

**Research Article**

## **IN VITRO DISSOLUTION STUDIES OF NIMESULIDE LOADED CELLULOSE ACETATE HYDROGEN PHTHALATE NANOPARTICLES BY SALTING OUT TECHNIQUE**

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### **ABSTRACT**

The aim of this study is to prepare nimesulide loaded cellulose acetate hydrogen phthalate nanoparticles by salting out technique. In this study Cellulose acetate Hydrogen phthalate was taken as polymer. Nimesulide was selected as a model drug. This technique is suitable for drugs and polymers that are soluble in polar solvents such as acetone or ethanol. The effect of drug concentration and polymer concentration on nanoparticle size, shape, uniform size distribution and stability was studied. Nanoparticles were evaluated for particle size, zeta potential and particle size distribution. Size of the particle was measured by SEM. (Scanning electron microscope). Surface charge and stability of the resultant nanoparticles was determined by Zetasizer. Particle size distribution was determined by Photon Correlation Spectroscopy (PCS) with a Malvern Zetasizer Nano-ZS. The cellulose acetate hydrogen phthalate concentration and nimesulide concentration was varied from 5mg/ml to 10 mg/ml. The effect of drug and polymer concentrations on nanoparticle size, shape, particle size distribution was studied. Increased drug concentration has no impact on the particle size. The size of the particle was found to be decreased with increased polymer concentration. Increased polymer concentration has resulted in uniform particle size distribution. Higher the polymer concentrations and lower the drug concentrations resulted in uniform particle size distribution.

**Keywords:** Cellulose Acetate Hydrogen Phthalate (CAHP) Nimesulide (NM) Zeta potential Scanning Electron Microscope (SEM), Photon Correlation Spectroscopy (PCS), Zetasizer (ZS)

### **INTRODUCTION**

Nanoparticles are sub nanosized colloidal structures made up of synthetic and semisynthetic polymers. Several methods exist for the preparation of nanoparticles from biodegradable polymers (Hoffman *et al.*, 1983). These include: emulsification solvent evaporation, monomer emulsion polymerization, salting out, and nanoprecipitation. Depending on the preparation method drugs or antigens can either be entrapped in the polymer matrix, encapsulated in a liquid core, surrounded by a shell-like polymer membrane, or bound to the particle surface by adsorption (Reddy *et al.*, 2004). For drug loading of nanoparticles, three major strategies can be employed: (1) covalent attachment of the drug to the particle surface or to the polymer prior to preparation, (2) adsorption of the drug to a preformed carrier system, and (3) incorporation of the drug into the particle matrix during particle preparation (Anne *et al.*, 2006).

**Nanoparticle preparation using polymer precipitation methods:** In these hydrophobic polymer and a hydrophobic drug is dissolved in an organic solvent followed by its dispersion in a continuous aqueous phase in which polymer is insoluble. The external phase also contains stabilizer. Depending upon solvent miscibility techniques they are designated as solvent extraction/evaporation method (Soppimath *et al.*, 2001).

The polymer precipitation occurs as a consequence of the solvent extraction/evaporation at which can be brought by (Swarnali *et al.*, 2011).

- a) Increasing the solubility of the organic solvent in the external medium by adding an alcohol (i.e. isopropanol)
- b) By incorporating additional amount of water into the ultra emulsion
- c) By evaporation of organic solvent at room temperature or at accelerated temperature or by using vacuum (Le *et al.*, 2009).

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d) Using an organic solvent that is completely soluble in the continuous aqueous phase-nanoprecipitation.

**Salting out:** It is one of the most commonly adopted methods to prepare nanoparticles. The method involves the incorporation of saturated aqueous solution of polyvinylalcohol into an acetone solution of polymer under magnetic stirring to form an o/w emulsion. The process differs from nanoprecipitation technique as in the latter the polymeric solution is completely miscible with the external aqueous medium. But in salting out technique, the miscibility of both phases is prevented by the saturation of external aqueous phase with PVA. The precipitation of polymer occurs when sufficient amount of water is added to external phase to allow complete diffusion of acetone from internal phase into aqueous phase (Sergio *et al.*, 2004). This technique is suitable for drugs and polymers that are soluble in polar solvents such as acetone or ethanol (Dash *et al.*, 2008).

## MATERIALS AND METHODS

### Materials

Cellulose acetate hydrogen phthalate Supplied by SD-Fine chemicals

Acetone Supplied by SD-Fine chemicals

Polyvinylalcohol supplied by Hi-Chem laboratories

Nimesulide Supplied by sigma laboratories

Magnesium chloride Supplied by SD-Fine chemicals

### Methodology

Cellulose acetate Hydrogen phthalate polymer and nimesulide were dissolved in acetone. Polyvinylalcohol was dissolved in aqueous phase. Magnesium chloride was added to aqueous phase. The aqueous phase was added to organic phase under magnetic stirring at 700 rpm. Stirring was continued for 8 hrs. Finally water was added to precipitate nanoparticles.

The emulsion was centrifuged at 13,000 rpm for 30 minutes. Finally the particles were dried at room temperature. Experiments were performed by changing the Drug concentration and keeping all the remaining parameters constant. The same experiment was repeated by changing the polymer concentration and keeping the remaining parameters constant.

The effect of drug concentration and polymer concentration on nanoparticle size, shape, particles distribution was studied.

