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FORMULATION DEVELOPMENT AND EVALUATION OF PYRAZINAMIDE-LOADED NANOSUSPENSIONS FOR ENHANCED ORAL DRUG DELIVERY

Balasubramanian Vallimanalan*[1] and Sebastin Varghese[2]

[1*]Department of Pharmaceutics, Malik Deenar College of Pharmacy, Seethangoli, Bela Post, Kasaragod, 671321, Kerala, India.

[2]Department of Pharmaceutical Chemistry, Malik Deenar College of Pharmacy, Seethangoli, Bela Post, Kasaragod, 671321, Kerala, India.

ABSTRACT

Pyrazinamide remains an essential antitubercular agent; however, its clinical application is often limited by rapid metabolism and dose-related toxicity. In the present investigation, a polymer-based nanosuspension system was designed to improve oral delivery and sustain drug release. Pyrazinamide nanosuspensions were formulated using cellulose acetate and a non-ionic stabilizer through a solvent displacement method, followed by chitosan surface modification. The formulations were evaluated for physicochemical properties, *in vitro* release behavior, compatibility and stability. The optimized formulation demonstrated nanoscale particle size, high drug encapsulation and prolonged release over 24 h, indicating its potential for improved oral therapy.

KEYWORDS

Pyrazinamide, Nanosuspension, Cellulose acetate, Oral drug delivery and Controlled release.

Author for Correspondence:

Balasubramanian Vallimanalan,

Department of Pharmaceutics,

Malik Deenar College of Pharmacy, Seethangoli,

Bela Post, Kasaragod, 671321, Kerala, India.

Email: manalanceutics@gmail.com

INTRODUCTION

Despite advances in chemotherapy, tuberculosis continues to demand prolonged treatment regimens that are often associated with adverse drug reactions. Pyrazinamide plays a vital role during the intensive phase of therapy; however, its pharmacokinetic limitations necessitate high dosing. Modern drug delivery approaches such as nanosuspensions offer opportunities to modulate release behavior and enhance systemic availability. The present study explores a polymeric nanosuspension strategy to address these challenges.

MATERIALS AND METHODS

Pyrazinamide was used as the model drug. Cellulose acetate was used as polymers, poloxamer 188 as stabilizer, ethanol as solvent, and low-molecular-weight chitosan as coating polymer. Nanosuspensions were prepared by solvent displacement method and evaluated for particle size, zeta potential, drug content, entrapment efficiency, morphology, *in vitro* drug release, release kinetics, compatibility and stability.

Formulation Design of Pyrazinamide Nanosuspensions

Pyrazinamide-loaded nanosuspensions were formulated with the objective of enhancing oral bioavailability and reducing dose-dependent hepatotoxicity. The drug concentration was maintained constant at 100mg per 10mL of formulation. Polymers cellulose acetate was used at three concentration levels (100, 200 and 300mg), while poloxamer 188 was employed as stabilizer at 15, 30 and 45mg. The formulations were prepared in pH 7.4 phosphate buffer to minimize premature drug metabolism.

Preparation of Nanosuspensions

Nanosuspensions were prepared by the nanoprecipitation technique. Accurately weighed quantities of polymer were dissolved in 3-5mL of ethanol at ambient temperature. Simultaneously, pyrazinamide was dissolved in pH 7.4 phosphate buffer containing poloxamer 188 under continuous stirring at 500rpm using a magnetic stirrer. The ethanolic polymer solution was injected dropwise into the aqueous drug solution using a 14-gauge needle. Formation of nanosuspensions was confirmed by the appearance of bluish opalescence. The system was stirred for 1 h to allow complete evaporation of ethanol. The dispersion was filtered through a 0.45µm membrane filter and stored at 2-8°C.

Chitosan Coating of Nanosuspensions

Optimized nanosuspensions were coated with LMW chitosan to achieve controlled drug release, protection from gastric environment, and enhanced mucoadhesion. Chitosan solutions (1%, 3% and 5% w/w) were prepared in acetate buffer (pH 4.4). One milliliter of the coating solution was slowly added to the nanosuspension under gentle stirring at 300rpm to ensure uniform coating. Among the tested concentrations, 5% w/w chitosan provided optimal coating characteristics and was selected for further studies.

