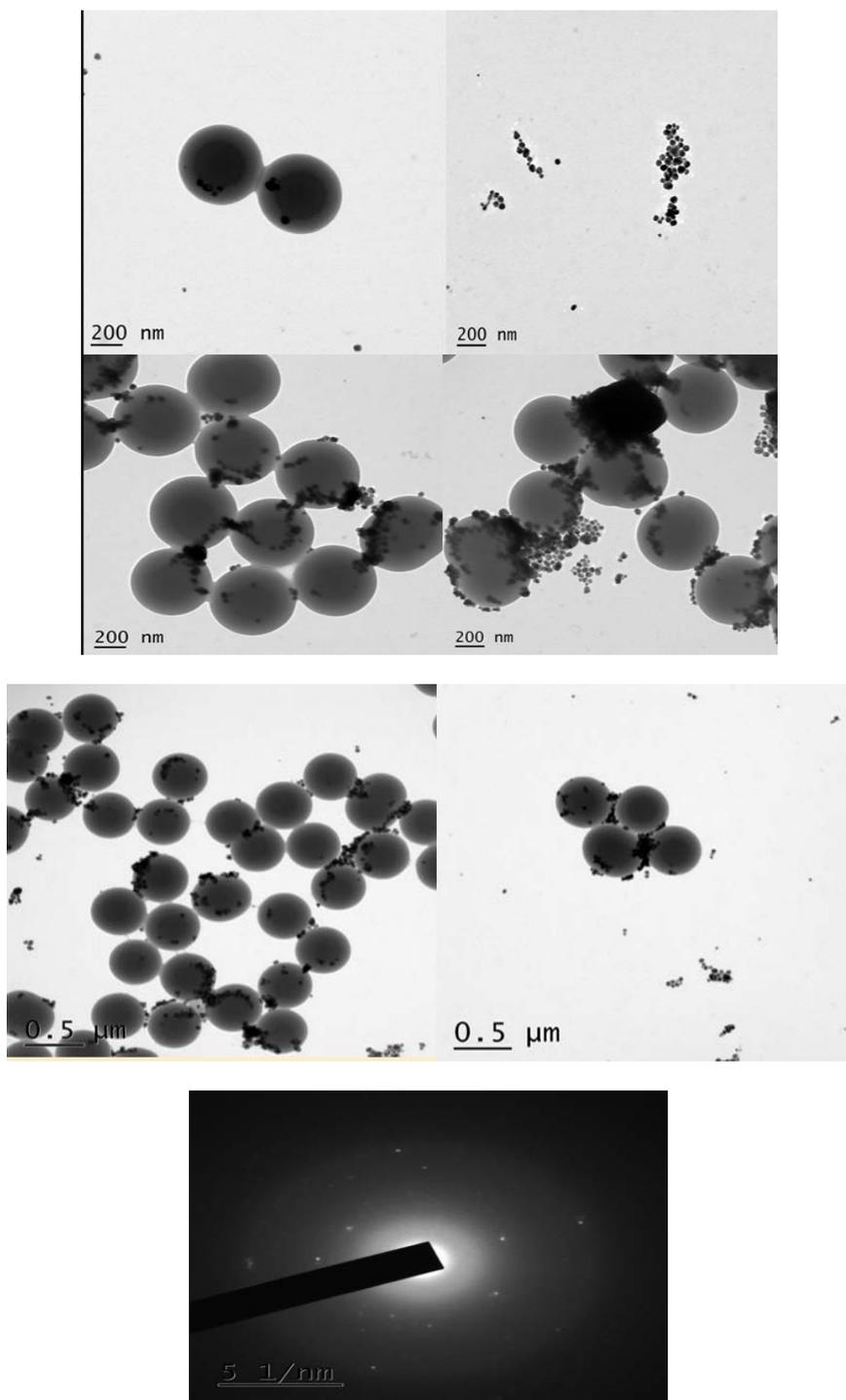


Figure 11: TEM images of Chloramphenicol Silver nanoshells

As described above, the images generated by HR TEM signifies the particle size ranges from 20 to several nanometers. The images reveal the presence of dielectric core and a proportionate increase in the shell thickness with respect to TEOS concentration and furthering it can be concluded that the nanoshells lack agglomeration and the average particle size remained constant. In continuation to the above, the surface morphology of silver nanoshells reveals that the particles are uniform, and spherical in nature which prevents the discrepancies in related to structural and morphological attributes.

Conclusion:

Nanoshells are considered as a specific category of nanocomposite materials in which one material is coated as a thin layer with the aid of another through selective techniques. They reveal superior functionalities with tailored properties and exhibit enhanced properties over existing nanoparticles through modifications in core to shell ratio. The current investigation is focused on development of chloramphenicol silver nanoshells comprising a dielectric silica core and modifying the resultant to metallo-dielectric nanocomposites. A total of 5 different formulations containing the altered ratio of TEOS to ethanol are generated and subjected to *in-vitro* analytical methods such as drug content, entrapment efficacy, particle size, and zeta potential, XRD, HR-TEM etc. for generating an optimized formulation that meets the pharmacokinetic and pharmacodynamic attributes. Hence, among the generated formulations F1 is found to be quite superior as it exhibits elevated drug content(95.2%), entrapment efficacy (82.3 %), particle size (50nm), and zeta potential (+48 mV) in comparison to other formulations and can serve as a basis for further investigations in the field of nanotechnology.

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