RESEARCH PAPER

A Comparative Study of Drying Methodologies for Generation of Free Flowing Powder of Polymeric Nanodispersions

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ABSTRACT

Stability of nanoparticles is a big issue for pharmaceutical applications due to aggregation. In these studies, we have studied and evaluated the effect of various drying methodologies like adsorption drying, freeze drying and vacuum drying along with excipients like adsorbents, cryoprotectants. The nature of formulation, its composition, conditions of drying and concentration of excipients used has found major effect in the drying processes. Results of screenings and evaluations of dried products obtained has suggested that freeze dried Eudragit®S-100 encapsulated retinol acetate nanoparticles (2/1, w/w, Drug (D) / Eudragit®S-100(E)) with mannitol 5%w/v as cryoprotectant was the best product. It was due to better redispersity with good flow properties. It has comparatively lower particle size of 200.1 ±15.8 nm, polydispersity index 0.446 ± 0.11, zeta potential -10.0 ± 0.39 mev and higher drug content 83.62 ± 0.5 %.

Keywords: Nanoparticles, Adsorption Drying, Freeze Drying, Vacuum Drying, Adsorbents, Cryoprotectants, Stability, Screenings, Evaluations.

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1. Introduction

Nanoparticles in dispersion form are vulnerable to instability problems like aggregation with increase in particle size and loss of drug from nanoparticles over a period of time. The objective of these studies was to evaluate and investigate the efficiency of various drying technologies on the transformation of polymer encapsulated drug aqueous nanodispersion into free flowing, stable, dried powder. These dried products obtained can be used in solid dosage forms like powder, tablets and capsules. During drying processes; water from materials / products is removed which improves their properties including stability. Hence drying is a significant process in the pharmaceutical and food industries to have quality products with longer shelf life. (Remington, 1995[1])

Adsorption is a surface phenomenon, whereby certain molecules of a fluid are preferentially attracted towards and attached to the surface of a solid. Such porous solids with very high specific surface areas which ranges for 300 to1000 m^2/gm are called as adsorbents. Adsorbed component of the fluid is known as an adsorbate. (Shyam B.*et al*. 2004[2]) For this labscale technique involves use of various commercially adsorbents like carbopol®71G, neusilin®US-2 and others. Use of adsorbents, embeds nanoparticles dispersion system into porous swellable system reducing moisture content and giving free flow powder after drying. Process involves spatulation mixing of suitable quantity of adsorbents with nanoparticles solution. Industrial adsorption process is important for dehydration of gaseous mixtures and liquid mixtures by the use of activated alumina as adsorbents. It involves process of selective adsorption at higher pressure and desorption process at lower pressure or under vacuum along with equilibrium and transport. (R. H. Ottawil *et al.*1983[3])

Vacuum combined with heat, is employed for removing bulk and absorbed water (or other solvents) from products. Mechanisms involved are shrinkage of particles with increasing void formation with drying. High degrees of dryness can be attained at relatively low temperatures. In this process pressure, temperature, cryoprotectant type and concentration are optimized to get fine quality products. Instrumentation consists of drying oven with heating system, vacuum pump assembly and process controls system. It gives high quality product with less energy consumption during drying of thermolabile substances or formulations. (V.Kutovoy *et al.* 2004[4])

Lyophilisation (freeze drying) is widely used process for pharmaceuticals and biological
mainly of solutions and suspensions to improve stability of products. In this process; removal of water from sample occurs by vacuum desorption or sublimation process. During freeze drying cryoprotectants such as sucrose, mannitol and dextrose are added if necessary to protect the sample from stresses for retention of homogeneity. Process of freeze drying involves freezing which is cooling the material until completely frozen. Primary drying involves sublimation of ice by reducing pressure in the chamber and providing heat to the product. Secondary drying involves desorption of residual moisture from the product. (Wasim A. *et al.* 2006[5]) Primary role of the adsorbent or cryoprotectant is to prevent irreversible coalescences during drying, thereby nanoparticles / products aggregates can readily re-disperse in an aqueous environment hence retaining their properties with therapeutic functions. (C.Schwarz *et al.*1997[6]*)*
In these experiment studies different industrially applicable drying methods like adsorption drying, freeze drying, vacuum drying with adsorbents and cryoprotectants were screened for optimized nanodispersions. Obtained products were evaluated for different *in-process* characterizations tests, flow properties evaluations and physiochemical evaluations tests.