Characterization of Nanosuspensions

Particle Size, Polydispersity Index and Zeta Potential

Mean particle size, polydispersity index (PdI) and zeta potential were determined using a Zetasizer (Malvern Instruments) by photon correlation spectroscopy at 25°C. Measurements were performed in triplicate.

Surface Morphology

Surface morphology of nanosuspensions was examined using scanning electron microscopy (SEM) (HITACHI TM 3030) at 60,000× magnification without prior metal coating.

pH Determination

The pH of nanosuspensions was measured using a calibrated digital pH meter. Measurements were performed in triplicate.

Drug Content Analysis

An accurately measured volume of nanosuspension equivalent to 10mg of pyrazinamide was lysed using methanol. Suitable dilutions were prepared with pH 7.4 phosphate buffer and analyzed spectrophotometrically at 268nm. All determinations were performed in triplicate.

Entrapment Efficiency

Entrapment efficiency was determined by ultracentrifugation. Nanosuspension samples were centrifuged at 11,000rpm for 30 min at 4°C. The supernatant containing unentrapped drug was analyzed spectrophotometrically at 268nm. Entrapment efficiency was calculated using the equation:

$$\text{EE}\ (\%) = \frac{(C_d - C)}{C_d} \times 100$$

where C_d is the total drug concentration and C is the concentration of unentrapped drug.

In Vitro Drug Release Studies

In vitro drug release was evaluated using the dialysis bag method. Dialysis membrane (MWCO 12,000 Da) was soaked overnight in pH 7.4 phosphate buffer. Nanosuspension equivalent to 50 mg of pyrazinamide was placed inside the membrane and immersed in 250mL of dissolution medium maintained at 37 ± 0.5°C with continuous stirring at 500rpm. Samples were withdrawn at predetermined intervals and replaced with fresh buffer. Drug content was analyzed at 268 nm using UV-visible spectrophotometry.

Drug Release Kinetics

Drug release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas kinetic models to determine release mechanism and kinetics. The release exponent (n) was used to characterize diffusion behavior.

Drug–Excipient Compatibility Studies

Compatibility between pyrazinamide and excipients was evaluated using Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC) and Powder X-ray Diffraction (PXRD).

Stability Studies

Short-term stability studies were conducted for the optimized formulation (FCA9) for six months under refrigerated (4°C), ambient (30°C/65% RH) and accelerated (40°C/75% RH) conditions. Samples were evaluated periodically for particle size, PdI and drug content.

RESULTS AND DISCUSSION

Physicochemical Characterization

All formulations exhibited high drug content (93-99%), indicating uniform drug distribution. Particle size analysis revealed nanoscale dimensions with low PdI values, confirming homogeneous dispersion. Increased stabilizer concentration resulted in reduced particle size and improved stability.

Cellulose acetate-based formulations showed superior entrapment efficiency, attributed to better polymer-drug interaction and higher surface area.

Zeta Potential and Stability

Zeta potential values ranged from -17 to -31mV, indicating sufficient electrostatic and steric stabilization. The optimized formulation FCA9 exhibited zeta potential close to -30mV, ensuring good physical stability.

Surface Morphology

SEM analysis revealed spherical, smooth-surfaced nanoparticles without aggregation, confirming the stability and uniformity of nanosuspensions.

In Vitro Drug Release

All formulations exhibited sustained drug release over 24 h. The nanosuspensions FCA9 showed significantly higher cumulative drug release. achieved nearly complete drug release (99.78%) within 24 h, attributed to smaller particle size and higher entrapment efficiency.

Drug Release Kinetics

Release kinetics followed first-order and Higuchi models, indicating diffusion-controlled release. Korsmeyer–Peppas analysis revealed Fickian diffusion as the predominant mechanism, with a minor contribution from polymer erosion due to chitosan coating.