## RESULTS AND DISCUSSION

The obtained formulations were evaluated for size, Product yield, Drug content, Entrapment efficiency, Loading capacity and drug release (Esmaeili *et al.*, 2008).

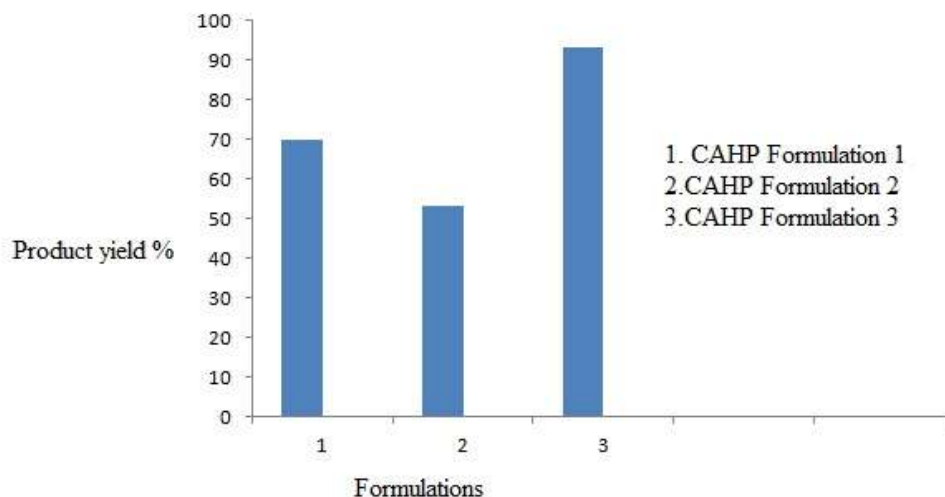


Figure 1: Comparison of Product yields of CAHP formulations

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**Percentage Yield:** The yields of the prepared nanoparticles were calculated. Nanoparticles dried at room temperature were weighed and the yield of nanoparticles was calculated using the formula:

Percent Yield = The amount of nanoparticles obtained (g) / The theoretical amount (g) x 100

The Product yields of Formulation 1, Formulation 2 and Formulation 3 prepared by salting out technique were found to be 70%, 53.3% and 93.3 % respectively. From the results it was found that product yield of Formulation 3 was more when compared with other two formulations.

### Fourier Transforms Infrared Spectroscopy (FT-IR)

Compatibility studies were performed using IR spectrophotometer. The IR spectrum of pure drug and formulations were studied. The characteristic absorption peaks of Nimesulide were obtained at wave numbers 3284.32  $\text{cm}^{-1}$ , 2929.6  $\text{cm}^{-1}$ , 1489.10  $\text{cm}^{-1}$ , 1340  $\text{cm}^{-1}$ , 1247  $\text{cm}^{-1}$ , 1564.32  $\text{cm}^{-1}$ . The character absorption peaks of CAHP were obtained at 3435  $\text{cm}^{-1}$ , 3414  $\text{cm}^{-1}$ , 1700  $\text{cm}^{-1}$ , 1271  $\text{cm}^{-1}$ . The peaks obtained in the spectra's of each formulation correlates with the peaks of drug spectrum. This indicates that the drug was compatible with the formulation components.

