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Application of Augmented Simplex-Mixture Design for Synthesis of Elemental Silver and its Effect on Insolubility of Ibuprofen

Kailas M. Karande[1] and Shivaji P. Gawade[2]

[1]*Department of Pharmaceutics, Satara College of Pharmacy, Satara, India*
[2]*Principal and Director, Veer Vijay Pharmacy College, Vill- Fatehpur Bhado Post - Chhutmalpur Dist, Saharanpur, India*

[*]***Corresponding author:*** *Kailas M Karande, Assistant Professor and Head, Department of Pharmaceutics, Satara College of Pharmacy, Satara, India. Tel: 9850346554; E-mail: karandekailas@gmail.com*

ABSTRACT

Metal nanoparticle-drug interaction is indeed an unexplored area of research and guarantees some effective outcomes pertaining to improvements in drug characteristics. This area of research would really bring glory to drug discovery owing to simplicity, efficiency, and organ targeting talent of metal nanoparticles. Various drugs get adsorbed on AgNps, thus increasing their solubility in water solution. This novel approach where the physicochemical properties of the drugs adsorbed on noble metal nanoparticles surface will allow us to deep in the design of new drug release systems with the potential to develop the clinical efficiency of the therapeutic effect of drug molecules. The present investigation reported a green method for synthesis of elemental silver i. e. Silver nanoparticles (AgNps) using gelatin and cow urine. Synthesized AgNps were subsequently evaluated for its effect on the insolubility of Ibuprofen. TEM of AgNps showed spherical shape and size distribution was significantly in the range of 10 to 20 nm. SAED pattern showed diffused rings pattern. XRD revealed the insignificant increase in the crystallinity of the AgNps. IR supports the evidence of the contribution of amide linkage in the process of reduction. DSC supports the evidence of elemental silver entrapped in helical strands of proteinous gelatin. Phase solubility study was carried out in and off a presence of AgNps which revealed that an increase in the concentration of AgNps may affect positively poor solubility of Ibuprofen. Instrumental analysis performed in the present investigation along with the SAED pattern may reveal the solubility enhancement was the consequence of adsorption phenomenon.

Keywords: Solubility enhancement, Silver nanoparticles, Augmented simplex mixture design.

INTRODUCTION

In recent years the subject of nanoparticles has received explicit interest in an exceedingly wide selection of fields [1]. The term "nano" comes from the Greek word "nanos" which means dwarf and denotes a measure on the dimensions of one-billionth (109) of a metre in size [2,3]. A strand of deoxyribonucleic acid is 2.5 nm in diameter [4], a typical virus is around one hundred nm wide [5] and a typical bacteria is around 1-3 µm wide [6]. The concept of nanotechnology was coined by scientist prof Richard Feynman during this historic speak "there is lots of space at the bottom" [7-13]. Within the current drive of developing green clean technologies for the nanomaterials synthesis, these aspects assume significant importance [14-18]. Nanoparticles are outlined as particulate dispersions of solid particles with a minimum of one dimension at a size threshold of 10-1000 nm [3,7]. Nanoparticles exhibit characteristic physical and chemical properties compared with their bulk materials. It's crucial to get well-dispersed, ultrafine and uniform nanoparticles to explain and utilize their distinctive properties [10]. The foremost vital feature of nanoparticles is their surface area to volume ratio, permitting them to interact with other particles easier [2,3]. This distinctive feature of nanometals may be utilized effectively in varied pharmaceutical arenas. Recently scientists become more and a lot of inquisitive about the interaction between inorganic molecules and biological species. Studies have found that a lot of microorganisms will turn out inorganic nanoparticles through either intracellular or extracellular routes [9]. Ample of methodologies are reportable within the past, to synthesize varied metal nanoparticles like silver, Au, Pt, Cu, Zn etc. [11-14]. Present investigation was narrowed on green synthesis of AgNps. Synthesis of metal nanoparticles using greenways (like plant extracts, bio-assisted synthesis etc.) are terribly price effective, and thus are often used as an economic and valuable alternative for the large-scale production of metal nanoparticles [15,16] and there's monumental demand for the nanomaterials within the industrial field [17].