Compatibility and Stability Studies

FTIR, DSC, and PXRD studies confirmed the absence of chemical interaction between pyrazinamide and excipients. Stability studies demonstrated no significant changes in particle size, drug content, or PdI under different storage conditions.

Table No.1: Compositions of nanosuspensions using Cellulose Acetate

S.No	Batch code	Amount of CelluloseAcetate (mg	Amount of Polaxamer (mg)
1	FCA1	100	15
2	FCA2	100	30
3	FCA3	100	45
4	FCA4	200	15
5	FCA5	200	30
6	FCA6	200	45
7	FCA7	300	15
8	FCA8	300	30
9	FCA9	300	45

Table No.2: Physico-chemical characterization of pyrazinamide with cellulose acetatenanosuspension

S.No	Batch Code	% Drug Content	Size (nm)	PdI	Zeta Potential (mV)	%EE	pH
1	FCA1	95.1 ± 0.5	325 ± 1.2	0.310 ± 0.1	17 ± 0.6	43 ± 1.5	7.4 ± 0.1
2	FCA2	96.2 ± 0.9	255 ± 0.1	0.296 ± 0.2	19 ± 0.2	51 ± 1.7	7.4 ± 0.3
3	FCA3	98.5 ± 0.1	147 ± 0.2	0.213 ± 0.1	22 ± 0.1	62 ± 3.0	7.4 ± 0.1
4	FCA4	95.4 ± 0.3	316 ± 0.1	0.315 ± 0.1	18 ± 0.4	58 ± 2.6	7.4 ± 0.2
5	FCA5	97.5 ± 1.0	271 ± 0.5	0.304 ± 0.1	21 ± 0.4	64 ± 1.7	7.4 ± 0.4
6	FCA6	98.2 ± 0.6	263 ± 1.2	0.242 ± 0.4	22 ± 0.6	68 ± 0.9	7.4 ± 0.2
7	FCA7	97.1 ± 0.7	341 ± 0.6	0.325 ± 0.1	24 ± 0.1	71 ± 2.0	7.4 ± 0.2
8	FCA8	96.1 ± 0.8	234 ± 0.2	0.254 ± 0.3	27 ± 0.2	84 ± 1.3	7.4 ± 0.1
9	FCA9	99.3 ± 0.8	142 ± 0.8	0.196 ± 0.1	31.4 ± 0.2	88 ± 1.5	7.4 ± 0.2

Each value represents Mean ± S. D. (n=3)

Table No.3: Drug release profile of cellulose acetate nanosuspensions

S.No	Time (hrs)	FCA1	FCA2	FCA3	FCA4	FCA5	FCA6	FCA7	FCA8	FCA9
1	0.5	12.26 ± 0.1	9.12 ± 0.9	10.37 ± 1.9	17.59 ± 1.6	18.31 ± 0.8	9.56 ± 0.6	15.32 ± 0.7	18.95 ± 0.3	21.56 ± 0.2
2	1	21.50 ± 0.9	19.83 ± 1.2	17.25 ± 1.2	25.58 ± 1.2	25.58 ± 0.9	17.25 ± 1.3	23.08 ± 1.3	25.58 ± 0.8	34.50 ± 0.9
3	2	36.95 ± 0.3	32.84 ± 0.8	25.22 ± 1.5	39.21 ± 1.5	37.70 ± 0.5	24.88 ± 1.5	27.98 ± 1.5	34.94 ± 0.9	44.44 ± 1.5
4	4	44.95 ± 1.3	45.85 ± 0.6	36.59 ± 0.9	48.56 ± 1.8	44.36 ± 1.2	36.84 ± 1.8	37.61 ± 1.2	44.10 ± 1.2	56.94 ± 0.8
5	6	53.57 ± 1.6	55.40 ± 0.9	40.64 ± 0.6	56.71 ± 1.4	53.74 ± 0.5	43.74 ± 1.6	43.76 ± 0.4	53.98 ± 0.8	66.34 ± 1.7
6	8	57.80 ± 0.8	59.98 ± 1.2	48.15 ± 0.5	63.72 ± 0.4	58.73 ± 1.6	51.44 ± 1.8	51.46 ± 1.7	60.05 ± 1.8	75.78 ± 0.2
7	10	62.06 ± 0.4	66.17 ± 0.2	53.70 ± 1.2	71.61 ± 1.8	65.67 ± 1.8	57.84 ± 1.2	60.12 ± 1.2	74.21 ± 0.2	84.60 ± 1.7
8	12	69.01 ± 1.2	73.48 ± 0.8	60.69 ± 0.9	79.46 ± 0.7	74.66 ± 1.4	65.52 ± 0.9	70.69 ± 1.8	85.84 ± 0.6	92.07 ± 1.3
9	16	73.26 ± 1.2	78.56 ± 0.9	64.36 ± 1.4	86.59 ± 0.6	81.64 ± 0.5	68.32 ± 0.8	77.89 ± 0.5	90.74 ± 0.2	95.65 ± 1.6
10	24	75.50 ± 1.4	83.68 ± 0.8	69.06 ± 1.1	92.54 ± 1.4	86.54 ± 1.8	71.41 ± 0.2	80.21 ± 1.1	92.17 ± 0.1	99.78 ± 1.0