2 Experimental

2.1 Materials and methods

Various nanoparticles like hydroxyl propyl methyl cellulose K-100M (H) encapsulated retinol acetate (Drug-D) nanoparticles (1:1,w/w) , polylactic coglycolic acid 50:50 (PLGA 50:50, (P)) encapsulated retinol acetate nanoparticles (1/1, w/w Drug / PLGA50:50) and Eudragit®S-100 encapsulated retinol acetate nanoparticles (2/1, w/w, Drug / Eudragit®S-100 (E)) obtained by modified rapid expansion of supercritical solution process(RESS) with mixing sonication at optimized conditions. All optimized nanoparticles were characterized for *in-process* characterizations and physiochemical tests.(C.B.Fernandes *et al.*2013[22]**)**
Adsorbents like carbopol®71G, dextrose (D-Glucose), starch (Wheat-Starch), pealitol®SD-200, microcrystalline cellulose (Avicel-102), neusilin®US-2 and cryoprotectants like mannitol (Pealitol®SD-200), sucrose **(**D(+)-Saccharose, Sugar**),** lactose (Pharmatose®200M Monohydrate) all were purchased from S. D. Fine Chemicals Ltd., Mumbai India. Double distilled water (DDW) obtained from Millipore Systems USA was used throughout these studies. 0.45 µm filters purchased from Millipore system USA.

2.2 Processing and evaluation of different drying techniques

Cryoprotectants and adsorbents were selected depending upon literature reports, availability and previous experimental trials. (S. Bozdag *et al.*2005[7]), (Qing-guo Hu *et al.*2006[8])
Most of the excipients selected were included in the FDA Inactive Ingredients Database for oral, parental route of applications and were GRAS listed. (Ridhi D. *et al*.2012[9])
Different drying methodologies such as adsorption drying, vacuum drying, freeze drying were screened for above mentioned all optimized nanoparticles obtained by modified RESS process with sonication and mixing at optimized process pressure of 290 bars, temperature 75°C and collection time of 45 minutes in double distilled water of 25 ml volume. These all dried nanoparticles products with optimized products were evaluated and characterized for particle size, polydispersity index (P.I.), drug content, zeta potential and physiochemical characterizations by thermoanalalytical Differential Spectroscopic Calorimetry (DSC) studies, crystal nature by X-ray diffraction studies and composition by Fourier Transform Infrared (FTIR) studies. (Filkova .*et al.*1995[10]) These all optimized nanoparticles were in the form of colloidal viscous dispersions.

2.3 Adsorption drying procedure

In this process; spatulation mixing of each nanodispersion was performed with sufficient amount of various adsorbents like neusilin®US-2, carbopol®71G, starch and others separately so as to get dried powder. Screenings of adsorbents were done depending upon redispersibility of dried nanodispersion in DD water and observing flow properties of obtained dried powder.

2.4. Freeze drying procedure

Freeze drying was performed using Labanco freeze dryer USA (WW-3339-97). Various
cryoprotectants such as mannitol, sucrose, lactose were screened in a concentration range of 1, 2 and 5 % w/v each for redispersibility of dried nanodispersion in double distilled water and observing flow properties. Process time, freezing temperature, pressure were set and monitored through the inbuilt Programmable Logic

Controller (PLC) system, for the various processes. Samples were kept in the flasks in a freeze drying chiller assembly. Initial chilling process was performed to remove the water vapours and to freeze the sample by evaporative cooling and freezing. This was performed by primary and secondary drying at freezing temperature of -40ºC for 12 hours and -15ºC for 6 hours respectively. Lyophilisation process was performed by application of vacuum and chilling. And allowed to sublimate ice into water vapour; this was kept for 20 hours till the complete removal of water from the flask. When temperature of flasks came to room temperature by stopping external condensation then flasks were removed. Each of the lyophilized material (in the form of powder) was removed from flasks.(Lee M.K. *et al*.2009 [11])

