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PREPARATION AND CHARACTERIZATION OF POLYMERIC NANOPARTICLES CONTAINING COMBINE DRUGS FOR ENHANCING OF COMBINATION THERAPY

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ABSTRACT

The present study aimed to develop and characterize polymeric nanoparticles containing Andrographolide and Aloin to enhance the efficiency of combination therapy. Initially, a pre- formulation study was carried out to evaluate the physical and chemical properties of both drugs. The UV absorption maxima (λmax) were found to be 225.0 nm for Andrographolide and 296.0 nm for Aloin, and calibration curves were constructed for quantitative analysis. Fourier Transform Infrared (FTIR) spectroscopy confirmed the presence of characteristic functional groups of both drugs, validating their chemical integrity and compatibility for nanoparticle formulation. Polymeric nanoparticles were prepared using the nanoprecipitation technique with biodegradable polymers to encapsulate both active pharmaceutical ingredients (APIs). The formulated nanoparticles exhibited a smooth and uniform physical appearance. Particle size analysis revealed sizes of 171.5 nm and 143.0 nm, suitable for efficient cellular uptake. The zeta potential of –12.9 mV indicated good nanoparticle stability with minimal aggregation. Scanning Electron Microscopy (SEM) confirmed spherical morphology with smooth surfaces, favorable for controlled drug release applications. The entrapment efficiency was high (95.21%), indicating effective encapsulation of both drugs. In vitro drug release studies demonstrated a controlled and sustained release over 16 hours for both Andrographolide and Aloin. Among all formulations (NPs1–NPs5), formulation F1 exhibited the best release profile and was selected for kinetic modelling. The release data for F1 followed Higuchi kinetics with a high regression coefficient (R^2 = 0.9961), confirming diffusion-controlled release. In conclusion, the developed polymeric nanoparticles showed optimal particle size, high stability, superior encapsulation efficiency, and sustained release behavior**,** making them a promising carrier system for combination therapy. The co-delivery of Andrographolide and Aloin through polymeric nanoparticles may offer improved bioavailability and enhanced therapeutic efficacy, supporting their potential use in advanced combination drug delivery systems.

KEYWORDS: Andrographolide; Aloin; Polymeric nanoparticles; Combination therapy; Nanoprecipitation; Controlled release; Entrapment efficiency.

1. INTRODUCTION

Polymeric nanoparticles are typically composed of biodegradable and biocompatible polymers such as poly (lactic-co-glycolic acid) (PLGA), chitosan, polycaprolactone (PCL), or polyethylene glycol (PEG)-based polymers. These materials not only protect the encapsulated drugs from premature degradation but also allow precise control over drug release kinetics through polymer composition, molecular weight, and formulation parameters **(Elmowafy *et al.*, 2023).** The surface modification of nanoparticles with ligands or targeting moieties further enhances site-specific delivery, enabling drugs to accumulate preferentially at the diseased site while minimizing systemic toxicity. The process of preparing polymeric nanoparticles can be achieved through various techniques, including solvent evaporation, nanoprecipitation, emulsification, or ionic gelation **(Pulingam *et al.*, 2022).** Each method has its

own advantages depending on the nature of the drugs and the desired nanoparticle characteristics. After formulation, thorough characterization is essential to determine particle size, polydispersity index (PDI), surface charge (zeta potential), morphology, encapsulation efficiency, and drug release profile. These parameters play a crucial role in defining the nanoparticles' stability, cellular uptake, and in vivo behaviour **(Danaei *et al.*, 2018).**

Andrographolide, a diterpenoid lactone isolated from *Andrographis paniculata* (commonly known as "King of Bitters"), is a well-known phytochemical recognized for its wide range of pharmacological activities. It exhibits potent anti-inflammatory, antioxidant, antiviral, anticancer, and hepatoprotective properties **(Mehta *et al.*, 2022).** Despite its broad therapeutic potential, the clinical application of Andrographolide remains limited due to its poor water solubility, low permeability, and short plasma half-life**.** Therefore, there is a growing need to develop an efficient delivery system that can overcome these limitations and enhance its bioavailability and therapeutic performance. Combination therapy**,** which involves the concurrent use of two or more therapeutic agents, has emerged as a powerful strategy to improve treatment outcomes, particularly in complex diseases such as cancer, inflammation, and infectious disorders **(Mokhtari *et al.*, 2017).** This approach helps achieve synergistic effects, minimize adverse reactions, and reduce the development of drug resistance. Incorporating Andrographolide into a polymeric nanoparticle-based combination therapy system could offer significant benefits—by enabling co-delivery with another complementary drug, ensuring synchronized release, and enhancing the overall pharmacological effect through synergism **(Sousa and Videira 2025).**

