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Formulation Of Nanoparticles Loaded With Using *Withania Somnifera* Extract, *Annona Squamosal* LINN Extract, And *Momordica Dioica* Extract

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Introduction:

Natural products and compounds have been used as herbal medicines from the Beginning of human history. Phytochemicals and natural products are plant derived secondary metabolites, which may exert many biological activities in humans, including anticancer properties. Certain natural and synthetic compounds that can modulate immune responses in a positive or negative manner are known as Immunomodulators. Plants have been used for the prevention and cure of various ailments including microbial and lifestyle diseases. According to the World Health Organization (WHO), approximately three quarter of the world‘s population relies on herbal medicine. The implementation of nanotechnology processes involving the improvement of the pharmacokinetics and pharmacodynamics of phytochemicals and natural compounds has gained the focus of researchers, who have developed several innovative delivery systems, including liposomes and polymeric nanoparticles. The biggest issues related to the use of natural products in the treatment of cancer and other diseases are their low solubility and bioavailability, which has caused problems in clinical trials. In this regard, nanotechnology and nanocarrier design may address this issue to improve drug delivery, biodistribution, biosolubility, and bioavailability of natural products and phytochemicals in order to extend the use of these substances in clinical practice. In recent years, studies have allowed the discovery of the specific molecular mechanisms responsible for the therapeutic effects of traditionally used phytochemicals and natural compounds, as well as potentiating their use with nanomedicine. The flexible surface chemistry of nano drug delivery systems also allows the ability to conjugate targeting ligands. Biological moieties such as peptides, nucleic acids, and antibodies can be attached to their surfaces to target drugs to specific diseased sites. Targeted nano drug delivery system (nano-DDS) can increase the drug payload while significantly reducing various risks of adverse side.

The best types of nanoparticles used for drug delivery and the biodistribution of phytochemicals and natural products include polymer nanoparticles, solid lipid nanoparticles (SLNs), crystal nanoparticles, liposomes, micelles, and dendrimers. Each of these nanoparticles has its own advantages and disadvantages as a drug delivery vehicle. Plant extracts and phytocompounds are found to fortify the host‘s immune system, and numerous plants have been listed in this category. Phytoimmunomodulatory agents can increase the body‘s immune-responsiveness against pathogens by activating the immune system in a specific or a non-specific manner that includes both the innate and adaptive immune systems. The use of plants for immunomodulation can be traced back to ancient Ayurveda of 6000 BC. This system describes medicinal plants as rasayanas having rejuvenating properties in terms of fortifying the immune system against various diseases. Currently, 34 plants have been identified as immunomodulators in rasayana. A number of medicinal plants are currently under high throughput screening for a quick assessment of pharmacologically important hit molecules that could be utilized as a lead molecule in drug development.

Collection and authentication of plant *Annona squmosa, Momordica dioica* and *Withania somnifera*
This plants are widely distributed in the region of Marathwada and rest of Maharashtra in the hilly and forest area. Generally fruiting in the month from June to December. The plants were collected from Latur Maharashtra. Further its taxonomic identification and authentication was done by Botanist.

EXTRACT PREPARATION OF *ANNONA SQUMOSA, MOMORDICA DIOICA* AND *WITHANIA SOMNIFERA* LEAVES

25g of the sample was weighed accurately shade drying of leaves and homogenization to coarse powder, Soxhlet extraction (successive solvent extraction) using following solvents Petroleum ether (40-60°c), chloroform, ethanol, water. Concentration of extracts using water bath and dried.

The extracts were screened for Phytochemical Screening of the active phytochemical compounds, the ethanol extract showed the presence of major phytoconstituents and used as successive ethanol extract (SEE) for further studies.

Preparation of Nanoparticle

- **1 Mm AgNO3 solution preparation**
  By combining 0.0169g of silver nitrate with 100 ml of distilled water, a one milimolar (mM) solution of AgNO3 was created and kept in an amber-colored container until usage.
- **Method of preparation of Nanoparticle**
  For the bioreduction procedure, the needed quantity of extract was added dropwise to a 1 mM aqueous AgNO3 solution in an Erlenmeyer flask at room temperature.

Procedure:
The reaction mixture was stirred with a magnetic stirrer at 200 rpm until the solution's colour changed from yellow to dark brown, signifying the creation of AgNPs. To get clear supernatant, the solution was centrifuged at 5000 rpm for 30 minutes. To get pure nanoparticles, the recovered particles were centrifuged many times with water after the supernatant was removed. Many silver nanoparticle formulations (F1-F6) were made using the extracts of *Withania somnifera*, *Annona squamosa*, and *Momordica dioica*. While not in use, the powders (nanoparticles) were lyophilized and stored at 40 degrees Celsius.

Characterizations of Nanoparticles:

1. Zeta potential study
The zeta potential (surface charge) of silver nanoparticles was investigated using a zeta sizer. To determine their zeta potential, the created nanoparticle formulations (F1–F6) were diluted with water (0.1 ml) and placed in an electrophoretic cell with a 15.5 V/cm electrical field. Three distinct measurements were made on each sample.

2. Scanning electron microscopy
We investigated the morphological characteristics of the nanoparticles using scanning electron microscopy. A 10mm glass slide was initially covered with 100l of the silver nanoparticle formulations (F1-F6) and allowed to dry overnight at room temperature in a vacuum desiccator before a SEM inspection. In a higher vacuum evaporator, nanoparticles were placed on the proper support and coated with gold using a gold sputter module. Observations were done at various magnifications at a voltage of 15kv.

3. Drug entrapment efficiency
The efficiency of silver nanoparticle formulations for drug entrapment was evaluated using the ultra-centrifugation method (F1-F6). AgNO3 containing the silver nanoparticle was isolated from AgNO3 by ultracentrifugation at 10,000 rpm for 30 minutes. Re-dissolving the pellets in distilled water allowed for the subsequent scanning of the supernatant with a UV-visible spectrophotometer in this particular parameter.
Using the connection in this equation, the effectiveness of drug encapsulation was calculated.

% Drug entrapment efficiency = experimental drug contentx100/Theoretical drug content

4. Production yield of nanoparticles
By comparing the total weight of the produced nanoparticles to the weight of the copolymer and drug combined, the yields of nanoparticles were calculated.