2.5 Vacuum drying procedure for experimentation

Vacuum drying was carried out using vacuum dryer OVA-11 from Jeio Tech Ltd., Korea. Cryoprotectants mainly; mannitol, sucrose, lactose were screened in the concentration range of 0.5, 1 and 1.5 % w/v each. Process time, temperature, pressure were set and monitored through the inbuilt Programmable Logic Controller (PLC) for the various processes. Samples were kept in trays inside vacuum drying oven. Vacuum drying was conducted at optimized temperature of 25ºC and pressure of -0.08 MPa for 8 hours by keeping in glass petric plates. After drying samples were removed. (Lin, Tein M *et al.*1998[12])

2.6 Evaluation of dry form of polymeric drug nanoparticles

Each resultant polymeric drug nanoparticles in the dried powder form were studied for various physicochemical parameters and *in-process* parameters along with flow properties, to check efficiency of drying products. These all tests procedures are described in details below. (Sujung Kim *et al.*2004[13])

2.6.1 Particle size and polydispersity index (P.I.) measurement

The particle size distributions were measured by redispersing dried sample in double distilled water (10 mg in 10 ml) by using dynamic light scattering ZetaSizer Nano ZS, Malvern Instruments, Worcestershire, U.K.

2.6.2 Zeta potential measurement

Was done in triplicate by dispersing dried nanoparticles in 0.45 μm filtered double distilled water (10 mg in 10 ml) using Malvern Zetasizer ZS, UK.

2.6.3 Scanning electron microscopy (SEM)

The morphological examination of the nanoparticles dispersion was performed by scanning electron microscopy (Environmental-SEM).The liquid sample (redispersed 10 mg in 10 ml DDW) was spread on a double adhesive tape previously adhered to SEM aluminium stubs and then sputtered with platinum in an ion sputter for 300 seconds. Images were collected at an acceleration voltage of 15 kV using a back scattered electron detector on Joel JSM®- 6360 SEM, Japan at 25 ± 2°C.

2.6.4 Drug content (%) determination

Dried nanoparticles (2.5 mg) were dissolved in 10 ml of ethanol /water (50/50 v/v) in amber coloured 10ml volume flask. This solution was further diluted by the same vehicle and analyzed by UV-Visible double beam (V-670, Jasco, Japan) spectrophotometrically at 326 nm. By comparing with the standard graph of retinol acetate drug content (%) was determined.

2.6.5 Angle of repose (°)

The angle of repose was measured by passing the powder from a funnel fixed to a stand and measuring the radius of the heap of powder formed (Remington *et al* .1995[1]).

It is determined by,

$$\tan\theta = \left[\frac{\text{Height from the tip of the funnel to the base of the heap (h)}}{\text{Radius of the cone (r)}}\right]$$

Angle of repose (θ) = $\tan^{-1}$ (h /r)

2.6.6 Bulk density

Bulk density is defined as a ratio of weight of powder mass to volume occupied by the same when it is poured. Determined by taking 10 g of dried powder in bulk-tapped densitometer from Scientific Ltd., India and by measuring its volume. The bulk density was calculated in g/ml by the formula,

Bulk density (ρo) = Mass of powder taken (M) / Apparent unstirred volume (Vo)

2.6.7 Tapped density

Tapped density is defined as a ratio of a weight of a powder to a volume occupied by the same after sufficient tapping has been done. Determined by taking 10 g of dried powder in

bulk-tapped densitometer from Scientific Ltd., India.
Tapped density was calculated in g/ml by the formula,

Tapped density (ρt) = Weight of sample powder taken (M) / Tapped Volume (Vf)

2.6.8 Compressibility index (% C.I.)

Compressibility index is defined as a 100 times the ratio of the difference between tapped density and bulk density to tapped density. Bulk density and tapped density was measured and compressibility index (Carr's Index) was calculated using the formula,

C.I = Tapped density (ρt) – Bulk density (ρo) / Tapped density (ρt) X 100

2.6.9 Water content (Loss on drying % w/w) Determined automatically by keeping 0.5 gm of sample in pan of the moisture analyzer (HB83 S) from Meter Toledo, India for 45 seconds.