According to ancient literatures, distillate of cow urine (CU) was the one to be used principally in various disorders as it was found to exhibit anti-oxidant effect [16]. An anti-oxidant could be a chemical that stops the oxidation of different chemicals and therefore the formation of free radicals [19-21]. Antioxidant potential is closely associated with the quantity of hydroxyls, the higher the amount, the less attackable the chain breaking antioxidant action of the compound [20]. Antioxidant property of copper is attributable to uric acid and allantoin present in CU [22].

Stabilization of metal nanoparticles is additionally an important throughout synthesis of NS, as poorly stable NS tends to grouping that is sign of instability. AgNps in aqueous solution have an overall weak charge and would take up to protein to a larger degree once pH was adequate to or slightly larger than the isoelectric point of the constituent proteins in a solution. This sorption considerably stabilizes metal nanoparticles [19].

Metal nanoparticle-drug interaction is indeed unexplored area of research and guarantees some effective outcomes pertaining to improvements in drug characteristics. This area of research would really bring glory to drug discovery owing to simplicity, efficiency and organ targeting talent of metal nanoparticles. Various drugs get adsorbed on Au nanoparticles, thus increasing their solubility in water solution. This is the novel approach where the physical-chemistry properties of the drugs adsorbed on noble metal nanoparticles surface will allow us to deep in the design of new drug release systems with potential to develop the clinical efficiency of the therapeutic effect of the anti-inflamatory molecules [20-37]. The scope of present investigation includes the use of green method for synthesis of silver nanoparticles.

Moreover, optimization of silver nanoparticles using cow urine is still unexplored. Having abundant traditional medicinal claims to cow urine, present investigation will ever first try to couple traditional substance (cow urine) in a more technological way. Augmented Simplex centroid design was used to delineate the optimum quantity of cow urine, gelatin and silver nitrate required to biosynthesize silver nanoparticles. Synthesized AgNps were evaluated for its effect on the solubility of Ibuprofen.

MATERIALS AND METHODS

Materials

Gelatin and $AgNO_3$ were procured from Sigma–Aldrich. All aqueous solutions were prepared in double distilled, membrane filtered water.

Collection of cow urine

Healthy virgin cow of around two years age was selected for procuring urine and first (morning) urine collected in a glass bottle was filtered through Whatmann filter paper and stored at cool temperature [28-37].

Biosynthesis of nanosilver and optimization

This investigations were focused on the effect of three formulation parameters (independent variables) using Augmented Simplex Centroid Design (ASCD), which were as follows: gelatin (A), cow urine (B) and water (C) on the synthesis of silver nanoparticles thereby optimizing the same. Experimental factors considered for the design were put on the scale of 0 to 1 (Coded values). The design comprising of 16 experimental runs with six replicates (Table 1). The experimental methodology was framed in accordance with the design where tests were executed with 250 ml Erlenmeyer flask containing gelatin solution in DM water, cow urine and silver nitrate solution. The flasks were autoclaved at 121 psi for 5 min. The response variable (Y), i.e., dependent variable selected was the surface plasma resonance (SPR) recorded at 420 nm. Response surface technique was employed to fit the experimental results of ASCD employing second order polynomial equation:

UV–visible Characterization of Nanosilver

UV-visible spectroscopy analysis was carried out on SHIMDZU 1700UV-visible absorption spectrophotometer with a resolution of 1 nm between 300 and 900 nm. A solution containing 2.0% gelatin and 1.5% cow urine was used as the blank [38,39].

Evaluation of the reducing power

1.0 ml of distillate was mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM), subsequently incubated at 50°C for 20 min. Thereafter, 2.5 ml of trichloroacetic acid (600 mM) was added to the reaction mixture, centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (6 mM) and absorbance was measured at 700 nm. The assays were carried out in triplicate and the results were expressed as mean values ± standard deviations. Tocopherol was used as standard [27].

Transmission electron microscopy

The size and shape of nanoparticles were analyzed using transmission electron micrographs. Tremendous increase in a surface area results in the increased adsorption efficiency of the Ibuprofen which could consequently improve the wetability of the drug; this may be owing to an increase in adhesion surface area between nanoparticles and the drug. Smaller particles have a larger surface area-to-volume ratio; therefore, most of the drug associated with small particles would be at or near the particle surface [36]. Micrographs were obtained using a Philips EM 208 from Bombay IIT (TEM operating at 200 kV) [28,29].