% Yield calculation = Amount of drug X100 / Amount of drug +polymer

5. In-vitro drug release study
It was investigated how manufactured silver nanoparticles released in vitro at 370°C in phosphate buffered saline (PBS) with a pH of 7.4. In a dialysis bag, silver nanoparticles were dialyzed for one hour against 50 ml of PBS with constant shaking. Aliquots were often taken out. A new volume of PBS is added to replace the destroyed sample volume. A UV-visible spectrophotometer was used to measure absorbance in order to determine how much medicine was discharged.

6. Transmission electron microscopy
A very thin item is used in TEM, a kind of microscopy, to create an electron stream that interacts with it along the route. Electrons travelling through a specimen produce an image that is magnified and focused onto an imaging device using a

fluorescent screen, photographic film, or a detecting sensor. Transmission electron microscopes are able to take pictures with a far higher resolution than light microscopes due to their short de Broglie wavelength. Physical and biological sciences, among other scientific fields, frequently employ TEM as a technique for analysis. As a result, it has several uses in a variety of industries, including semiconductor research, materials science, virology, pollution, cancer research, and nanotechnology.
The morphology of AgNPs was examined using transmission electron microscopy (TEM) (JEOL-JEM 2100, 1.4 Angstrom Unit, Tokyo, Japan). The samples were created using drops of diluted AgNP solutions, which were subsequently air dried on carbon sheets supported by copper grids. As the voltage reached 120 kV, TEM pictures could be seen below the microscope.

Stability of Nanoparticle

The best formulation was kept in a stability chamber (at 4°C) for three months in order to assess the stability of the created nanoparticles. At various times of one, two, and three months, the particle size, zeta potential, entrapment effectiveness, and physical appearance were assessed. (In line with ICH Q1A).

Results:

Characterization of Nanoparticles:

1. Particle size determination by Zeta Sizer

The particle size of nanoparticles (F1-F6) made from extracts of *Withania somnifera*, *Annona squamosa*, and *Momordica dioica* were determined. The nanoparticles particle size were determined to be between a range of 1-100nm.

2. Scanning electron microscopy

This method was used to look at the silver nanoparticles surface morphology. As a consequence of their investigation, the researchers were better able to comprehend the morphological characteristics of the nanoparticle. There were several approximately spherical nanoparticles present, and they will tear apart from one another. A tiny, spherical nanoparticle with a small size can be seen in the SEM image of a freeze-dried silver nanoparticle with a prolonged cross-linking duration.
The vast majority of the nanoparticles were round (Figure F1-F6). The size of the nanoparticles produced by the Withania somnifera extract was around 58 nm, which is consistent with the findings of light scattering for these nanoparticles. The average size of nanoparticles made from the extract of Annona squamosal is 47.2 nm, whereas that of the extract of Momordica dioica is 38.5 nm. After being freeze-dried, the nanoparticle dispersion formed sponge-like structures. This method was utilised to ascertain the sponge's morphology.

3. Drug Entrapment Efficiency

For the "F1 to F6" formulation, greater drug entrapment efficiency was reported using Withania somnifera, Annona squamosa, and Momordica dioica extract loaded silver nanoparticle, ranging from 50% to 80%.

4. Production yield of nanoparticles

It was found that the yield increased as the concentration of AgNO3 increased. We used a same amount of polymer, which led to a consistent production of extracts coated with silver nanoparticles from Withania somnifera, Annona squamosa, and Momordica dioica.

5. In-vitro release study:

The in vitro drug release of *withania somnifera, anona squamosa,* and *momordica dioica* loaded silver nanoparticles of all batches was investigated. The maximum medication release for batches F1–F6 was shown to be between 91–93 percent. It was investigated how manufactured silver nanoparticles released in vitro at 370°C in phosphate buffered saline (PBS) with a pH of 7.4. Dialyzing silver nanoparticles took 60 minutes. A UV-visible spectrophotometer was used to measure absorbance in order to determine how much medicine was discharged.

6. Transmission electron microscopy

The TEM micrograph of each nanoparticle sample (F1–F6) is shown below. The average particle size is 45 nanometers, while the range is 30 to 60 nanometers. The particles are round in shape. To confirm that no further types of metal oxide were present, the same sample was also examined using electron diffraction.

Stability of Nanoparticles:

By analysing its absorption spectra, the stability of the silver nanoparticles in the improved formulations F1, F3, and F5 was assessed after three months. The silver nanoparticles did not agglomerate and had no significant changes during storage, suggesting that they were more stable.
For prepared nanoparticles, the pattern of change in entrapment effectiveness, particle size, and zeta potential was the same. There is little increase in the size of the nanoparticles (1.5%) was detected after three months of storage at 4°C. Zeta potential was found to be lowered by 4%, whilst the entrapment effectiveness of nanoparticles was reduced by around 1-2%. The few modifications that were seen during storage.

Discussion:

The nanoparticles particle size were determined between a ranges of 38.2 to 41.3nm. In SEM analysis, the size of the nanoparticles produced by the Withania somnifera extract was around 58 nm, which is consistent with the findings of light scattering for these nanoparticles. The average size of nanoparticles made from the extract of Annona squamosal is 47.2 nm, whereas that of the extract of Momordica dioica is 38.5 nm. For the "F1 to F6" formulation, greater drug entrapment efficiency was reported using Withania somnifera, Annona squamosa, and Momordica dioica extract loaded silver nanoparticle, ranging from 50% to 80%. It was found that the yield increased as the concentration of AgNO3 increased.
In-vitro release showed, the maximum medication release for batches F1–F6 was shown to be between 91–93 percent. In TEM analysis, the average particle size is 45 nanometers, while the range is 30 to 60 nanometers. The particles are round in shape.

Conclusion:

The formulated silver nanoparticles of extracts of Withania somnifera, Momordica dioica and Annona squamosal were prepared according and characterized for the tests and found to be match the standard nanoparticles formulation. The silver nanoparticles did not agglomerate and had no significant changes during storage, suggesting that they were more stable.