2.6.10 Determination of contact angle (º)

Dynamic contact angle between powder compacts and water was measured by the sessile drop method by Kruss DSA100 instrument with drop shape analysis Kruss DSA1 software both are from Kruss Ltd., Hamburg, Germany. The static contact angle between powder sample 10 µg and water was measured in triplicate.

2.6.11 Fourier Transform Infrared (FTIR) spectroscopy study

1mg of dried nanoparticles powder was triturated with 0.1g of potassium bromide (KBr).
The pellet was prepared in a KBr press (model-15) from Scientific Ltd., India and FTIR spectrum was recorded in the range between 4000 and 400 cm^{-1}, for 4 mm/s with a resolution of 2 cm^{-1} on FTIR Spectrum RXI spectrometer.(Model-LM 500) Perkin Elmer Ltd., Germany. KBr pellet was used as a reference sample.

2.6.12 Differential scanning calorimetric (DSC) study

DSC thermoanalytical measurements were carried of dried nanoparticles on the Themofischer PVT. Ltd.,(DSCPT-100) India instrument. DSC thermogram scans were recorded with 5 mg of sample with nitrogen flow of 20 ml/minute at a scanning rate of 10°C /minute from temperature of 35°C to 310°C. An empty standard aluminium pan was used as reference.

2.6.13 X -ray Diffraction (XRD) studies

It was carried out for dried nanoparticles powder 3 to 5 mg, in order to clearly elucidate solid state of nanoparticles, using a Bruker D-8 advanced diffractometer made by Bruker Biosciences Ltd., Spain. The X-ray diffractogram pattern was scanned with diffraction angle 2Ø range of 20 to 80° at a scan rate of 0.02°/min. and angle with a step angle 0.02° and count time 1 second at a constant room temperature 25°C.

3.0 Results and discussion

Experiments were carried out as screening of suitable drying method for optimized polymer encapsulated nanoparticles. Each dried product obtained was evaluated and characterized by the tests enlisted above to observe the *in-process* characteristics with flow properties; results are given below.

3.1 Adsorption drying

Screening of adsorbents for all three polymeric nanoparticles systems were done by observing dried products for redispersity in DDW and flow property. From the experiments; it was observed that the adsorbents like pealitol®SD-200 and neusilin®US-2 could contribute to the adsorption drying process of various polymer encapsulated drug polymeric nanoparticles exhibiting fair to possible and good flow of dried products. Also redispersibility of pealitol®SD-200 and neusilin®US-2 adsorbent dried nanoparticles in double distilled water were good with somewhat free flow nature. Among the two adsorbents, neusilin®US-2 was found good in terms of flowability and the redispersibility. Neusilin®US-2 is an amorphous, synthetic form of magnesium aluminum metasilicate. It is being a fine ultra light granule, due to its large surface area and porous nature, adsorbs high loads of water and could result into the free flowing powder. Due to large surface area with porous nature of neusilin®US-2 act synergistically allowing the powder to swell and absorb many times it weight in water. This was also reported that its high adsorption capacities are helpful for formulating lipophilic oils or drug substances and useful as an anti-caking agent**.** Thus neusilin®US-2 acted as a carrier, filler and stabilizer. (Chakraborty *et al.* 2010[14]) Selective observations of the best output adsorb dried products characterizations results are given in the table 1.

3.2 Freeze drying

Screening of suitable cryoprotectant at optimized freeze drying conditions for all three polymers encapsulated nanoparticles systems using freeze dryer were done. And observed product for the redispersity and flow property. Mannitol 5% w/v was found to be the effective cryoprotectant showing good redispersity and good free flow of obtained dried product for all three nanoparticulate systems. During drying of nanodispersions, the ceaseless motion of polymeric chains and the subsequent steric repulsion mechanism became inactive. Removal of water between nanoparticles induced entanglement of polymeric chains and the entanglement or particle fusion resulted in irreversible aggregation. Fast drying has provided better conditions for the prevention of chain entanglement; as noted by S.R. Schaffazick *et al.*2003.[15] For all nanoparticles dispersion mannitol 5% w/v acted as the best cryoprotectant agent giving lower particle size with narrow particle size distribution due to excellent protective, carrier and tonicity adjusting property with moisture adsorbing nature. Similar results were noted by Yu L.Milton *et al.*1999[16] regarding mannitol as a cryoprotectant. Observations of the optimized freeze dried products characterizations are given in the table no.1.Figure 1.0 have represented the comparative particle size and P.I. of freeze dried products with optimized nanoparticles before drying. This has also shown that dried nanoparticles of Eudragit®S-100 (2D/E) encapsulated retinol acetate nanoparticles with 5% w/v mannitol have lower particle size with narrow polydispersity index (P.I.).