Phase Solubility

An excess amount of drug (Ibuprofen) was placed into 50 ml volumetric flask containing different volumes of AgNPs. Simple 2^2 factorial designs was used to optimize the quantity of AgNps for solubility enhancement. All flasks were closed with stopper and covered with cellophane membrane to avoid solvent loss. The flasks were kept in a Remi shaker and continued stirring for 48 hrs. After 48 hrs content of each flask was filtered through filter paper. Filtrate was diluted and analyzed spectrophotometrically. All solubility measurements were performed in duplicate, in and off presence of silver nanoparticles [30].

X- ray diffraction analysis

The aqueous solution of gelatin embedded with silver nanoparticle was spray dried in a Labultima Lab spray drier (Model – LU 222Advanced) at 100^0C. Spray drying was performed at an inlet air temperature of 135^0C, corresponding to an outlet temperature 90^0C. The powder was collected from I and II cyclones of the spray dryer. The powder X-ray diffraction was performed using a Philips, Holland –PW3710 Diffractometer. The diffracted intensities were recorded from 10^0 to 65^0 2θ angles [31,32].

Differential thermal analysis

Differential thermal analysis of the pure ingredient and solid dispersion were carried out at IIT, Mumbai. Samples weighing around 5 mg were kept in the aluminium pans and heated at the scanning rate of 50°C/minute from 300°C to 2100°C. Pure nitrogen gas, i.e., without water vapour was purged through the sample cell continuously [33].

FTIR study

The prominent IR peaks (Wave numbers, cm^{-1}) of mixture of silver nanoparticle, soluble starch and silver nitrate and gelatine were recorded using Alpha-E, Bruker FT-IR spectrophotometer. Spray dried and freeze dried samples were used for IR studies [34].

RESULTS AND DISCUSSION

A mixture design is appropriate when the response depends on the component *proportions* of the mixture and not on the component quantities. Present investigation was carried out keeping quantity of $AgNO_3$ (1 ml of 1 mM) constant. Mixture proportion was defined by the gelatin solution, cow urine and amount of water. Proportion of water was already determined by the proportion of cow urine and gelatin.

Typically the experiment begins with preparation of 100 mL mixture of 2% gelatin and 1 ml of 1 mM $AgNO_3$. To this mixture 2.5 mL of cow urine was added and whole mixture was kept at 120°C at 15 lbs for 5 mins in an autoclave. No reports were found where silver nanoparticles were synthesized using application of cow urine, gelatin as a formulation variables and moist heat as a process variable. The reduction process was preliminarily was confirmed by the color change of solution which turns pale yellow. The reaction product was subjected to a scanning UV-visible analysis which shows the absorbance in the range of 300 to 700 nm wavelength. Monodisperse nature of the AgNps was selected response factor for the experimentation [35]. The end objective of above experiment was to identify and confirm the levels of influencing variables. ASCD was used to decide experimental runs which were shown in Tables 1 and 2.

Table 1: Experimental runs by augmented simplex design.

Std	Run	A:Gelatin	B:CU	C:Water	Abs
6	1	0	0.5	0.5	0.317
9	2	0.166667	0.666667	0.166667	0.497
1	3	1	0	0	0.312
2	4	0	1	0	0.617
13	5	0	0	1	0
15	6	0.5	0	0.5	0.152
16	7	0	0.5	0.5	0.32
11	8	1	0	0	0
12	9	0	1	0	0.619
10	10	0.166667	0.166667	0.666667	0.198
7	11	0.333333	0.333333	0.333333	0.719
5	12	0.5	0	0.5	0.25
4	13	0.5	0.5	0	0.819
3	14	0	0	1	0
14	15	0.5	0.5	0	0.817
8	16	0.666667	0.166667	0.166667	0.517

Table 2: ANOVA for Quadratic model.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	1.07	5	0.2142	17.75	0.0001
[(1)]Linear Mixture	0.7293	2	0.3647	30.23	< 0.0001
AB	0.3157	1	0.3157	26.17	0.0005
AC	0.0369	1	0.0369	3.06	0.1109
BC	0.001	1	0.001	0.0861	0.7753
Residual	0.1206	10	0.0121		
Lack of Fit	0.0672	4	0.0168	1.88	0.2327