Aloin, a naturally occurring anthraquinone glycoside extracted from *Aloe vera* and *Aloe ferox* species, has demonstrated diverse pharmacological activities, including anti-inflammatory, antioxidant, anticancer, antimicrobial, and hepatoprotective effects. Despite its strong therapeutic potential, the clinical application of Aloin is largely limited by its poor water solubility, low permeability, instability under physiological conditions, and rapid elimination from the body **(Merino *et al.*, 2025).** Therefore, developing a suitable delivery system capable of improving the pharmacokinetic profile of Aloin is essential to maximize its therapeutic benefits. The formulation of polymeric nanoparticles containing Aloin typically involves methods such as solvent evaporation, nanoprecipitation, emulsification-solvent diffusion, or ionic gelation, depending on the solubility characteristics of both the drug and the polymer **(Chauhan and Malik 2025).** Biocompatible and biodegradable polymers like poly(lactic-co-glycolic acid) (PLGA)**,** chitosan**,** polycaprolactone (PCL)**,** and polyethylene glycol (PEG) are commonly employed to achieve stable formulations. These polymers not only encapsulate and protect Aloin from degradation but also allow for precise modulation of release kinetics through polymer composition and molecular weight **(Salah Othman *et al.*, 2025).**

The present study focuses on the preparation and characterization of polymeric nanoparticles containing Andrographolide and Aloin.

2. MATERIALS AND METHOD

2.1 Chemical

Aloin and Andrographolide was obtained from a sigma-aldrich. 95 % Alcohol, Ammonia, and 1% Copper Sulphate Solution were procured from Clorofilt ind, while Magnesium was supplied by Himedia. Chloroform was obtained from Rankem. Merck provided the Methanol. All other reagents and solvents used were of analytical grade.

2.2 Pre-formulation study of drugs

It is the investigation of a drug substance's physical and chemical properties both alone and in combination with excipients. Pre-formulation testing's primary purpose is to generate data that formulators can use to build stable, bioavailable dosage forms that are simple to mass produce. Pre formulation studies are designed to offer all important information, particularly on the physicochemical, physicomechanical, and biological properties of medicinal substances, excipients, and packaging materials **(Borkar *et al.*, 2022).**

2.2.1 Organoleptic evaluationof both drugs

Organoleptic evaluation of a drug refers to the assessment of its sensory properties, such as taste, smell, color, texture, and appearance **(Patil *et al.*, 2018).**

2.2.2 Solubility study of both drugs

To perform a solubility study by visual inspection, add (1 mg) amount of the drug to a suitable solvent (1 ml each) methanol, ethanol, chloroform, acetone, and water and stir or shake the mixture. Observe the solution for any visible signs of dissolution, such as cloudiness or undissolved particles, over a period of time. If the drug dissolves completely, it indicates good solubility; if particles remain, it suggests limited solubility. The process can be repeated at different temperatures or with varying solvent types for further insights **(Jain and Verma 2020).**

2.2.3 pH determination of both drugs

A digital pH meter was used to measure the pH of the drugs andrographolide and aloin **(Elmataeeshy *et al.*, 2018).**

2.2.4 Melting point determination of both drugs

Melting point was analyzed by melting point apparatus. The procedure is repeated for accuracy, and the average melting point value is calculated to ensure precision **(Johnson and Zhang 2025).**

2.3 Determination of Maximum Wavelength (λ max)

2.3.1 Preparation of Andrographolide and Aloin standard stock solution in methanol

A standard solution of Andrographolide and Aloin pharmaceuticals was generated by dissolving precisely weighed 10 mg of Andrographolide and Aloin in 5 ml of methanol solvent in a 10 ml volumetric flask (a separate volumetric flask). To create a 1000 µg/ml stock solution, add 10 ml of methanol to the original amount. To create a standard stock