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Tables & Figures:

Table 1: Formula of different formulations of *Withania Somnifera* extract, *Annona squamosal* LINN extract, and *Momordica dioica* extract

Sr. No	Name of Ingredients	F1	F2	F3	F4	F5	F6
1	*Withania Somnifera* extract	5 ml	10 ml	-	-	-	-
2	*Annona squamosal* LINN extract	-	-	5 ml	10 ml	-	-
3	*Momordica dioica* extract	-	-	-	-	5 ml	10 ml
4	AgNO3	95 ml	90 ml	95 ml	90 ml	95 ml	90 ml

Table 2: Particle size and zeta potential of extract of *Withania somnifera*, *Annona squamosa*, and *Momordica dioica.*

Sr. No.	Sample	Nanoparticle Size (nm)	Zeta Potential (mV)
1	Extract of *Withania somnifera* -F1	41.3±11	-28.61
2	Extract of *Withania somnifera* –F2	39.1±11	-29.42
3	Extract of *Annona squamosal* –F3	43.8±11	-49.61
4	Extract of *Annona squamosal* –F4	41.2±11	-48.34
5	Extract of *Momordica dioica* –F5	39.5±17	-32.24
6	Extract of *Momordica dioica* –F6	38.2±17	-29.46

Values are shown as the mean ±standard deviation;n=5.

Table 3: % Entrapment Efficiency of formulation F1-F6

Formulations	Entrapment Efficiency (%)
F1	84.61
F2	71.52
F3	82.49
F4	70.82
F5	81.48
F6	71.34

Table 4: Production yield of all formulation (F1-F6)

Formulation	Production Yield (%)
F1	82.31
F2	79.49
F3	84.62
F4	79.96
F5	83.16
F6	78.17

Table 5: In-vitro release study of *withania somnifera*, *anona squamosa*, and *momordica dioica* loaded silver nanoparticles

Time (Min)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
10	10.15±0.14	15.46±0.02	15.96±0.74	13.51±0.10	13.87±0.10	14.61±0.49
15	22.47±0.31	29.25±1.03	25.81±0.16	22.72±0.19	21.91±1.03	19.49±0.44
30	79.46±0.12	58.72±0.16	62.94±0.24	78.97±0.24	68.49±1.22	44.61±0.02
45	88.72±0.24	79.61±1.24	89.52±0.72	81.47±0.15	84.67±1.06	72.73±0.23
60	92.51±0.31	89.86±0.26	93.16±0.25	89.42±0.27	91.73±1.03	88.61±0.17

Table 6: Impact of storage on the zeta potential, entrapment effectiveness, and particle size of nanoparticles (n=3). Values are displayed as mean ±SD.

Storage time		0 Month	1 Month	2 Month	3 Month
Particle size (nm)	F1	41.3±11	41.4±11	40.6±12	40.1±13
	F3	43.8±11	43.2±12	44.4±11	44.3±12
	F5	39.5±17	39.1±17	40.4±17	40.6±16
Zeta potential (mV)	F1	-28.61	-28.60	-27.74	-27.25
	F3	-49.61	-49.21	-49.24	-48.94
	F5	-32.24	-32.21	-32.06	-31.61
Entrapment efficiency (%)	F1	84.61	84.52	84.49	84.23
	F3	82.49	82.41	82.39	81.29
	F5	81.48	80.46	81.41	81.32

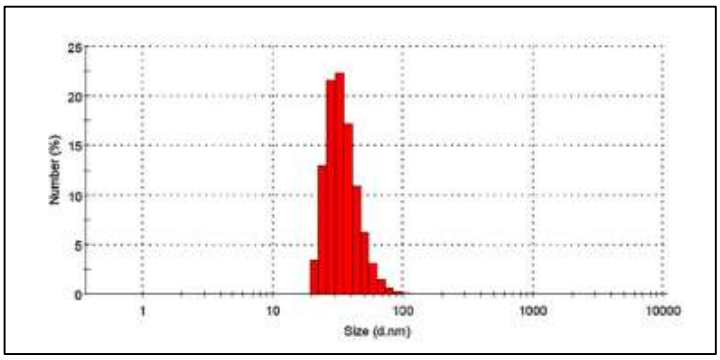

Figure 1: Particle density index of extracts derived extract of *Withania somnifera* -F1

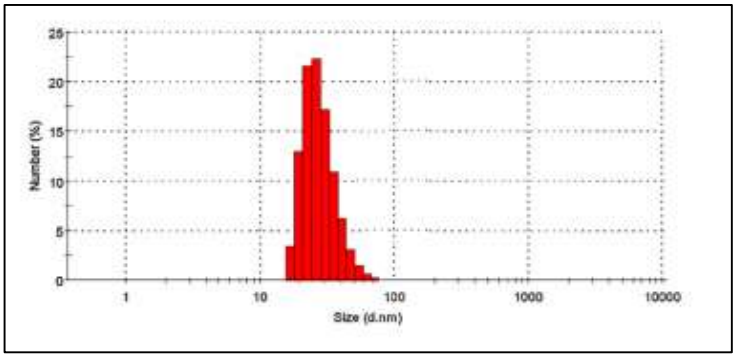

Figure 2: Particle density index of extracts derived extract of *Withania somnifera* –F2

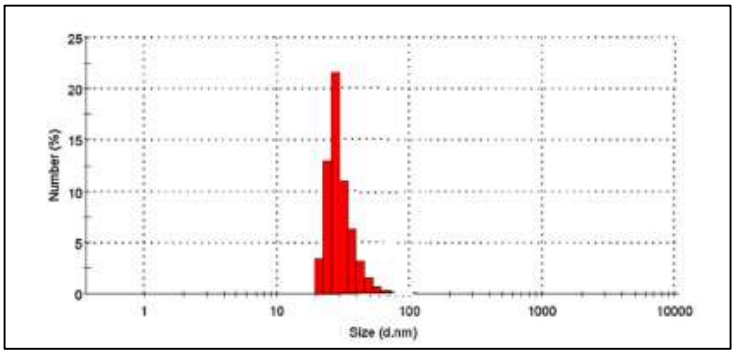

Figure 3: Particle density index of extracts derived extract of *Annona squamosal* –F3