Each value represents Mean ± S. D.(n=3)

Table No.4: Stability Data showing drug content at different temperatures

S.No	Time Period (in months)	Temp. in Range		
		4° C	30± 2° C/ 65 ± 5%RH	40± 2° C/ 75 ± 5%RH
1	0	71.32±0.4	71.32±0.4	71.32±0.4
2	1	70.4 ± 1.3	70.2 ± 1.0	70.9 ± 1.1
3	2	70.2 ± 1.1	69.9 ± 1.3	70.1 ± 1.0
4	3	69.6 ± 1.0	69.1 ± 1.1	69.2 ± 1.7
5	6	69.3 ± 1.6	68.7 ± 1.0	68.1 ± 1.4

Table No.5: Stability Data showing drug content at different particle size

S.No	Time (in months)	4° C		30± 2° C/ 65 ± 5%RH		40± 2° C/ 75 ± 5%RH	
		Particle Size(nm)	PdI	Particle Size(nm)	PdI	Particle Size(nm)	PdI
1	0	0.204	0.315	0.204	0.315	0.204	0.315
2	1	0.208	0.311	0.210	0.314	0.207	0.312
3	2	0.210	0.314	0.214	0.316	0.210	0.310
4	3	0.207	0.317	0.206	0.314	0.211	0.319
5	6	0.205	0.312	0.208	0.318	0.214	0.316

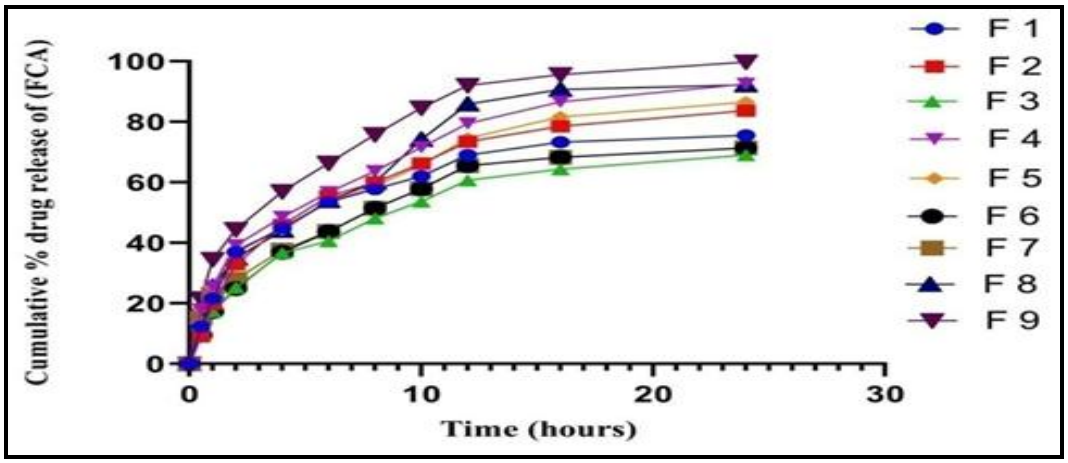

Figure No.1: *In vitro* cumulative percentage drug release profiles of PYZ of FCA nanosuspensions