2.3.2 Lambda max Determination

Andrographolide stock solutionto make a 20 µg/ml Aloin stock solution, 2 ml of Andrographolide drug was added to a 10 ml volumetric flask and the content was marked with methanol. To prepare a concentration of 10 µg/ml, 1 ml of Aloin medication was put into a 10 ml volumetric flask and the volume was marked with methanol. The drug's working standard solution was scanned in the UV range of 200 to 400 nm in normal mode, with methanol serving as a blank. The resulting peaks were observed, and the absorption **(Kumbhar and Salunkhe 2013).**

2.3.3 Calibration curve determination of both drugs

Dilutions of Andrographolide were prepared from the working standard solution of 100 µg/mL to 5, 10, 15, 20, 25, and 30 µg/mL. In separate volumetric flasks, add 30, 40, 50, 60, 70, and 80 µg/mL of aloin. After accurately transferring the Andrographolide and Aloin working standard stock solution into a series of 5 mL calibrated flasks, the volume was adjusted using methanol. The absorbance of the resulting solutions was measured at 225.0 and 296.0 nm for Andrographolide and Aloin medicines, respectively, in comparison to a methanol blank. A calibration curve was developed by plotting the drug's absorbance against concentration. A six-point calibration curve was created for Andrographolide and Aloin medication concentrations from 5 to 30 µg/ml and 30 to 80 µg/ml **(Patidar and Ramteke 2024).**

2.3.4 Functional group identified by FTIR

The FTIR spectra of medicines were acquired using the KBr press pellet technique and scanned from 400 to 4000 cm-1. The KBr disc was made by combining 1 mg of Andrographolide and Aloin with 100 mg of spectroscopic grade KBr and drying it under an infrared light source. To produce a disc, KBr and drug were mixed together and subjected to hydraulic pressure. This disc was placed in the FT-IR chamber **(Lakshminarayanan and Balakrishnan 2020).**

2.4 Formulation of Polymeric Nanoparticles

Solvent evaporation method was used for the preparation of Andrographolide and Aloin nanoparticles. Firstly, the emulsification of polymeric solution was done in an aqueous solution containing a surfactant. Then the evaporation of polymeric solution was done by precipitation of the polymer. In the solution of Acetone, methanol drug (Andrographolide 30mg and Aloin 30 mg) was dissolved. With constant stirring using a magnetic stirrer, the organic solution was added into an aqueous phase containing polyvinyl alcohol. The emulsion was sonicated using sonicator for 6 min to get nano size of the emulsion. The organic solvent was then evaporated using constant stirring on a magnetic stirrer for about 4-5 hrs. After centrifugation (30 min, 10000 rpm), the nanoparticles were collected. The prepared emulsion was then kept for lyophilization for 48 hrs (**Saharan *et al.*, 2019).**

Table 1: Composition of Polymeric Nanoparticles.

Formulation Code	Eudragit RS 100 Polymer Concentration (mg)	Polyvinyl alcohol Surfactant Concentration (%)	Drugs (Andrographolide 30mg and Aloin 30 mg)	Aqueous solution Water (ml)	Stirring Time (Hrs.)
NPs 1	250	0.3	1:1	10	4
NPs 2	200	0.3	1:1	10	4
NPs 3	150	0.3	1:1	10	4
NPs 4	100	0.3	1:1	10	4
NPs 5	50	0.3	1:1	10	4

2.5 Evaluation parameter of drug loaded Nanoparticle formulation

2.5.1 Physical appearance

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are used for microscopy studies. These techniques are used to observe the Physical appearances such as size, shape, crystallinity, and surface topography of a plain drug and formulated Polymeric Nanoparticles **(Shringirishi *et al.*, 2014).**

2.5.2 Particle size

The size of Polymeric Nanoparticles was measured using Malvern Zeta sizer (Malvern Instruments) **(Singh and Vingkar 2008).**