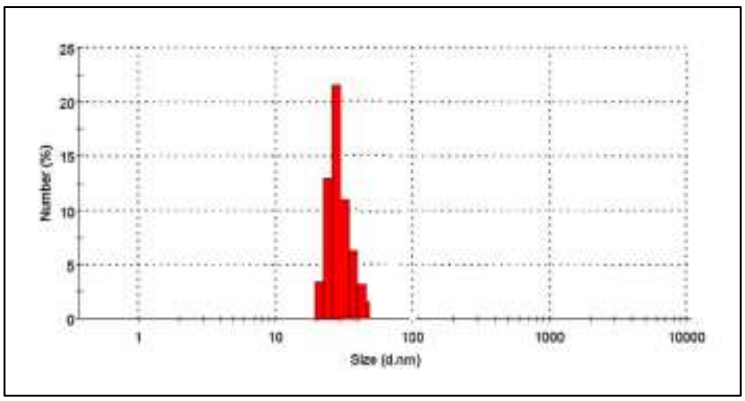

Figure 4: Particle density index of extracts derived extract of *Annona squamosal* –F4

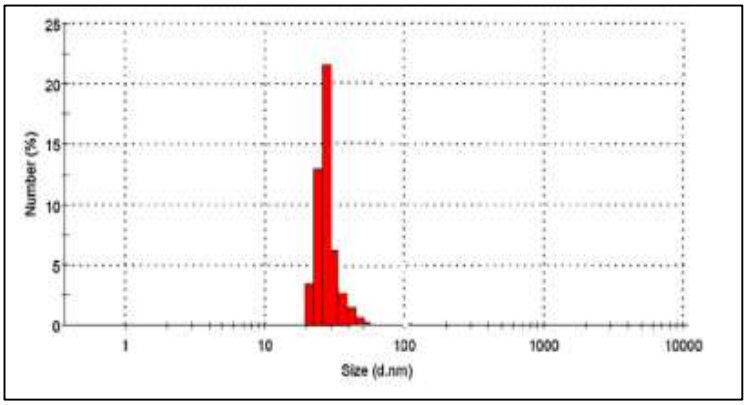

Figure 5: Particle density index of extracts derived extract of *Momordica dioica* –F5

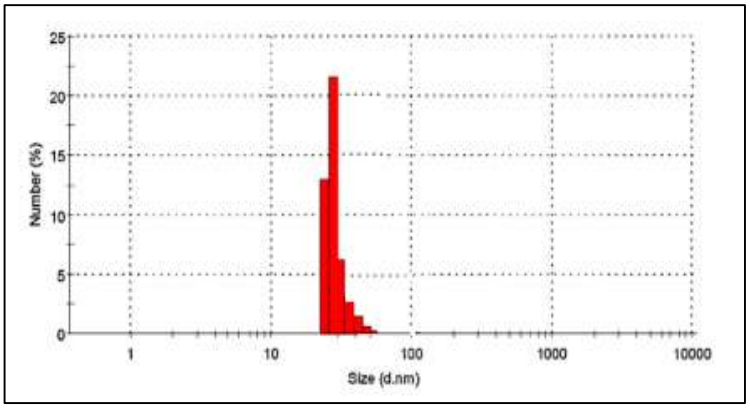

Figure 6: Particle density index of extracts derived extract of *Momordica dioica* –F6

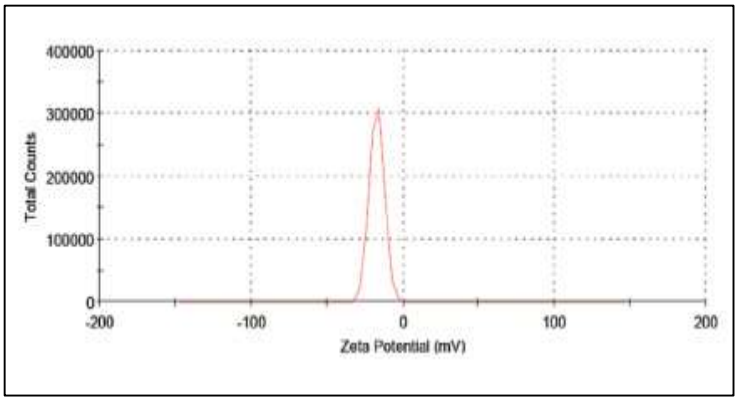

Figure 7: Zeta Particle size distribution peak of nanoparticles of extract of *Withania somnifera* -F1

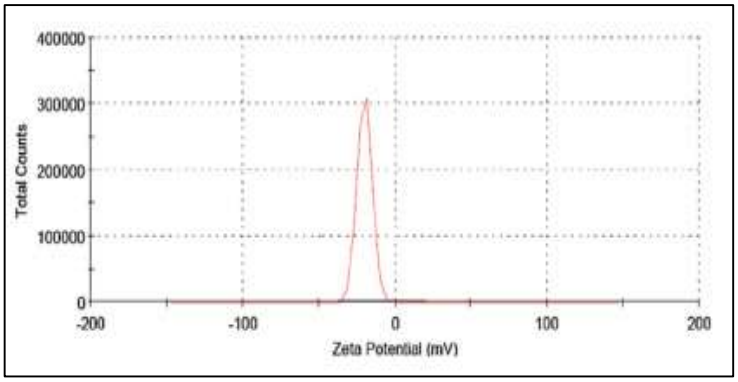

Figure 8: Zeta Particle size distribution peak of nanoparticles of extract of *Withania somnifera* –F2

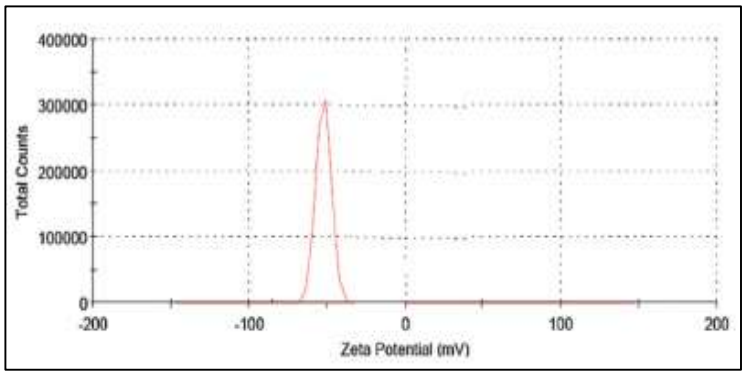

Figure 9: Zeta Particle size distribution peak of nanoparticles of extract of *Annona squamosal* –F3