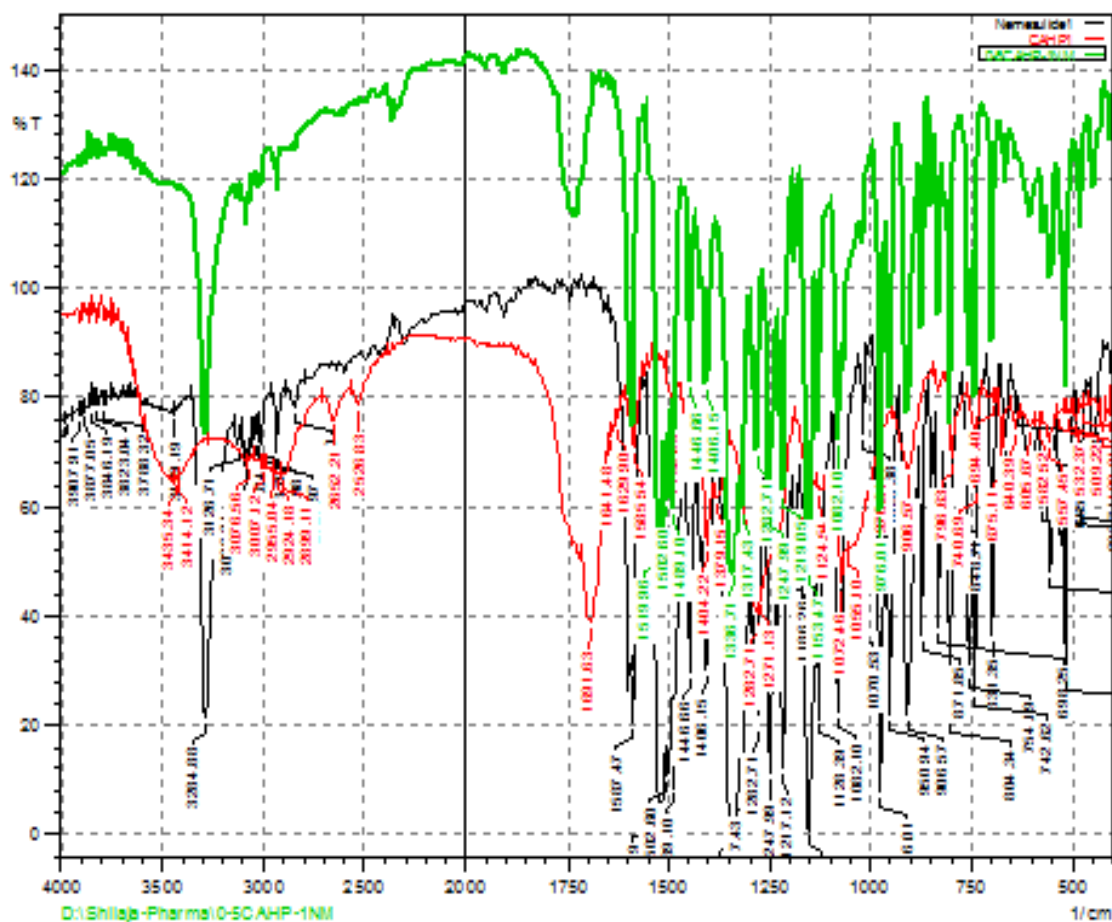
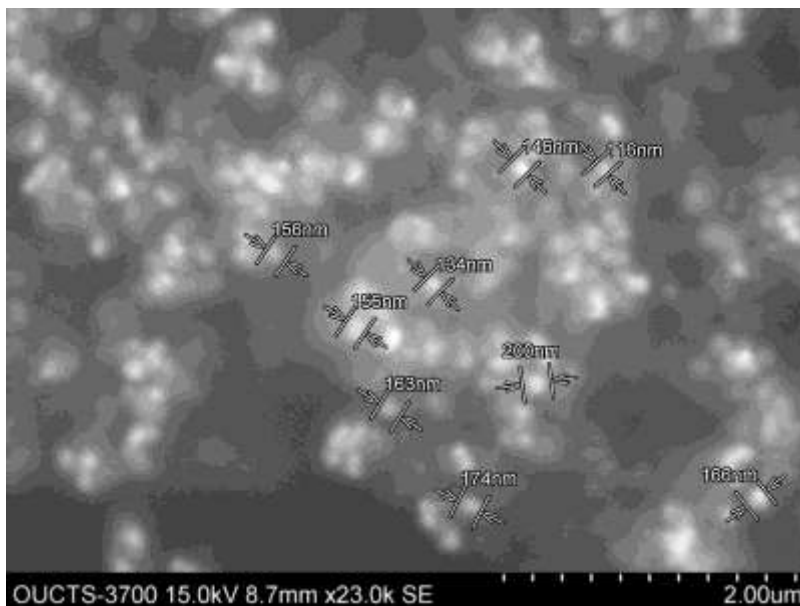


Figure 2: FTIR Spectra of Cellulose Acetate Hydrogen Phthalate nanoparticles

### Scanning Electron Microscopy (SEM)

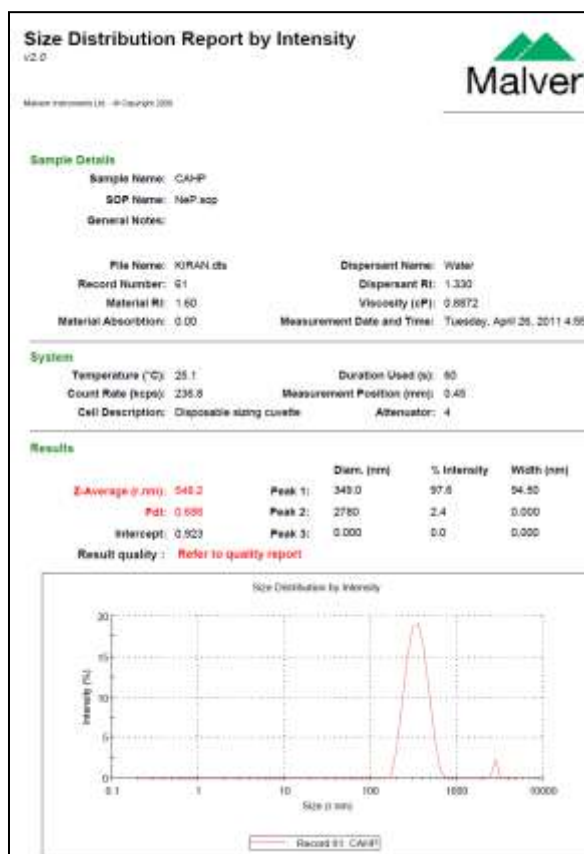
Morphological characterization of the nanoparticles was carried using scanning electron microscopy (SEM-S-3700N). For SEM the double – sided sticking tape, and coated with gold film (thickness 200nm) under the reduced pressure (0.001torr) fig No3.4. The sample for the SEM analysis was prepared by sprinkling the nanoparticles on one side of double adhesive stub. The nanoparticles were viewed at an accelerating voltage of 15-20kv.