2.5.3 Zeta potential

Zeta potential was measured using a zeta potentiometer (Malvernzeta seizer). To determine zeta potential, SLN samples were diluted with double distilled water and placed in the electrophoretic cell in a cuvette. Each sample was analyzed in triplicate **(Luo *et al.*, 2006).**

2.5.4 Scanning Electron Microscopic (SEM)

The morphological properties of the Andrographolide and Aloin-loaded Polymeric Nanoparticles were obtained using the electron beam from a scanning electron microscope. The Polymeric Nanoparticles were coated with a thin layer (2-20 nm) of metal(s) such as gold, palladium, or platinum using a sputter coater in vacuum. The pretreatment specimen was then attacked with an

electron beam, which resulted in the creation of secondary electrons known as augers. From this interaction between the electron beam and the specimen's atoms, only the electrons scattered at 90° were selected and further processed based on Rutherford and Kramer's Law for acquiring the images of surface topography **(Anwer *et al.*, 2019).**

2.5.5 Quantitative analysis (Entrapment Efficiency)

Entrapment efficiency was determined by indirect estimation. Drug -loaded Polymeric Nanoparticles were centrifuged at 15,000 rpm for 30 min using REMI Ultra Centrifuge. The non-entrapped drug (free drug) was determined in the supernatant solution using UV spectrophotometer. The peak area was determined and amount of free drug is determined by extrapolating the calibration curve. And drug entrapment calculated by using below equation **(Balla and Goli 2020).**

Entrapment efficiency % = Total drug conc. - Supernatant drug conc. / total drug conc. ×100

2.5.6 Drug release study

Firstly, formulation was dissolved in phosphate buffer solution 7.4. Then put into the Franz diffusion cell such that the cell's drug releasing surface remained towards the receptor compartment; which containing 50ml of phosphate buffer pH 7.4 at 37±0.5°. The cell was placed on a magnetic stirrer, and the solution in the receptor compartment was continuously stirred using magnetic bead at 50rpm at 37±0.5°C. 5ml solution was withdrawn at predefine time intervals and changed with same volume of phosphate buffer pH 7.4. Finally test solutions were quantified for drug by uv spectrophotometer.

Zero order release F = K0.t F = drug release, K0 = release rate constant, t = release time. The plot of percentage drug release versus time was linear.

First order release Log (100 – F) = K.t F = drug release, K = release rate constant, t = release time. A plot of log % drug release versus time was linear.

Higuchi model F = K.t1/2 F = drug release, K = Higuchi constant, t = release time. A plot of percentage drug release versus square root of time was linear.

Korsmeyer-Peppas model Mt/M∝ = K.tn M = fraction of drug released, K = release constant, t = release time, n = diffusion exponent.

The value of n indicates release mechanism. When n = 1 means release rate is independent of time (zero-order) (case II transport), n = 0.5 stands for Fickian diffusion, 0.5 < n < 1.0 stands for diffusion and nonFickian transport (swellable and cylinder Matrix), n > 1.0 shows super case II transport is apparent. n is the slope value of log Mt/M∝ vs. log time curve **(Pintu and Subas 2011, Patel, P. M. 2017).**

3. RESULTS AND DISCUSSION

3.1 Pre-formulation study of Andrographolide and Aloin

3.1.1 Organoleptic evaluation

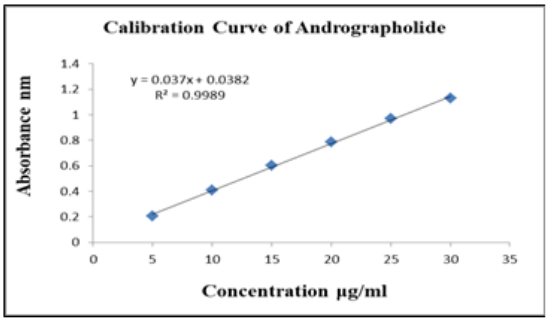
Table 2: Organoleptic evaluation of Andrographolide and Aloin

Physical parameter	Observation (Andrographolide)	Observation (Aloin)
Color	Off-white	Yellow-brown
Odor	Odorless	Bitter odor
State	Crystallinesolid	CrystallinePowder
Appearance	White square prisms or flaky crystals	Yellow crystals or powder