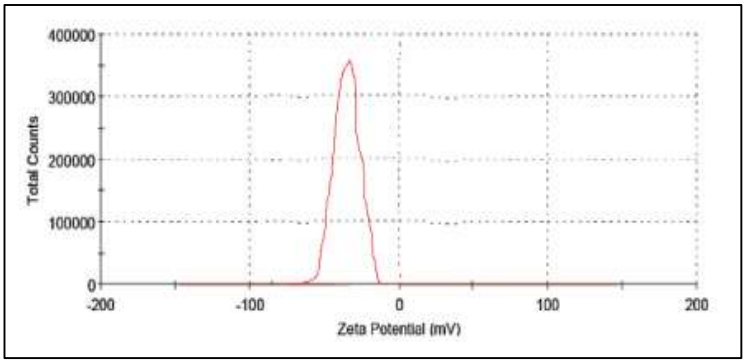

Figure 10: Zeta Particle size distribution peak of nanoparticles of extract of *Annona squamosal* –F4

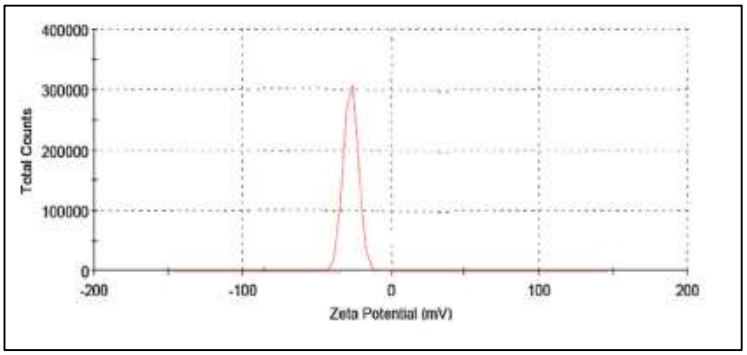

Figure 11: Zeta Particle size distribution peak of nanoparticles of extract of *Momordica dioica* –F5

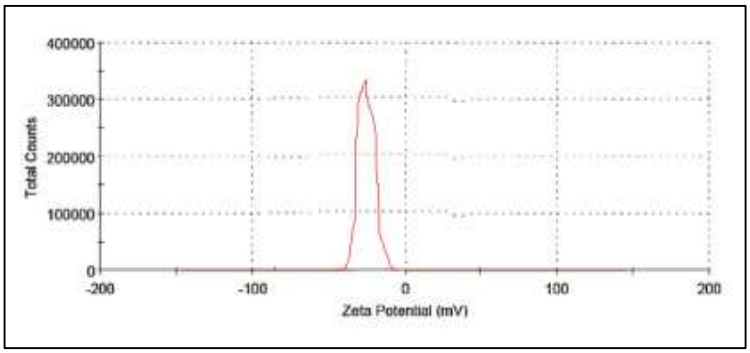

Figure 12: Zeta Particle size distribution peak of nanoparticles of extract of *Momordica dioica* –F6

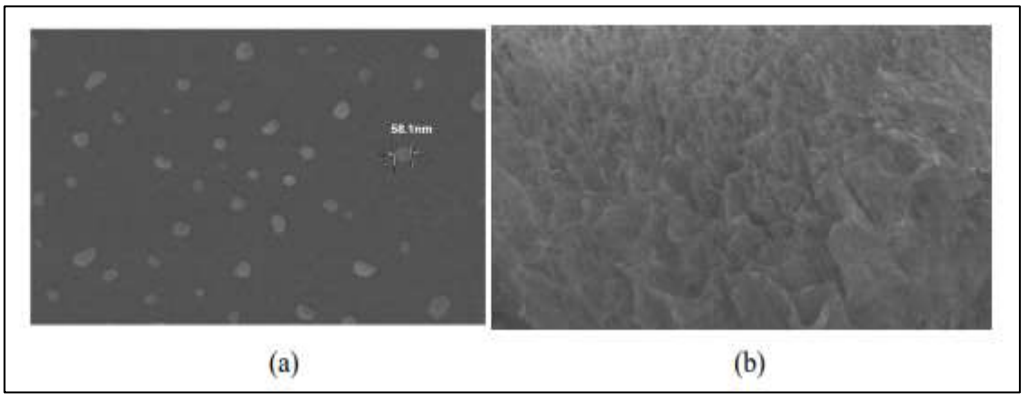

Figure 13: Scanning electron micrograph of nanoparticles (F1) obtained by extract of *Withania somnifera* (a) and freeze-dried *Withania somnifera* nanoparticles (b).

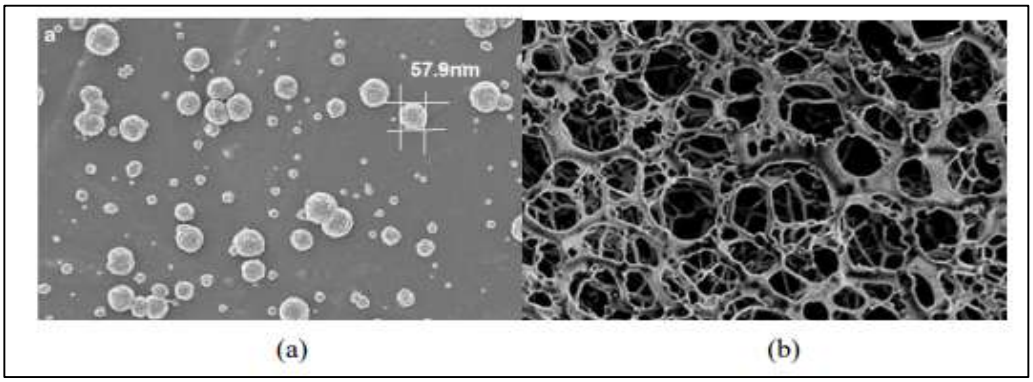

Figure 14: Scanning electron micrograph of nanoparticles (F2) obtained by extract of *Withania somnifera* (a) and freeze-dried *Withania somnifera* nanoparticles (b).