**Figure 3: SEM Images of Cellulose Acetate Hydrogen Phthalate nanoparticles**

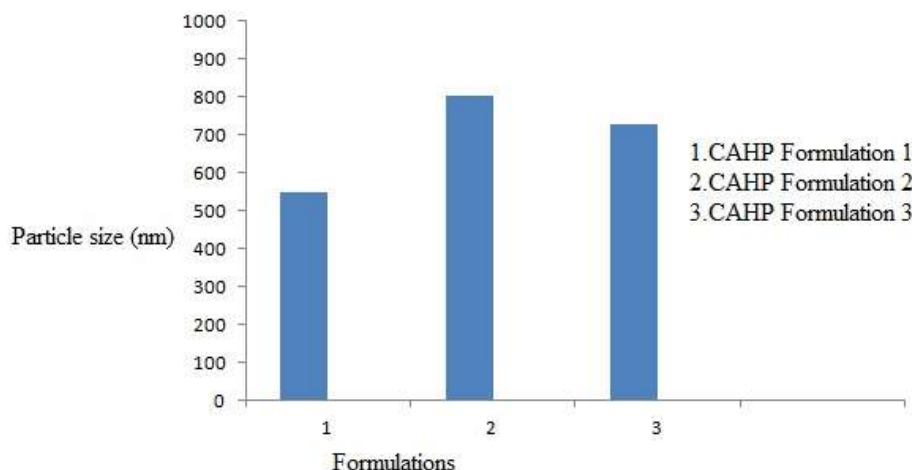
#### Particle Size Analysis

Mean particle size of the nanoparticles was determined by Photon Correlation Spectroscopy (PCS) with a Malvern Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK).



**Figure 4: Particle size distribution report of CAHP Formulation 1**

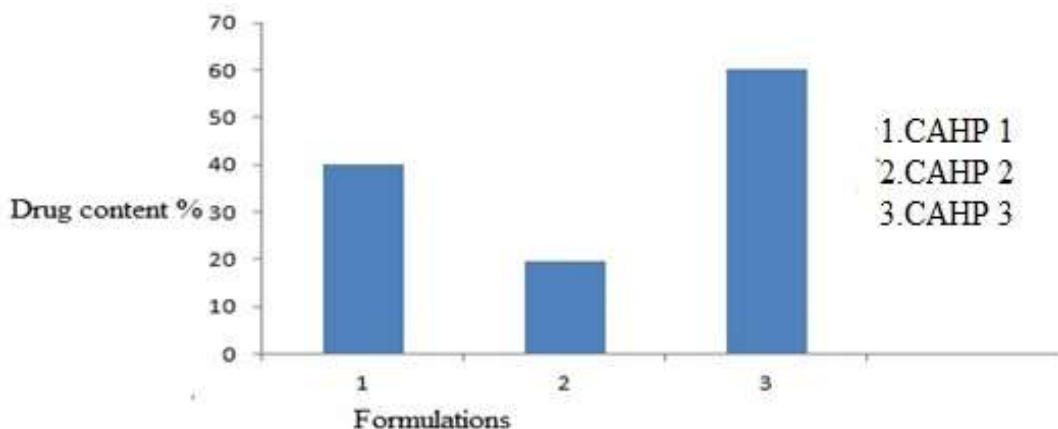
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**Figure 5: Comparison of particle sizes of CAHP formulations**

Measurements were realized in triplicate at a 90° angle at 25°C under suitable dilution conditions. Particle size distribution was expressed as mean diameter (nm)  $\pm$  standard deviation and polydispersity index. The Mean Particle diameters of Formulation 1, Formulation 2 and Formulation 3 prepared by salting out technique were found to be 548 nm and 804 nm and 727 nm respectively. From the results it was found Formulation 1 resulting particles in the nanorange when compared with other two formulations. This may be because of the molecular weight and concentration of the polymer which affect the size of the nanoparticles. Concentration of the polymer has opposite effects on nanoparticle size. Increased polymer concentration has increased the size of the nanoparticles. In Formulation 3 the concentration of the polymer was doubled. So particle size has been increased in comparison with Formulation 1. When compared Formulation 1 and 2 the particle size was increased in Formulation 2. In Formulation 2 the drug concentration was doubled. Greater the amount of drug results in a more viscous dispersed phase, which makes mutual dispersion of the phases more difficult and results in the origin of larger particles.

**Drug content:-** Drug loaded nanoparticles were weighed, then grinded to fine powder and dissolved in a solvent in which the drug is completely soluble. It was subjected to stirring around 700 rpm for 3 hrs. Amount of drug in the supernatant was determined by UV-Spectrophotometric method. The Drug contents of Formulation 1, Formulation 2 and Formulation 3 prepared by salting out technique were found to be 40.04%, 19.5% and 60.20% respectively. From the results it was found that drug content of Formulation 3 was more when compared with other two formulations.



**Figure 6: Comparison of Drug contents of CAHP Formulations**

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### Encapsulation Efficiency (EE)

For determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation was determined (w) by UV-spectrophotometry. A standard calibration curve of concentration versus absorbance was plotted for this purpose.