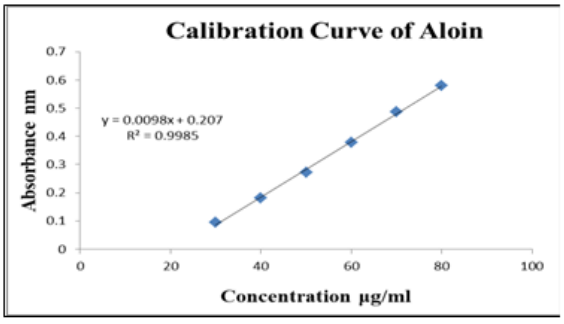
3.1.2 Melting Point and pH determination of Andrographolide and Aloin

Table 3:- Melting Point and pH determination of Andrographolide and Aloin.

Drug	Observed (pH)	Observed (Melting point)	Reference (Melting point)
Andrographolide	6.8	230 °C	229–232 °C
Aloin	4.5	148 °C	148–149 °C

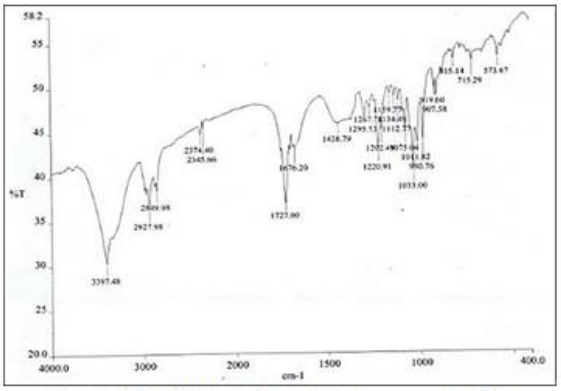


Graph 1: Calibration curve of Andrographolide

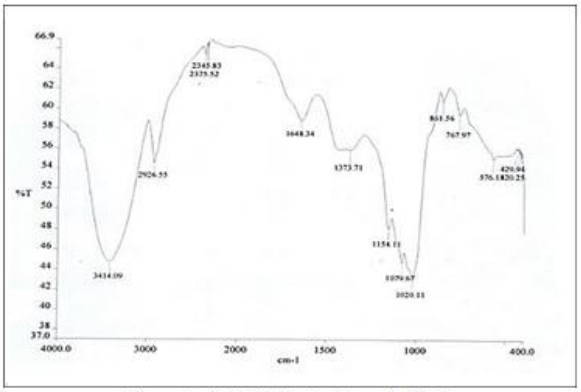


Graph 2: Calibration curve of Aloin

3.2 Functional group identified by Infra-Red spectroscopy



Graph 3: FTIR study of Andrographolide



Graph 4: FTIR study of Aloin

Table 4: Interpretation of IR spectrum of Andrographolide.

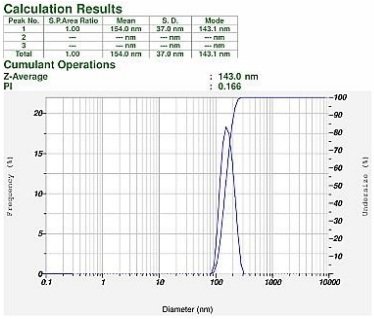
Peak obtained	Reference peak	Functional group	Name of functional group
3397.48	3500–3200	H–bonded	Phenols
2849.98	2900-2800	-CHO stretching	-C-H aldehydic
1676.20	1678-1668	C=C stretching	Alkene
1438.79	1440-1395	O-H Bending	Carboxylic acid
1220.91	1275-1200	C-O stretching	Alkyl Aryl Ether
1033.00	1070-1030	S=O stretching	Sulfoxide
815.14	840-790	C=C Bending	Alkene

Table 5: Interpretation of IR spectrum of Aloin.