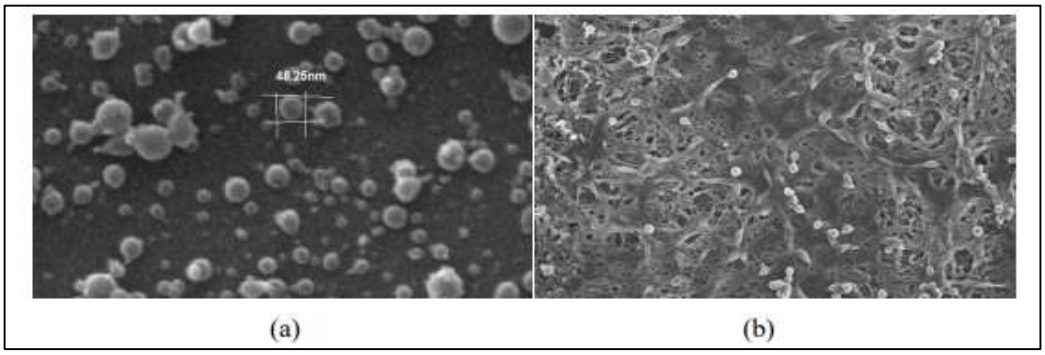

Figure 15: Scanning electron micrograph of nanoparticles (F3) obtained by extract of *Annona squamosal* (a) and freeze-dried *Annona squamosal* nanoparticles (b).

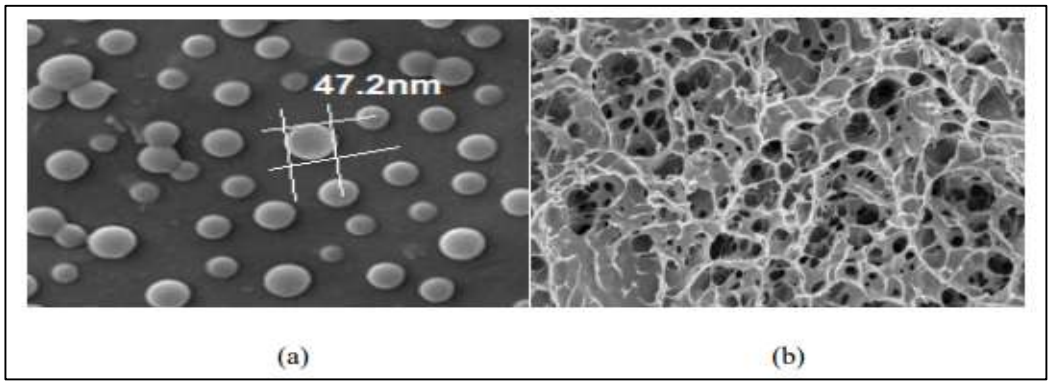

Figure 16: Scanning electron micrograph of nanoparticles (F4) obtained by extract of *Annona squamosal* (a) and freeze-dried *Annona squamosal* nanoparticles (b).

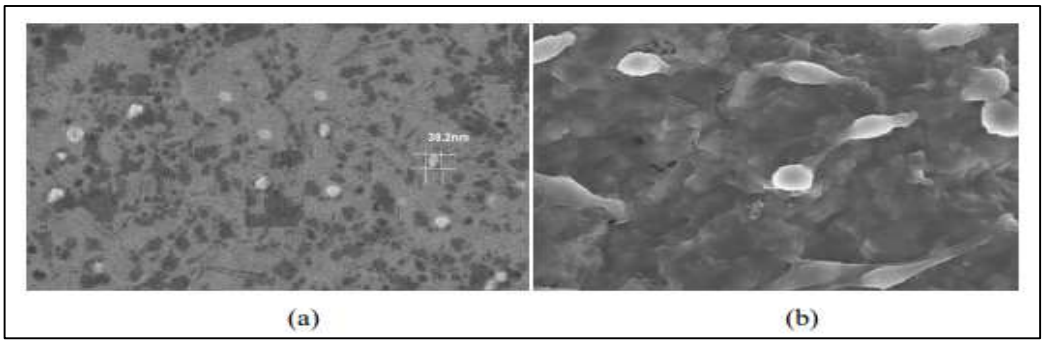

Figure 17: Scanning electron micrograph of nanoparticles (F5) obtained by extract of *Momordica dioica* (a) and freeze-dried *Momordica dioica* nanoparticles (b).

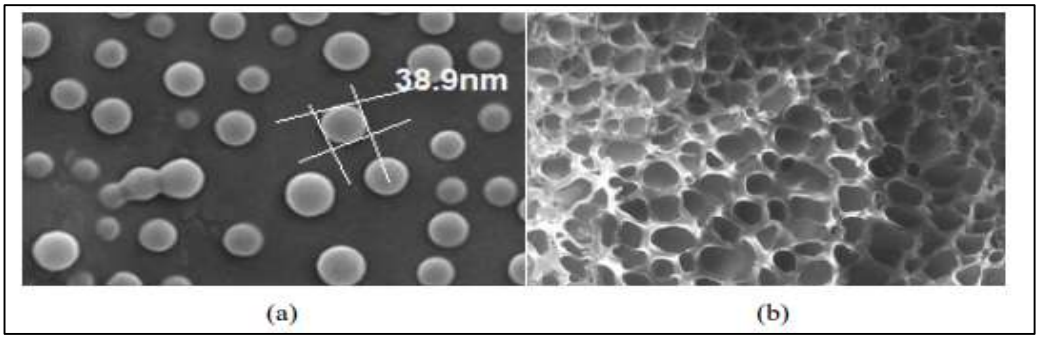

Figure 18: Scanning electron micrograph of nanoparticles (F6) obtained by extract of *Momordica dioica* (a) and freeze-dried *Momordica dioica* nanoparticles (b).