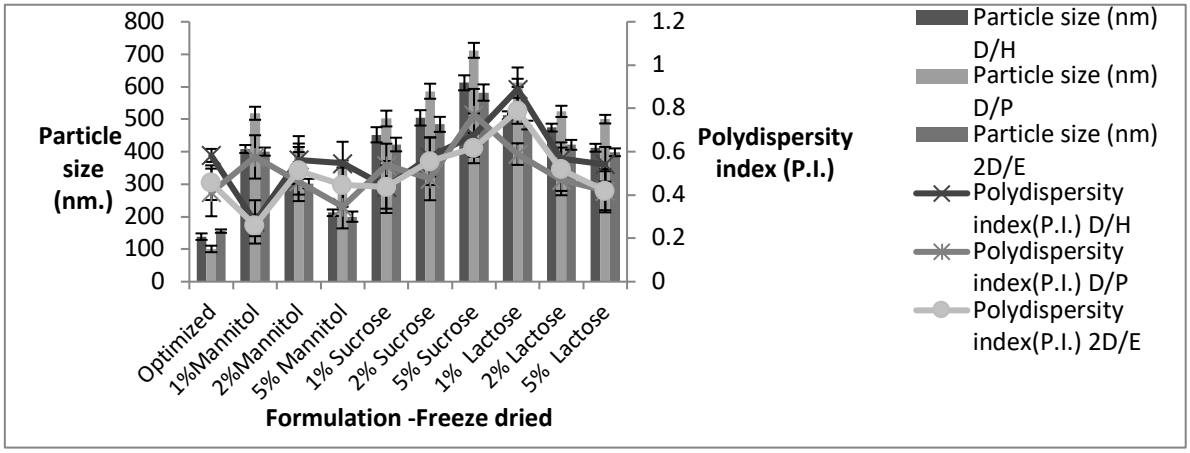

Figure 1.0: Freeze dried products with optimized nanoparticles particle size and P.I.

3.3 Vacuum drying

Screening of suitable cryoprotectant system at optimized vacuum drying conditions for all three polymers encapsulated nanoparticles systems using vacuum dryer were done successfully. Vacuum dried polymer encapsulated polymer encapsulated retinol acetate nanoparticles with different cryoprotectants were tested for redispersity in double distilled water and observed for particle size, polydispersity index with flow property manually.

Graphs (figure:2) and observations showed that lactose 1.5% w/v was found to be the effective cryoprotectant giving good particle size and P.I. after redispersing. Observations of the optimized vacuum dried products characterizations are given in the table no.1. Limited aggregation due to heat and mass transfer parameters, changes in conditions like pressure, temperature, air flow, weight and volume changed during drying process caused, increase in particle size as compare to previous particle size similar observations were observed for C. Schwarz *et al.*1997.[6]

Lactose at higher concentration of 1.5%w/v acted as cryoprotectant and diluent. It also played an important role to increase plug size and aid cohesion during freeze drying process, similar observations were noted by Ridhi D. *et al*.2012[9] and Medeni *et al*.2001.[17] Figure 2.0 represented the comparative vacuum dried products with optimized nanoparticles particle size and P.I. This has shown that vacuum dried PLGA50:50 (D/P)

encapsulated retinol acetate nanoparticles with 1.5%w/v lactose with lower particle size and narrow P.I.