ANOVA for Quadratic model showed the model F-value of 17.75 which implies the model was significant. There was only a 0.01% chance that an F-value this large could occur due to noise. p-values less than 0.0500 indicate model terms were significant. In this case B, AB were significant model terms. Coefficients of model terms were shown in Table 3. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 was desirable. Ratio of 12.507 indicated an adequate signal which revealed that the applied model can be used to navigate the design space. Although the suggested design space was not explored in the present study, however the design was validated by conducting experiment in the suggested region. Surface curvature above the scale for gelatin and cow urine strongly supports the interaction between them during the synthesis of AgNps. 3-D surface response and contour plot was shown in Figures 1 and 2 respectively. The Lack of Fit F-value of 1.88 implies the Lack of Fit is not significant relative to the pure error. There is a 23.27% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit was found to be good. Response obtained during the validation was analogous to suggested response. The characteristic peak obtained due to Surface Plasmon Resonance (SPR) [40-43] was shown in Figures 3 and 4. AgNps obtained during validation were considered for the further study.

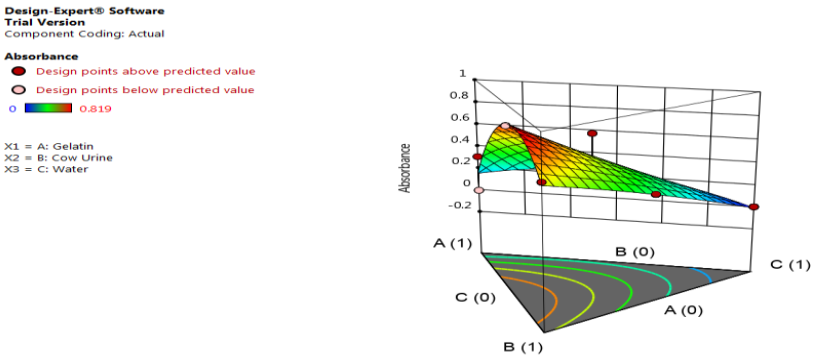

Figure 1: 3-D surface response curve.

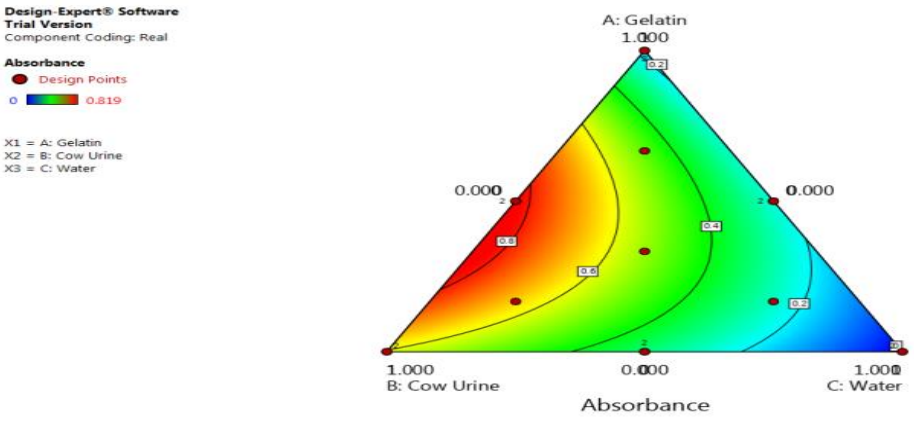

Figure 2: Contour plot.

Table 3: Coefficients in terms of coded factors.

Coefficients	Equal to
0.1612	A
0.5912	B
-0.0099	C
1.82	AB
0.6223	AC
0.1044	BC

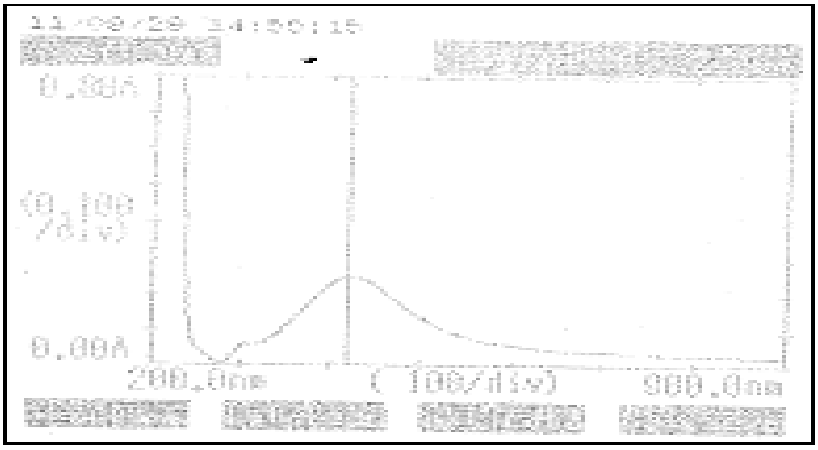

Figure 3: SPR at 437 nm.