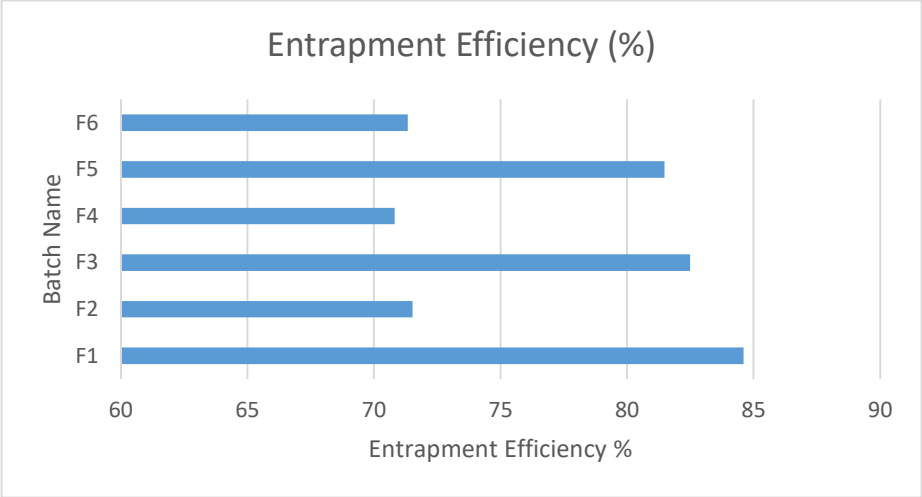

Figure 19: Graphical representation of entrapment efficiency

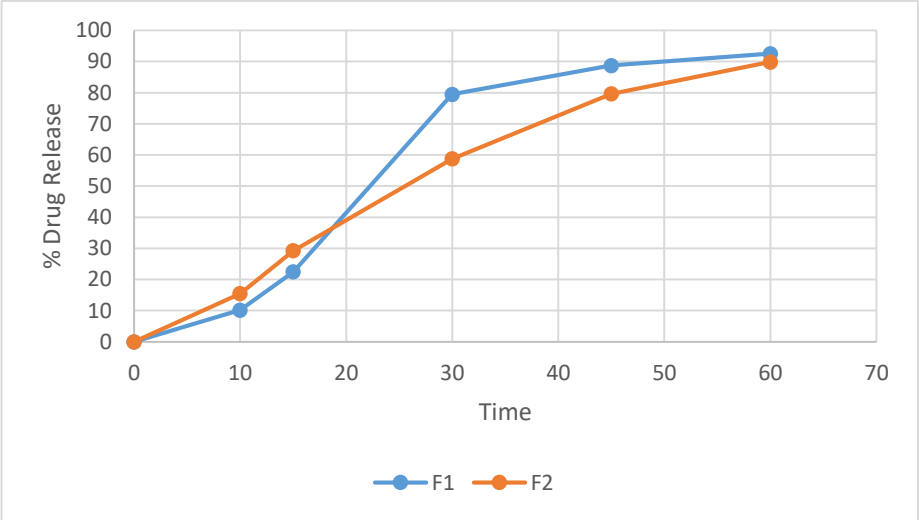

Figure 20: % Cumulative drug release (F1-F2)

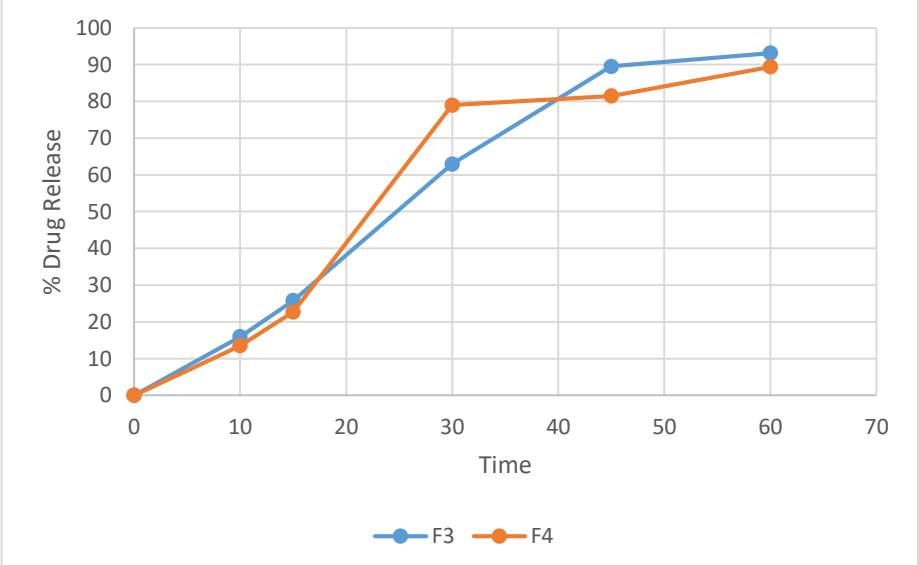

Figure 21: % Cumulative drug release (F3-F4)

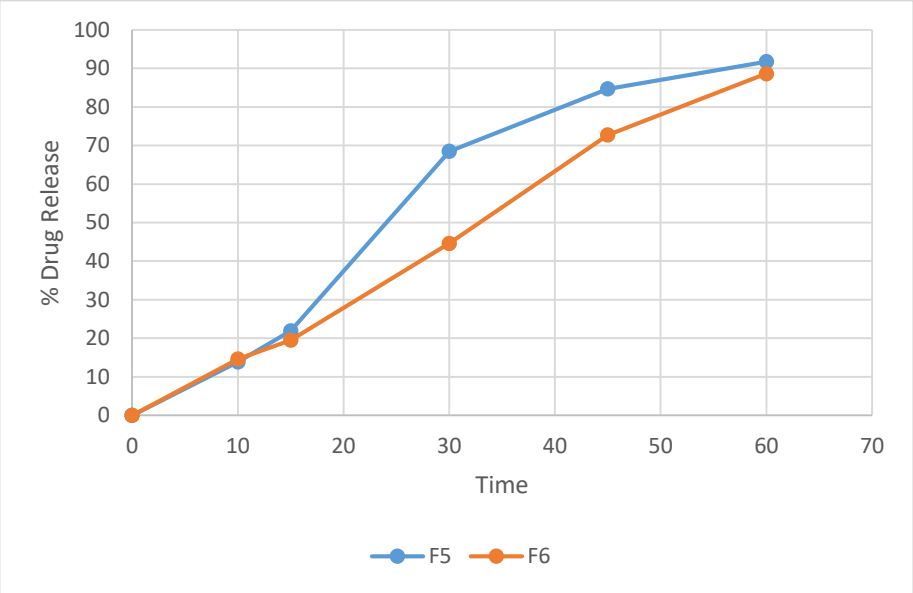

Figure 22: % Cumulative drug release (F5-F6)