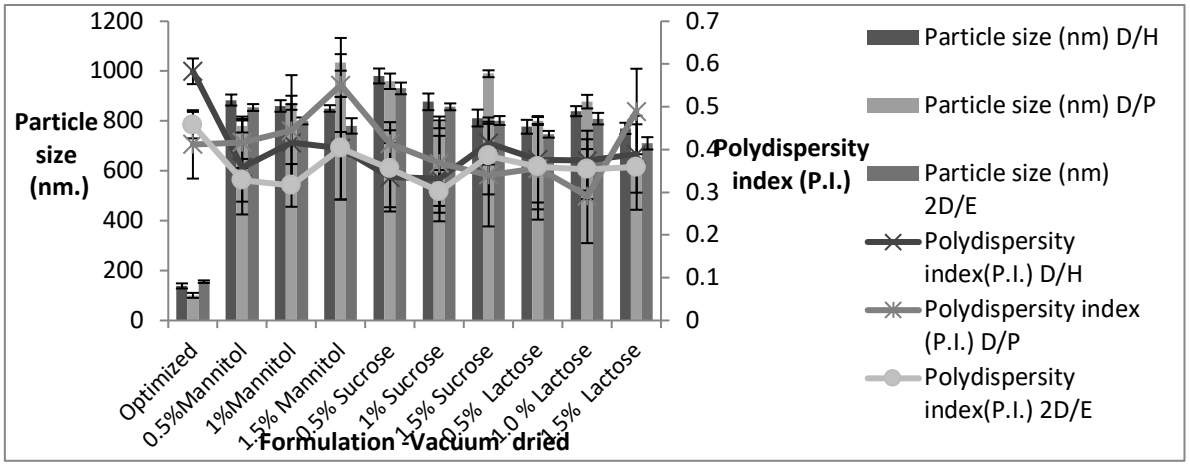

Figure 2: Vacuum dried products with optimized particles size and P.I.

3.4. Zeta potential

Comparative particle size, polydispersity index, zeta potential of optimized polymer encapsulated and each optimized dried nanoparticles system is given in the table no.1. It has shown that drug encapsulated by Eudragit®S-100 (2D/E) freeze dried nanoparticles with mannitol 5%w/v have relatively lower value of particle size, narrower polydispersity index with lower zeta potential value which was considered as a relatively stable dried product. For all neusilin®US-2 adsorb dried products, comparatively higher particle size, wider P.I. and higher zeta potential vales were observed. Reasons may be adsorption drying was a manual process, so due to adsorption and desorption of moisture aggregations were occurred. Obtained results were complied with Chakraborty S.*et al*.2010.

With decreasing vehicle from nanodispersion reduced zeta potential of dried product. Another reason for change in zeta potential after drying was the change in solvating shell at

the particle surface and adsorbent / cryoprotectant nanoparticles interaction. This has confirmed stabilizing effect of cryoprotectans / adsorbents during drying. Similar process results were obtained by W. L. J. Hinrichs *et al.*2006[18] for polymeric nanoparticles.

3.5 Scanning electron microscopic imaging

SEM analysis of redispersed dried nanoparticles were done in double distilled water 0.45 µm filtered. As shown in the figure 3, 4 and 5, nanoparticles were observed different in shapes, dispersion was found viscous. Particle size of redispersed all dried products were in the desired range for oral administration with major nanoparticles range around 1000 nm size, with some aggregates. During drying of the nanosuspension, dissolved polymers precipitated out of solution in added adsorbent/ cryoprotectants adsorbed onto the surface of nanoparticles. It was lead to increased size; these results were complied to experiments of Ridhi Dave *et al.*2012 and others.

Table 1.0: Particle size, P.I., zeta potential for dried nanoparticles

Formulation nanoparticles	Particle size (nm) with std .dev.	Polydispersity index(P.I.) with std .dev.	Zeta Potential (mev) with std .dev.	Drug Content % with std .dev.
HPMC K-100 M encapsulated drug (1/1,w/w)	138.6±10	0.583±0.03	-14.9±2	80 ± 3%.
Neusilin®US-2 adsorbed HPMC K-100M encapsulated drug	812.5 ± 45	0.671 ± 0.12	-13.8 ± 1.4	77.5±1.1
Freeze dried HPMC K-100M encapsulated retinol acetate with 5% w/v mannitol	212.1 ± 10.1	0.546 ± 0.1	-11.3 ± 1	78.72 ±1.2
Vacuum dried HPMC K-100 M encapsulated retinol acetate with 1.5% w/v lactose	770.5 ± 23.5	0.389 ± 0.09	-13.3 ± 1.1	79.1±0.6