Transmission electron microscopy

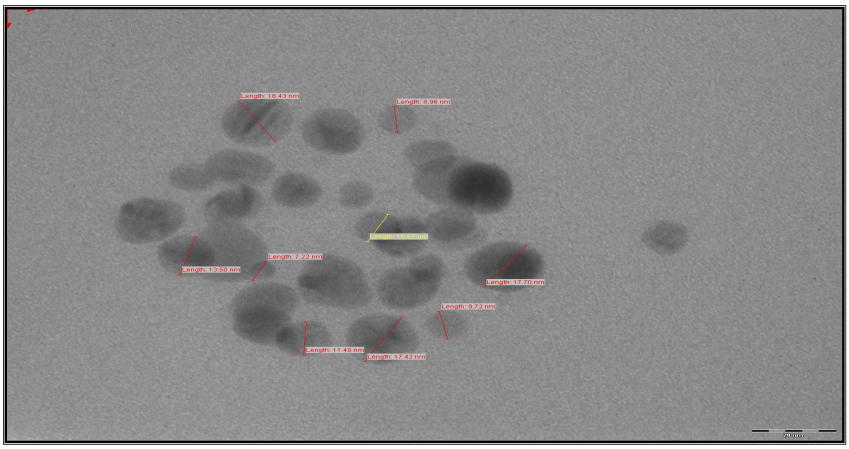

Figure 4: TEM of Silver nanoparticles.

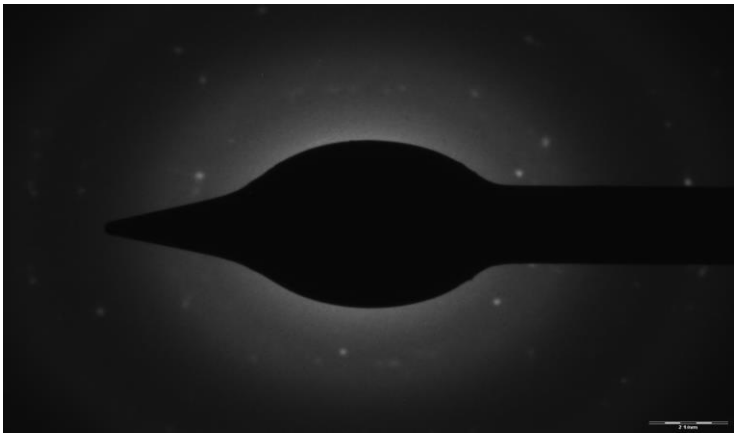

Figure 5: SAED (selected area electron diffraction suggested) pattern.

Transmission electron microscopy image discovered that the AgNPs generally existed in the spherical and roughly hexagona. The average particle sizes of silver nanoparticles were estimated to be 7-28 nm. If we consider the three dimensional space vectors for a specific nanomaterial and length scales are in the critical regime of 1-100 nm, they can be known as 0-dimensional particles or quantum dots, typical examples being spherical (1-10 nm diameter) nanoparticles of Au, Ag, CdS, CdSe etc. [44]. Agnps synthesized in present investigation ranges in the region of quantum dots. This may be due to ability of gelatin to catalyze the rate of reduction of ionic silver to elemental silver [45]. The size distribution was shown in Table 4 and Figures 4-6. SAED (selected area electron difraction suggested) pattern suggested that sample was amprohous (diffuse rings) in nature [46]. Results of which were shown in Figure 5.

Table 4: Size distribution tables of nanoparticles.