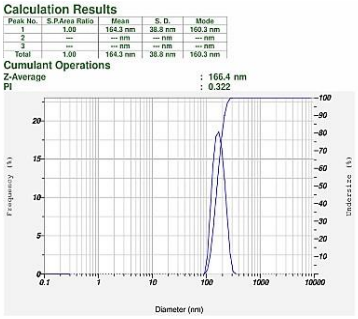
Peak obtained	Reference peak	Functional group	Name of functional group
3414.09	3500–3200	O–H stretch	Alcohols
2926.55	3000–2850	C–H stretch	Alkanes
1648.34	1600–1585	C–C stretch	Aromatics
1373.71	1390-1310	O-H bending	Aliphatic Amines
1020.11	1320–1000	C–O stretch	Alcohols, carboxylic acids
861.56	900–675	C–H “oop”	aromatics

3.3 Characterization of drug loaded Polymeric Nanoparticle formulation

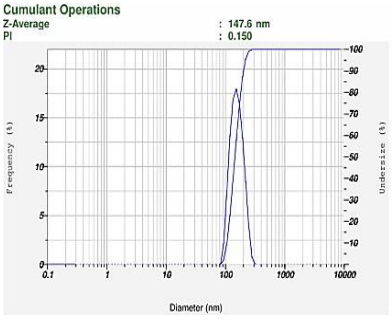
3.3.1 Particle Size



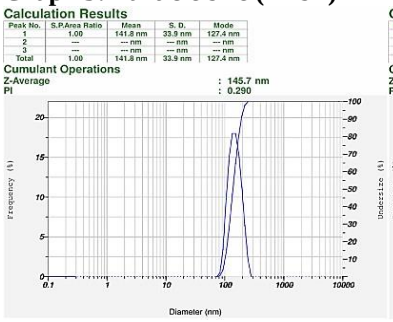
Graph 5: Particle size (NPs 1)



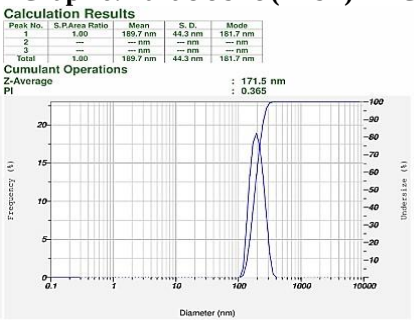
Graph 6: Particle size (NPs 2)



Graph 7: Particle size (NPs 3)



Graph 8: Particle size (NPs 4)

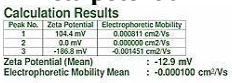


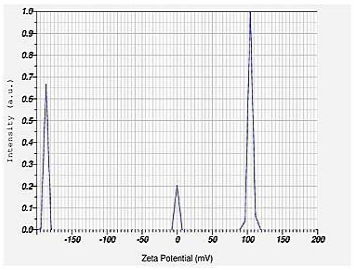
Graph 9: Particle size (NPs 5)

Table 6: Particle size.

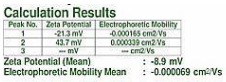
Formulation code	Particle size (nm)	PI Value
NPs 1	**143.0 nm**	**0.166**
NPs 2	166.4 nm	0.322
NPs 3	147.6 nm	0.150
NPs 4	145.7 nm	0.290
NPs 5	171.5 nm	0365

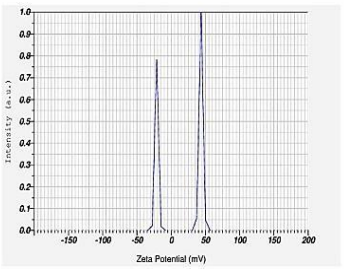
3.3.2 Zeta potential



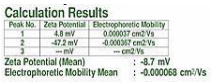


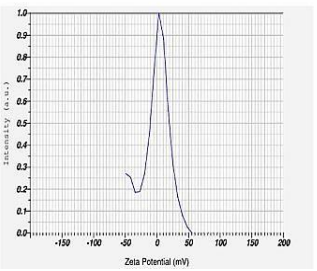
Graph 10: Zeta potential (NPs 1)



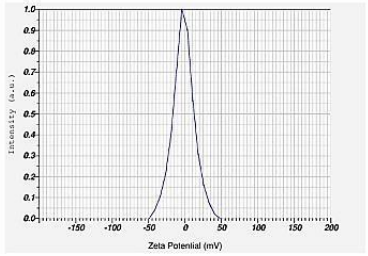


Graph 11: Zeta potential (NPs 2)

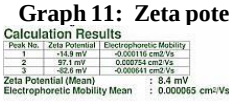


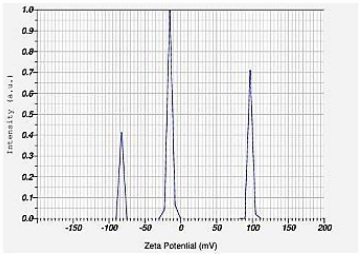


Graph 12: Zeta potential (NPs 3)



Graph 13: Zeta potential (NPs 4)





Graph 14: Zeta potential (NPs 5)

Table 7: Zeta potential.