The amount of drug in supernatant was then subtracted from the total amount of drug added during the preparation (W). Effectively, (W-w) will give the amount of drug entrapped in the pellet. Then percentage entrapment is given (Govender *et al.*, 1999).

by

$$\frac{(W - w)}{W} \times 100$$

Loading capacity was calculated by the Following equation

$$\frac{(W - w)}{\text{Nanoparticle weight}} \times 100$$

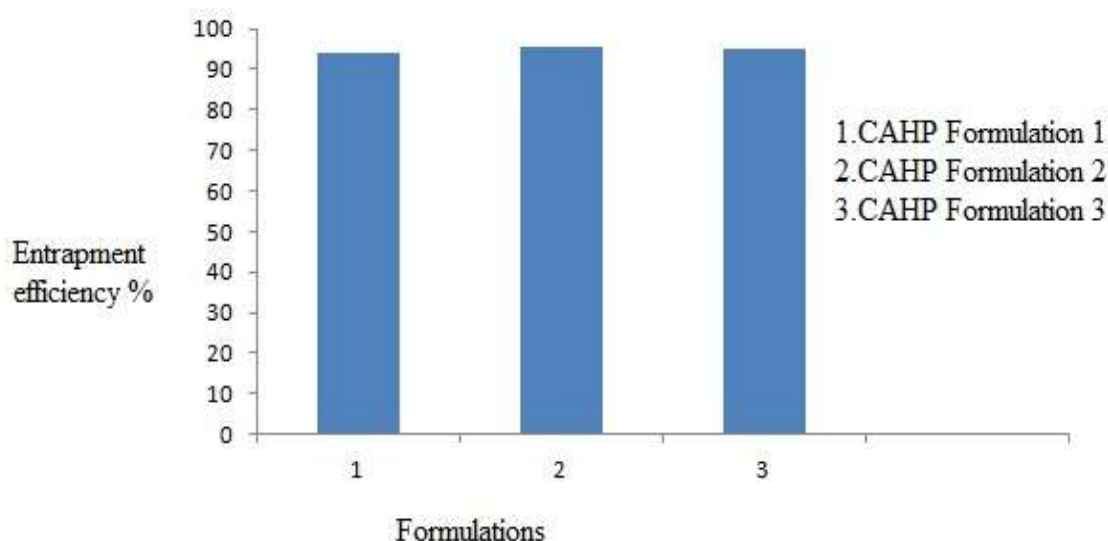
### Nanoparticle weight

Entrapment efficiencies of Formulation 1, Formulation 2 and Formulation 3 prepared by salting out technique were found to be 94%, 95.4 % and 95.25% respectively. From the results it was found that entrapment efficiency of Formulation 2 was more when compared with other two formulations.

The lower encapsulation efficiencies obtained with the smaller particles could be explained by the longer surface area of smaller droplets for a given volume of organic phase.

Hence, during the emulsification step, a more direct contact between internal and external phases occurred, resulting in a higher drug loss by diffusion towards the external medium.

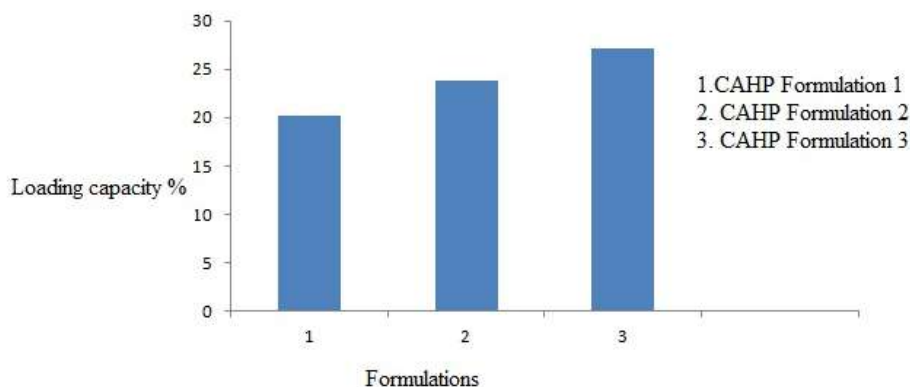
Loading capacities of Formulation 1, Formulation 2 and Formulation 3 prepared by salting out technique were found to be 20.21 % , 23.85 % and 27.2 % respectively. From the results it was found that loading capacity of Formulation 3 was more when compared with other two formulations.



**Figure 7: Comparison of entrapment efficiencies of CAHP formulations**



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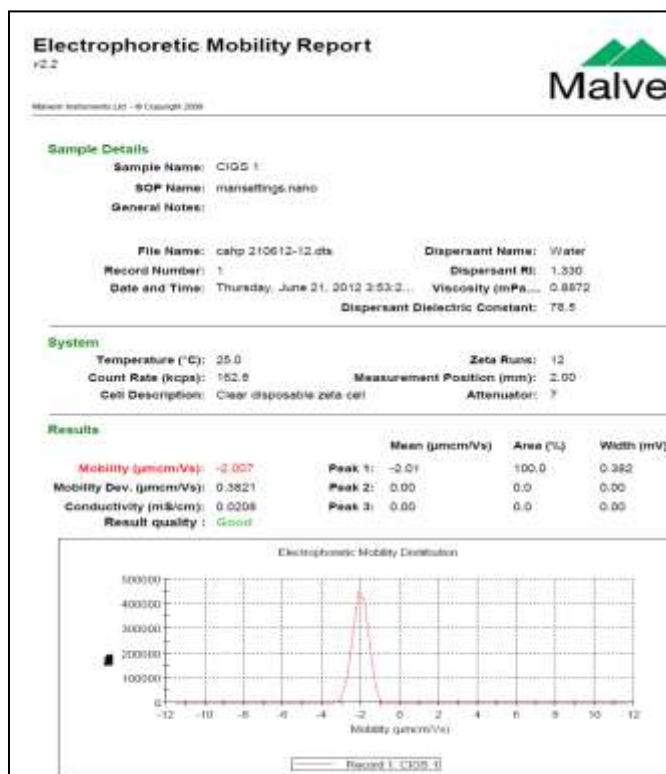