S. No.	Particle range (nm)	Particles (No.)
1	6-8	5
2	8-10	7
3	10-12	21
4	12-14	17
5	14-16	28
6	16-18	5
7	18-20	1

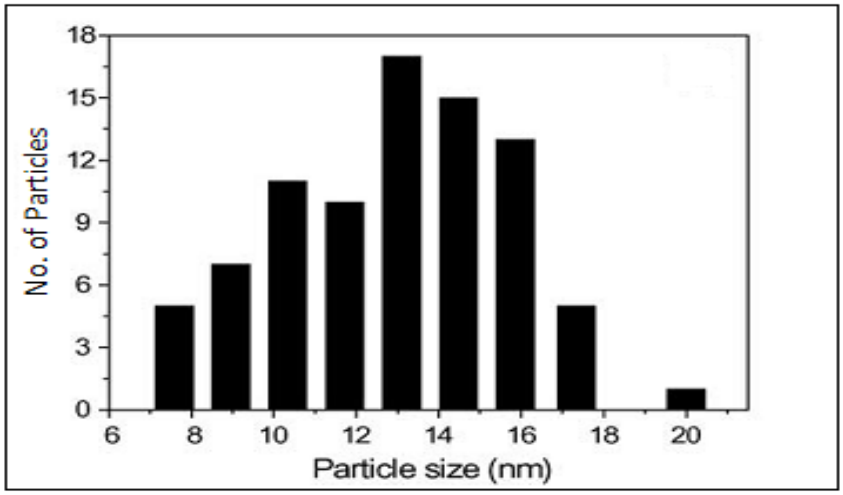

Figure 6: Particle size distribution of silver nanoparticles.

Evaluation of reducing power of cow urine

The reducing capability of the cow urine was measured by the transformation of $Fe^{3+}$to Fe^{2+} in the presence of different concentrations of cow urine at 700 nm. Increased absorbance of the reaction mixture was indicative of increased reducing power of cow urine. This study may reveal that cow urine may have antioxidant potential and may involve in the process of reduction of silver ions, results of which were shown in Table 5.

Table 5: Readings of different concentrations of cow urine at 700 nm.

Sr.	CU (ml)	λ_{max}	Absorbance			S.D.
1	0.1	700	0.621	0.609	0.605	0.008
2	0.2	700	0.607	0.61	0.617	0.005
3	0.3	700	0.613	0.616	0.614	0.001
4	0.4	700	0.65	0.654	0.657	0.003

Phase solubility of Ibuprofen

Aqueous phase solubility of Ibuprofen in presence of silver nanoparticle solution was carried out using shake flask method. The study revealed that increase in volume of nanoparticles solutions increases the phase solubility of Ibuprofen. AgNps proved to have efficiency to adsorb drug molecules [37]. The increase in solubility of Ibuprofen may be attributed to drug adsorption phenomenon. In addition, the diffusion distance on the surface of drug nanoparticles is decreased, causing an increased concentration gradient. An increase in surface area and concentration gradient leads to a more pronounced increase in dissolution rate compared to the micronized product. Saturation solubility and dissolution rate are important parameters affecting the bioavailability of orally administered drugs. Drug nanonization can reduce erratic drug absorption so the adhesion process of drug nanoparticles to mucosal surface can be improved AgNPs [38]. In present work, enhancement of solubility of Ibuprofen was

observed in presence of AgNP solution. Increase in concentration of the silver nanoparticles revealed proportionate increase in the solubility of the Ibuprofen. This may be due to the increase in the wetability of Ibuprofen after adsorption on the AgNps. The results of phase solubility were shown in Table 6.

Table 6: Phase solubility of Ibuprofen with AgNps.

S. No	Concentration of AgNps (ml)	λ_{max}	Abs	Concentration of product in (µg/ml)
1	0	223	0.2397	5.46
2	2.5	223	0.2661	6.13
3	5	223	0.2855	6.62
4	7.5	223	0.4412	10.59
5	10	223	0.5184	12.55
6	12.5	223	0.5277	12.79
7	15	223	0.6423	15.70
8	17.5	223	0.8801	21.75
9	20	223	0.5289	12.82

X-ray diffractogram

X-ray diffractogram of gelatin showed number of diffused peaks. On the other hand, first diffraction peak at approx. 10.3^0 (2θ) (sharp and intense) is directly related to the diameter and the rate of the triple helix, respectively [46]. The diffractogram of gelatin impregnated with silver nanoparticles showed long range peaks, suggesting probable transformation of amorphous form into crystalline state. Increase in crystallinity was determined by comparing some representative peak heights in the diffraction patterns of pure gelatin (reference) and gelatin impregnated with AgNPs (sample). However, there was not significant change in the crystallinity. This was also shown in the selected area electron diffraction pattern obtained TEM investigations which revealed diffused rings with mere crystal spots (Figure 5). X-Ray defractogram of gelatin and gelatin with AgNps was shown in Figures 7 and 8 respectively**.**