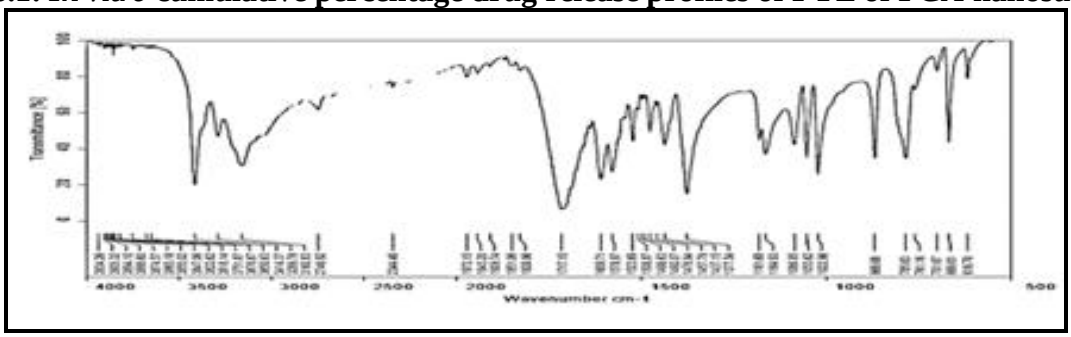

Figure No.2: FT-IR Spectrum of Pyrazinamide

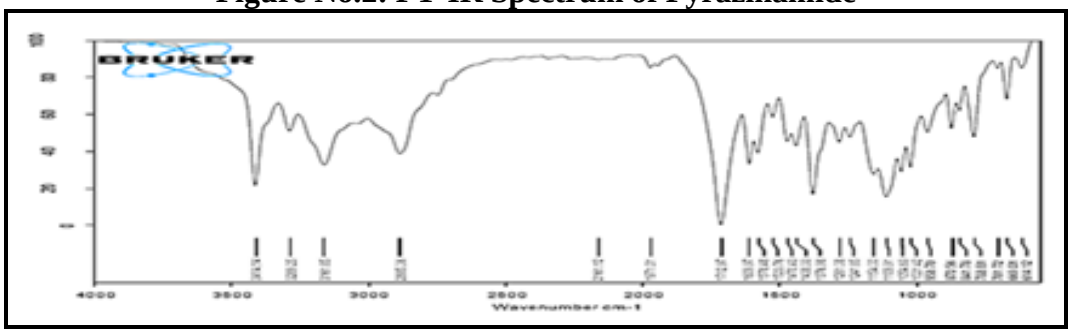

Figure No.3: FT-IR Spectrum of FCA9 formulation

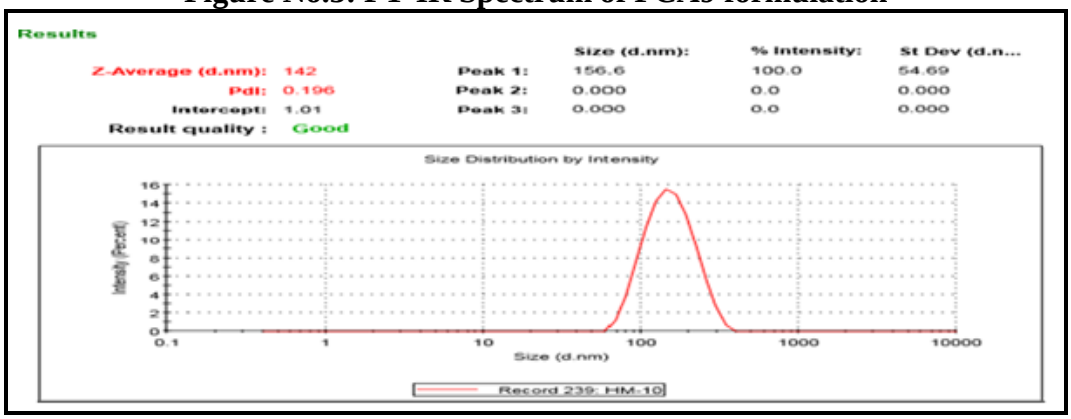

Figure No.4: A graph showing particle size distribution of FCA9 nanosuspensions

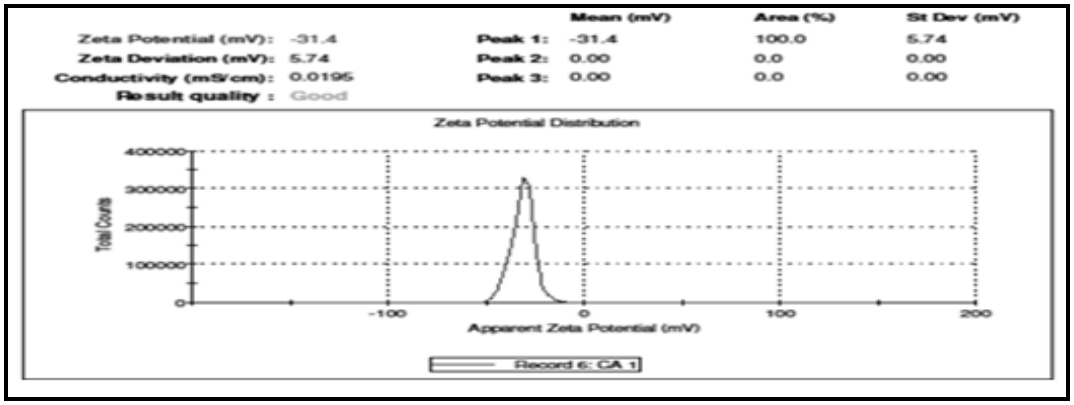

Figure No.5: Zeta potential (mV) graph showing optimized FCA9 formulation

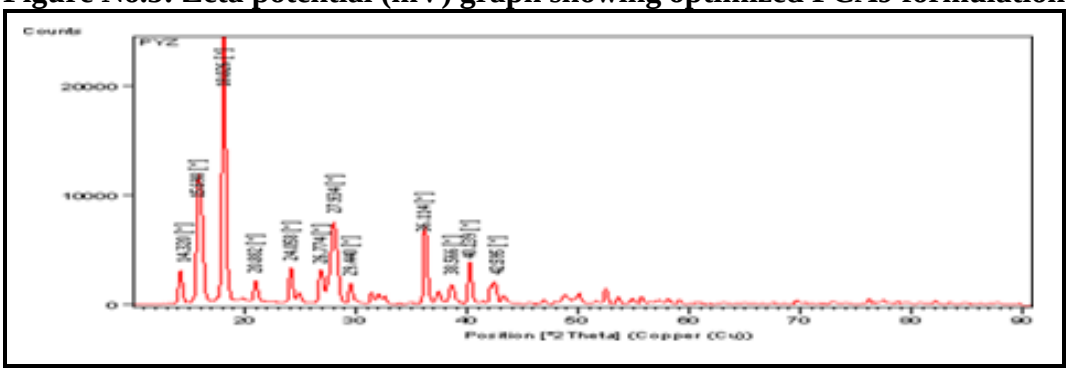