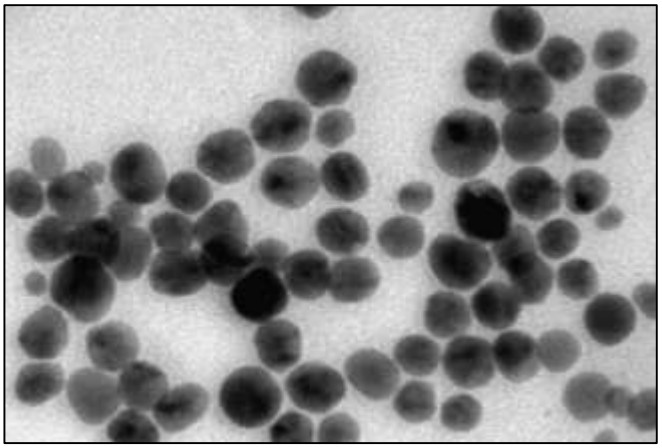

Figure 23: TEM of formulation F1

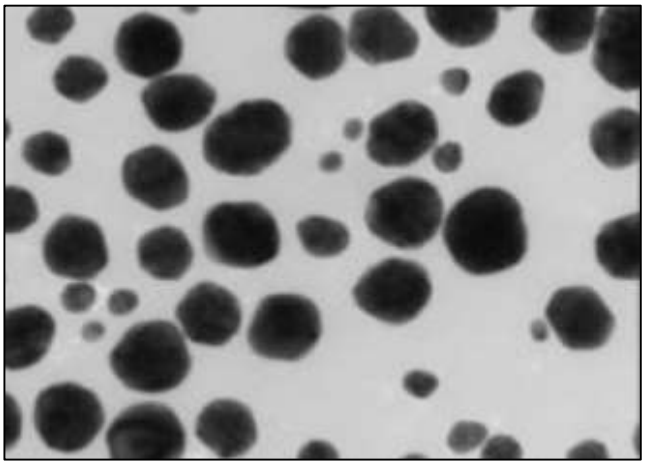

Figure 24: TEM of formulation F2

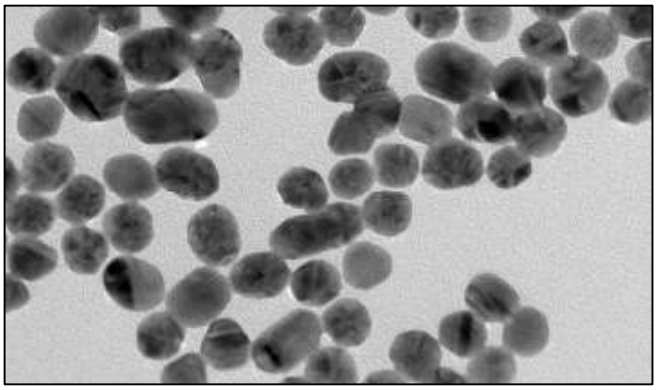

Figure 25: TEM of formulation F3

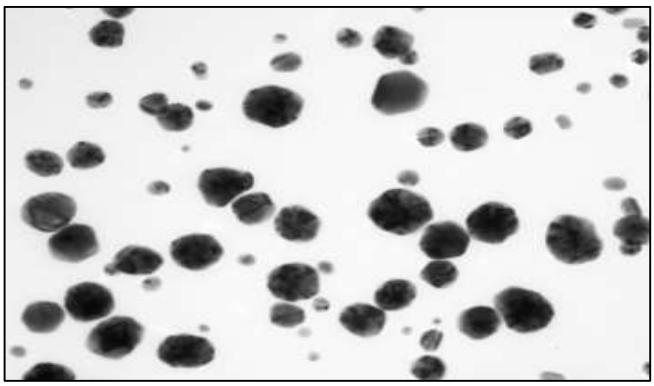

Figure 26: TEM of formulation F4

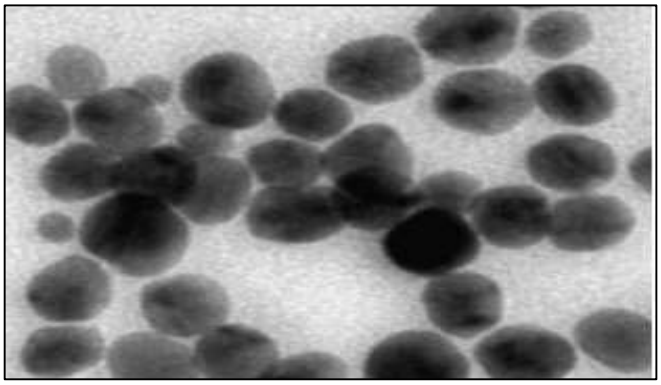

Figure 27: TEM of formulation F5

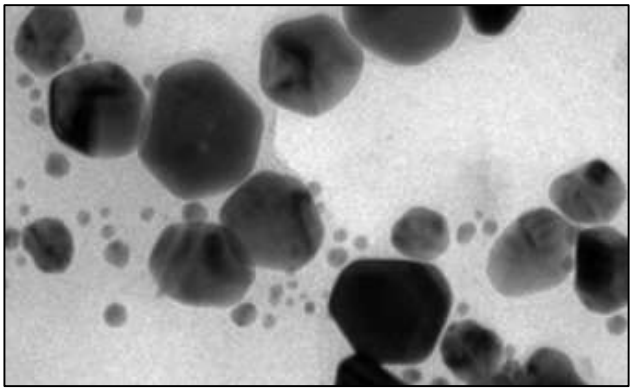

Figure 28: TEM of formulation F6