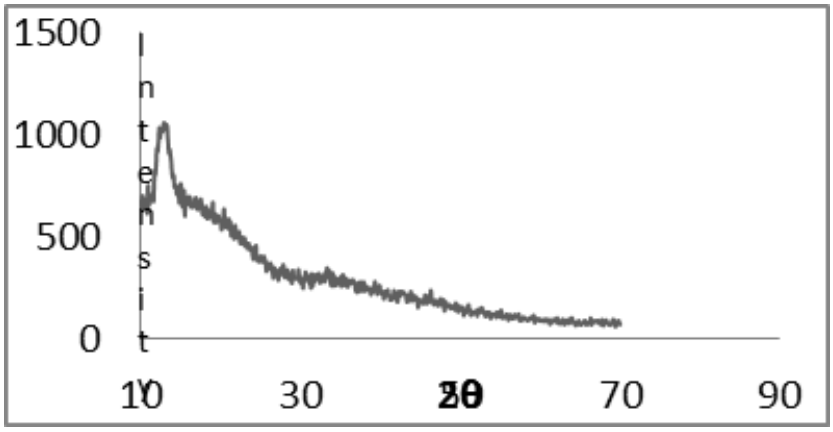

Figure 7: XRD of gelatin.

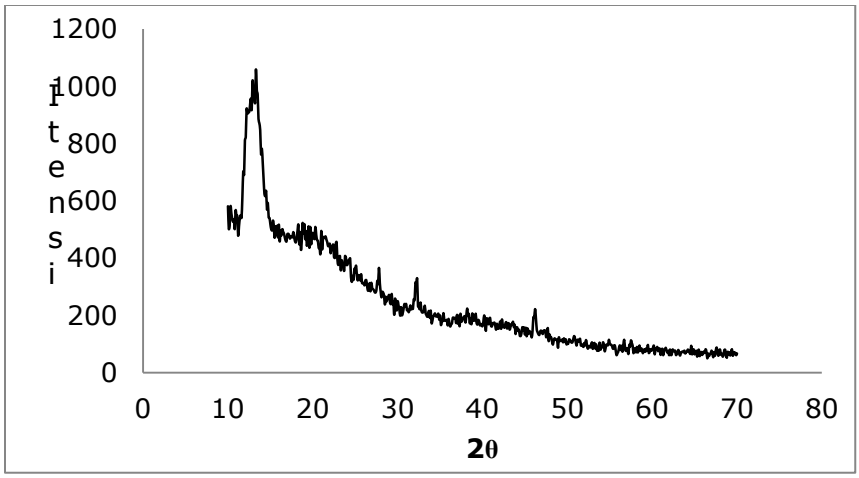

Figure 8: XRD of gelatin with AgNps.

Differential Scanning Calorimetry

The DSC plot of raw gelatin exhibits an endothermic peak associated with the helix–coil transition of collagen. The rate of the denaturation enthalpy associated to this peak is related to the relative amount of triple helical structure, and is significantly lower for gelatin with respect to collagen. Figure 9 reported the DSC plots recorded from films prepared with gelatin and gelatin with AgNps. It is evident the partial greater denaturation enthalpy associated with the transition of the sample at higher bloom value. The increase in bloom value may be attributed to stabilization of AgNps in the helical structure of gelatin.

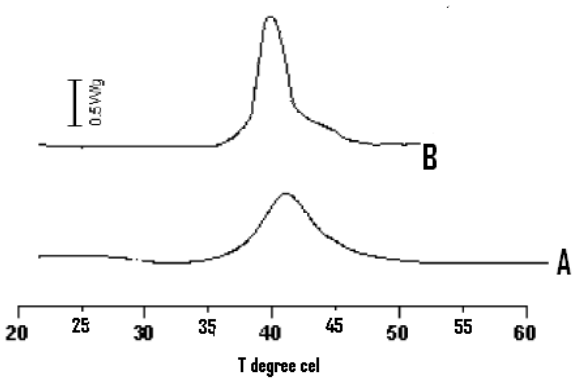

Figure 9: DSC, A, gelatin. B. AgNps with gelatin

FTIR

Proteins has the stronger ability to bind metal indicating that the proteins could possibly stabilize metal nanoparticles and may prevent agglomeration & thereby stabilize the medium [46]. The stabilization of AgNPs could be owing to functional groups available on the molecular structure of gelatin. The roles of these functional groups in stabilization of AgNPs were confirmed using FTIR measurements, the spectrum of which is shown in Figures 10 and 11. Change in intensity at 1514.4 indicates the involvement of hydrogen of amide group in the process of reduction.