Figure No.6: XRD Data of Pyrazinamide

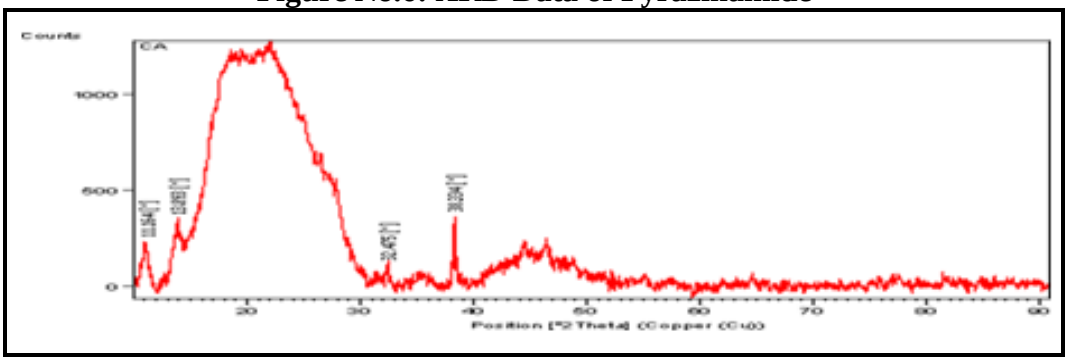

Figure No.7: XRD Data of Cellulose Acetate

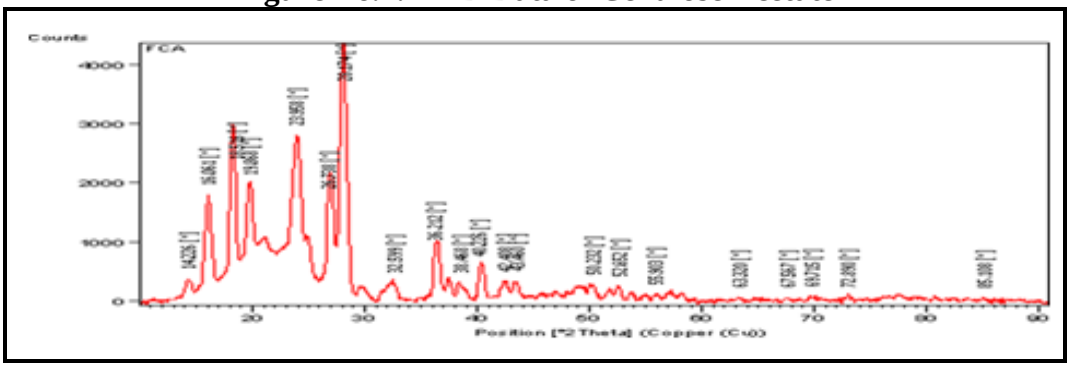

Figure No.8: Formulation with CA (FCA9)

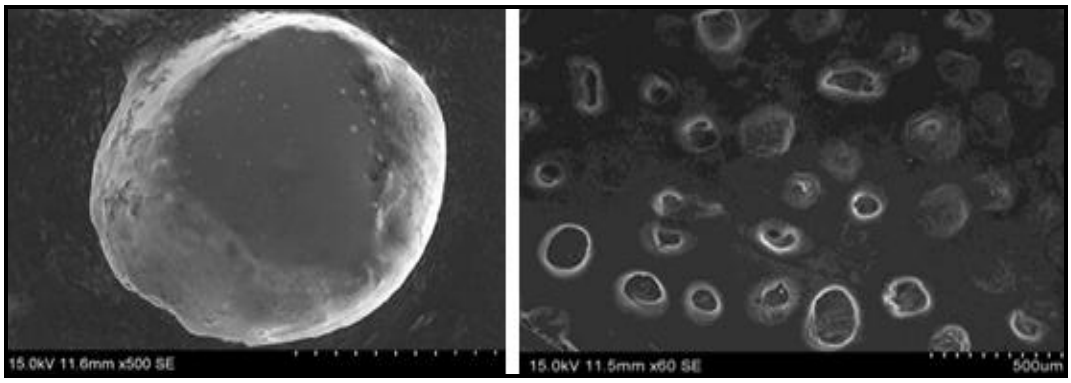

Figure No.9: Surface morphology of dendritic nanoparticles

CONCLUSION

Pyrazinamide-loaded nanosuspensions were successfully formulated using nanoprecipitation technique. Among all formulations, cellulose acetate-based nanosuspension FCA9 showed optimal particle size, high entrapment efficiency, sustained drug release, and good stability. The developed nanosuspension system offers a promising approach to improve oral bioavailability, reduce dosing frequency and minimize hepatotoxicity associated with conventional pyrazinamide therapy.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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