PLGA50/50 encapsulated drug (1/1,w/w)	100.8 ± 10	0.412 ± 0.08	-17.2 ± 1.2	82±5%
Neusilin ®US-2 adsorbed drug PLGA50/50	671.5 ± 23.2	0.589 ± 0.11	-14.4 ± 1.12	79.52±1
Freeze dried drug PLGA50/50 with mannitol 5%w/v	362.1 ± 11.0	0.346± 0.1	-11.01±1.1	80.12 ±1
Vacuum dried drug / PLGA 50/50 with 1.5% w/v lactose	620.0 ±17.8	0.489 ± 0.1	-10.2 ± 0.47	80.2 ±0.72
Eudragit®S-100(1/1,w/w) encapsulated drug	155.6 ±5.2	0.457±0.031	-16.3±1.42	85±3.2%
Neusilin®US-2 adsorbed drug Eudragit®S-100	871.1± 34.6	0.651± 0.12	-13.2 ± 1.12	81.92±1.02
Freeze dried drug/ Eudragit®-S -100 with 5% w/v mannitol	200.1 ±15.8	0.446 ±0.11	-10.0 ±0.39	83.62 ±0.5
Vacuum dried drug/ Eudragit®-S-100 with 1.5%w/v lactose	710.5±24.7	0.359 ±0.1	-10.10 ±0.50	82.64 ±1.15

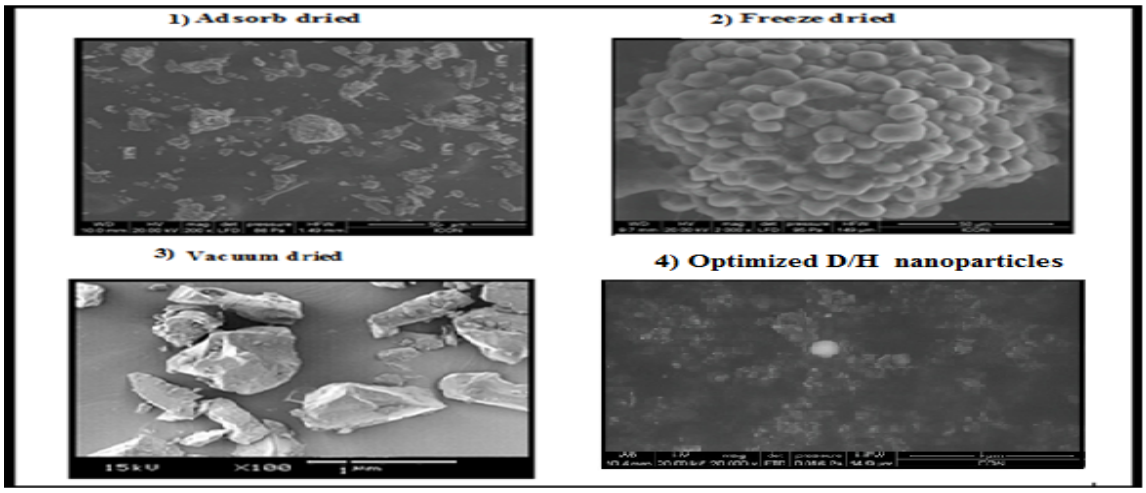

Figure 3: SEM images of dried nanoparticles (Drug / HPMC K-100M)