Formulation Code	Zeta potential
NPs 1	**-12.9 mV**
NPs 2	-8.9 mV
NPs 3	-8.7 mV
NPs 4	-2.2 mV
NPs 5	-8.4 mV

3.3.3 Scanning electron microscope (SEM)

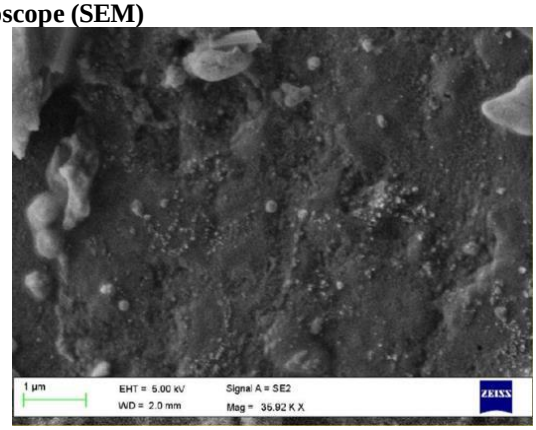


Figure 1: Scanning electron microscope (SEM)

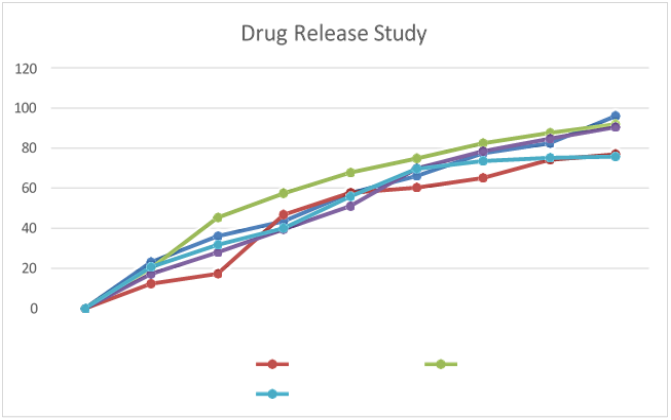
3.3.4 Quantitative analysis (Entrapment Efficiency)

Table 8: Entrapment Efficiency.

Formulation code	Entrapment Efficiency (%)
NPs 1	**95.21**
NPs 2	69.02
NPs 3	63.61
NPs 4	79.28
NPs 5	90.77

3.3.5 Drug release study of all formulations (F1 to F5) Table 9:- Drug release study of all formulations (F1 to F5).

Time (Hr)	% drug released (F1)	% drug released (F2)	% drug released (F3)	% drug released (F4)	% drug released (F5)
0	0	0	0	0	0
1	23.18	12.45	20.11	17.26	20.72
2	36.09	17.36	45.36	28.00	31.85
4	43.63	46.86	57.46	39.60	40.12
6	57.6	57.80	67.86	51.10	55.89
8	66.13	60.31	74.88	69.82	69.74
10	77.56	65.23	82.47	78.47	73.66
12	82.45	74.22	87.63	84.73	75.21
16	96.13	76.96	92.01	90.48	75.89



Graph 15: Drug release study of all formulations (F1 to F5).

4. CONCLUSION

In conclusion, the developed polymeric nanoparticles demonstrated significant potential for improving the efficiency of combination therapy. The nanoparticles exhibited favorable characteristics, including optimal size, stability, high entrapment efficiency, and a controlled release profile, which are crucial for enhancing the bioavailability and therapeutic outcomes of Andrographolide and Aloin. This drug delivery system could provide a promising strategy for more effective treatment regimens, particularly in conditions where combination therapies are essential for better clinical outcomes.

5. REFERENCES

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