**Figure 8: Comparison of loading capacities of CAHP formulations**

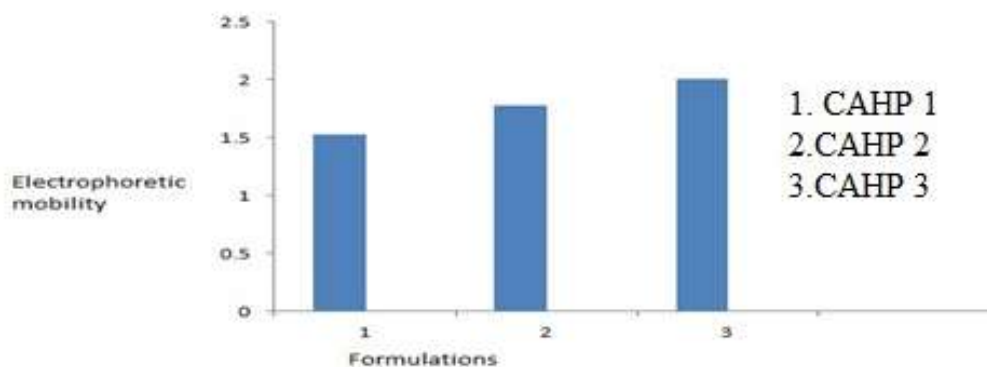
### Zeta Potential Measurement

Zeta potential of nanoparticle dispersions was measured in mV by Malvern Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK) in triplicate to determine the surface charge and the potential physical stability of the nanosystem. Zeta potential of nanoparticles was measured in aqueous dispersion. Measurements were realized in triplicate at a 120° angle at 25°C (Murakami *et al.*, 1999).

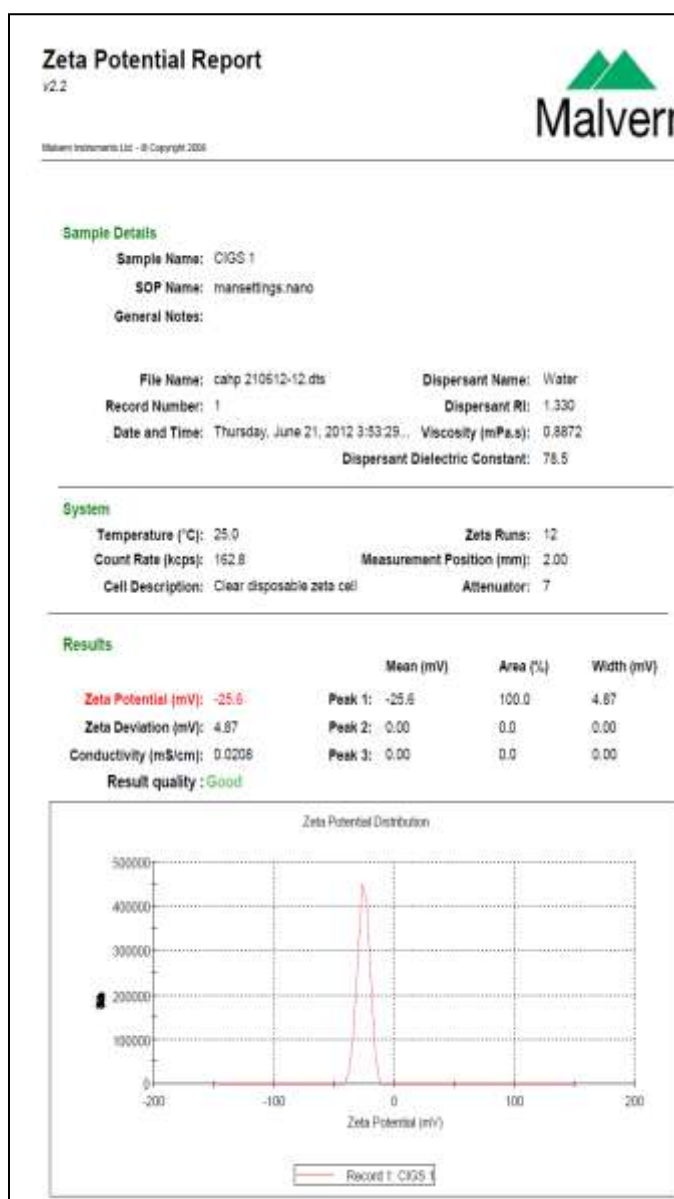
The Electrophoretic mobilities of Formulation 1, Formulation 2 and Formulation 3 prepared by salting out technique were found to be -1.529, -1.782 and -2.007 respectively. From the results it was found that Electrophoretic mobility value of Formulation 3 was higher when compared with other two formulations. Zetapotential values of Formulation 1, Formulation 2 and Formulation 3 prepared by salting out technique were found to be -19.8, -23.1 and -25.6 respectively. Zetapotential value of Formulation 3 was higher when compared with other two formulations indicating greater stability.