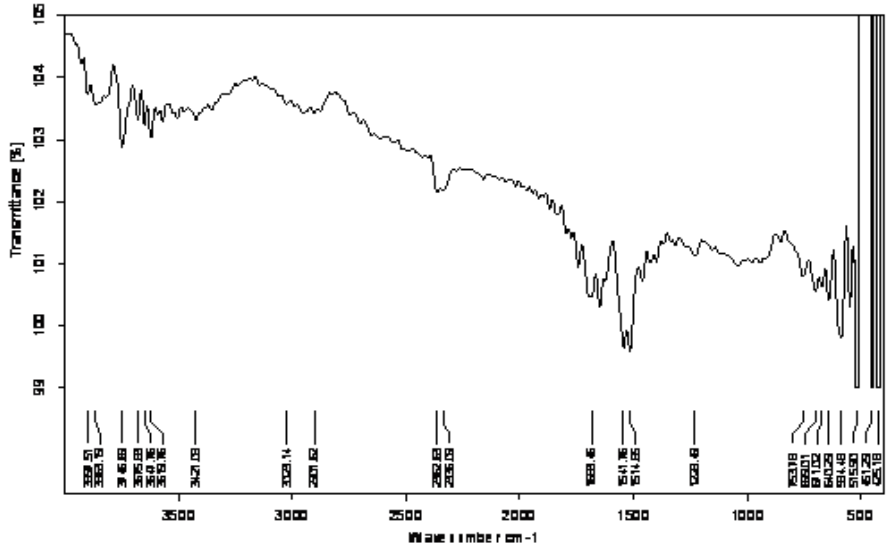

Figure 10: IR spectra of gelatin.

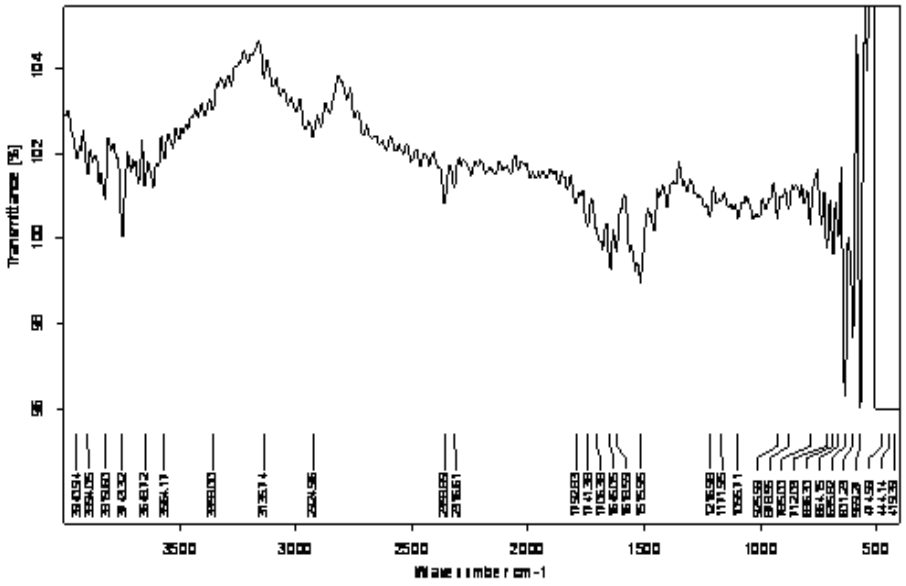

Figure 11: IR spectra of silver nanoparticles using gelatin.

CONCLUSION

Silver nanoparticles were successfully synthesized in the present investigation. Simultaneous use of cow urine and gelatin for the synthesis of AgNps can have better interactive effect. Phase solubility study in and off presence of AgNps revealed that the increase in concentration of AgNps in the reaction mixture can enhance the solubility of Ibuprofen by many folds. SPR, IR, XRD study shown favorable interactions between gelatin and AgNps for the effect on the solubility of Ibuprofen.

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