**Figure 9: Electrophoretic mobility report of CAHP Formulation 3**



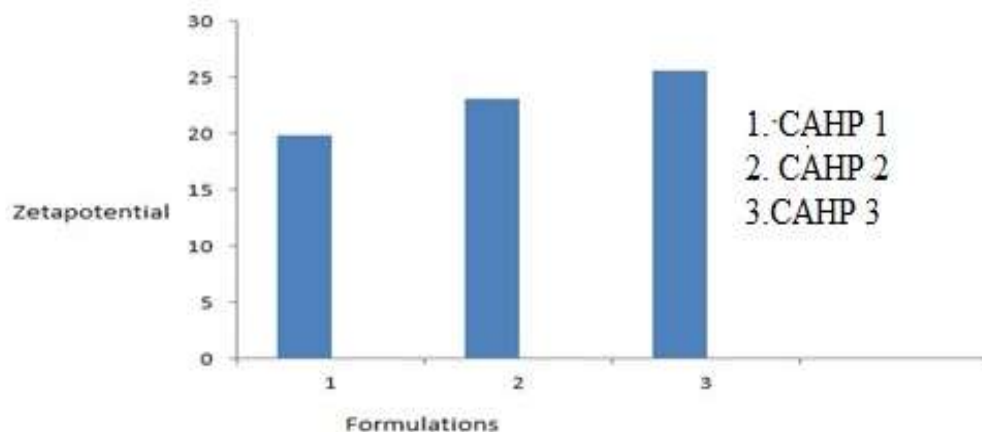
**Figure 10: Comparison of electrophoretic mobility values of CAHP formulations**



**Figure 11: Zetapotential report of CAHP Formulation 3**



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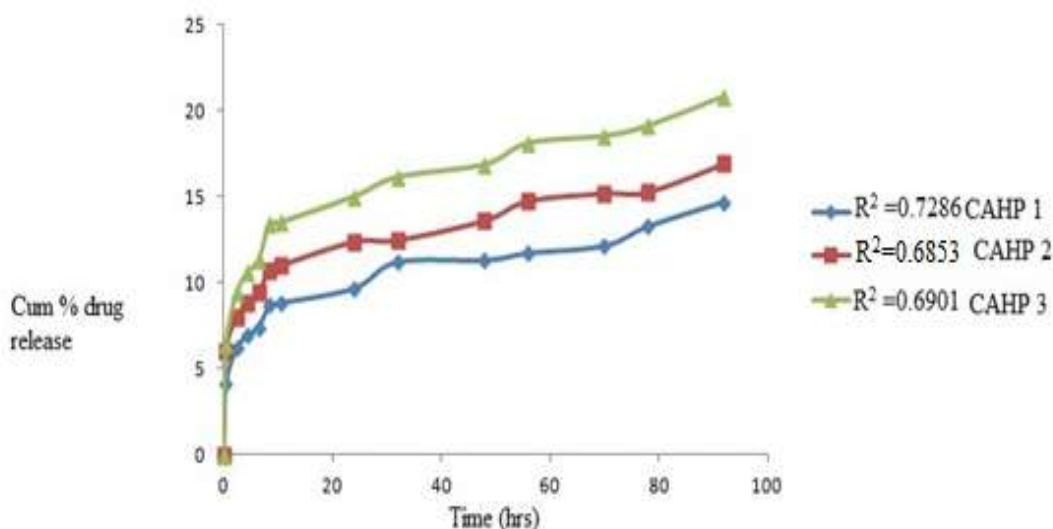


**Figure 12: Comparison of zeta potential values of CAHP formulations**

**Drug release studies:-** Drug release studies were performed by means of orbital shaker. Drug release from polymeric nanoparticles was determined as follows. A known amount of nanoparticles was transferred to a conical flask and 50 mL of the Phosphate buffer pH 7 was added to the tube. The temperature and rotation were adjusted to 37°C and 90 rpm, respectively.

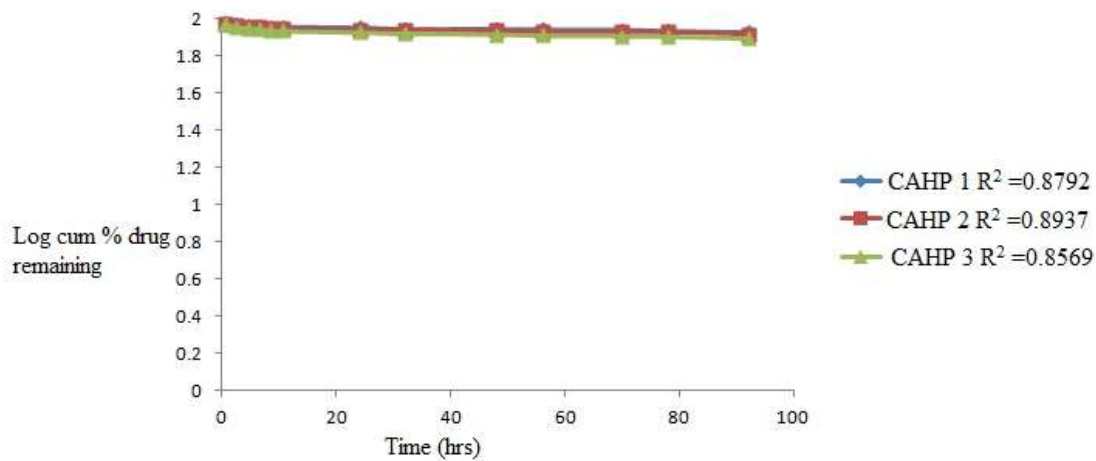
At predetermined time of 0.5, 2, 4, 6, 8, 10, 12, and 24, 36, 48 hours. 5mL of sample was removed and ultracentrifuged at  $15,000 \times r$  for 60 minutes, and 5mL of the supernatant were replaced by fresh medium. The samples were further analyzed using UV Spectrophotometer (David-Quintanar-Guerrero *et al.*, 1998). This experiment was continued for a period of 92 hrs.

In all CAHP Formulations the drug release was slow, extended over a period of several days . In a time period of 92 hrs 14.7 %, 16.9 % and 20.85 % of drug of has been released from CAHP Formulation 1, Formulation 2 and Formulation 3 respectively. When compared Drug release was more in Formulation 3. On comparison because of the higher drug content in CAHP Formulation 3 it was showing maximum drug release in a sustained manner.

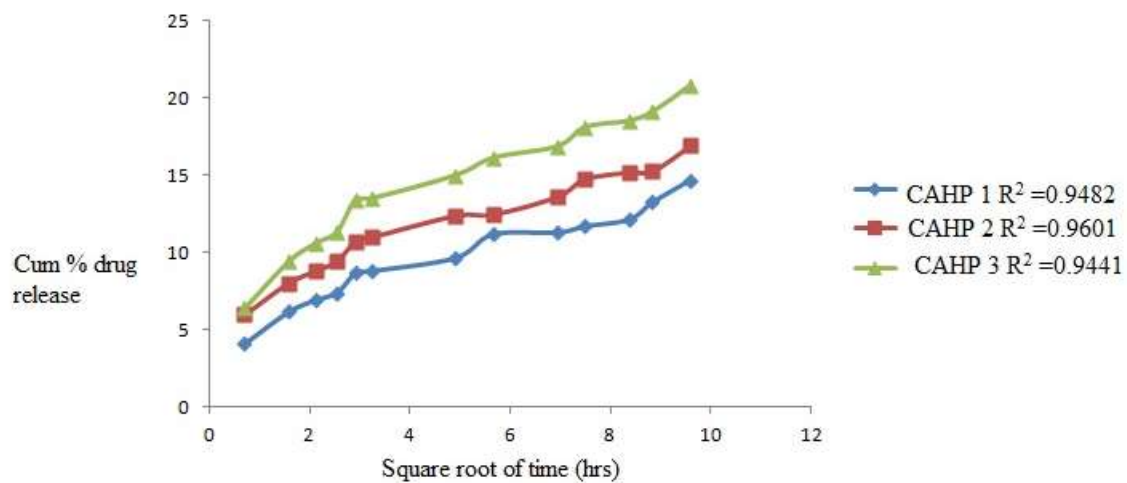


**Figure 13: Comparison of Invitro drug release profiles of CAHP formulations**

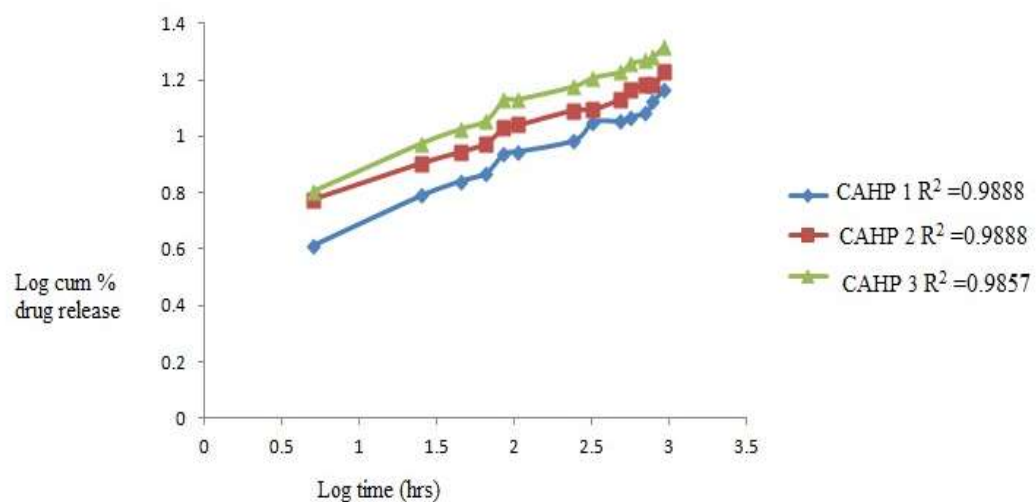
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**Figure 14: Comparison of First order release of CAHP formulations**



**Figure 15: Comparison of Higuchi square root time dependent plots of CAHP Formulations**



**Figure 16: Comparison of Peppas double log plots of CAHP Formulations**

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### Conclusion

By taking polymer and drug at equal concentrations, best nano formulations were obtained with mean diameter of 548 nm. By increasing the polymer concentration, product yield, drug content and loading capacity were also increased. By increasing the drug concentration, Entrapment efficiency was slightly increased. Entrapment efficiency was found to be 95.4 %. By observing Electrophoretic mobility and Zeta potential values of formulation 3 (-2.007,-25.6) it can be concluded that with increased polymer concentration, more stable formulation was obtained. When compared invitro drug release profiles for a period of 92 hrs, the drug release was more in Formulation 3 (20.85 %). It may be because of the higher drug content and loading capacity. From the results it can be concluded that Formulation 3 can be considered as best Formulation because of small particle size, good stability and sustained release effect.

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