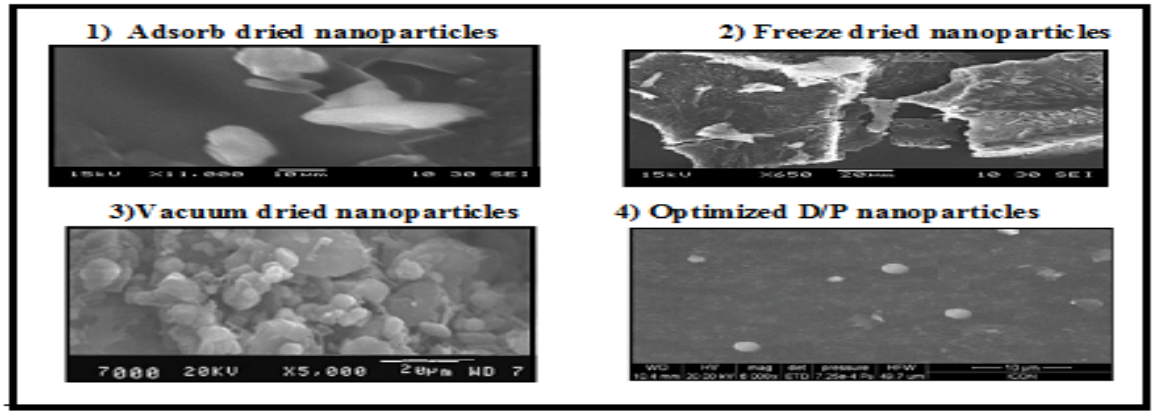

Figure 4: SEM images of dried nanoparticles (Drug / PLGA50:50)

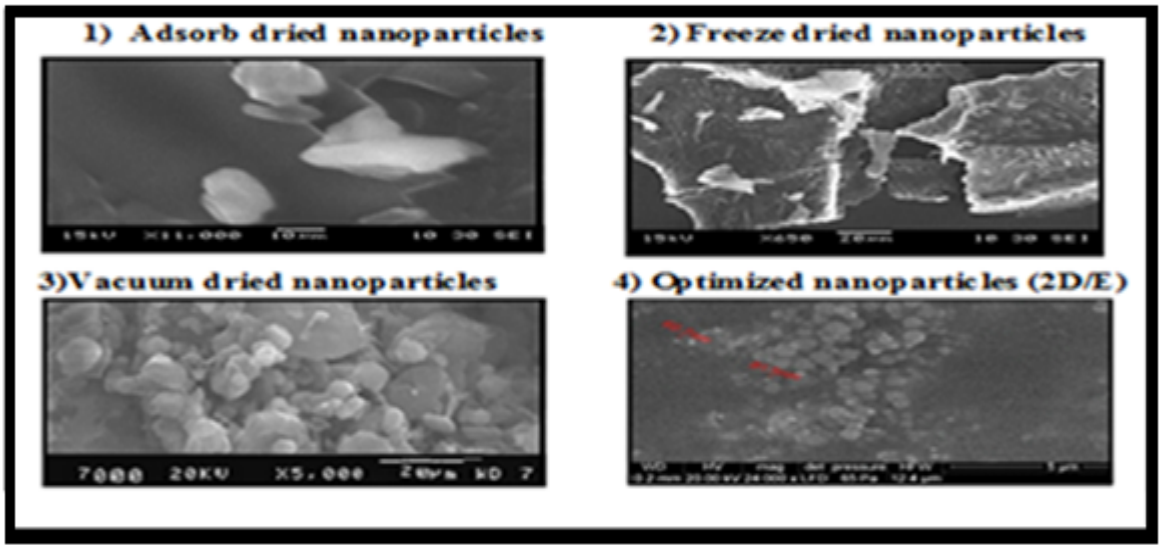

Figure 5: SEM images of dried nanoparticles (2D/E)

3.6 Characterization of dried products Bulk density, tapped density, Hausner's ratio and Carr's index, contact angle, moisture content, drug content were determined by the methods described to evaluate the efficacy of drying processes. Results are described below;

3.6.1 Drug content (%)

From the observations results in the table1, it was found that drug content (%) were relatively low for all dried nanoparticles as compared to optimized polymer encapsulated nanoparticles. This decrease in drug content % could be attributed to the experimental losses, handling errors and leaching of drug from the nanoparticles due to the smaller batch sizes. During drying of the nanosuspension, dissolved polymers precipitated out of solution due to addition of adsorbent /cryoprotectants adsorbed on to the surface of nanoparticles. The cryoprotectant coat formed over the surface of nanoparticles prevents its aggregation and rehydration dehydration shocks during drying processes. Due to this, bursting of drugs from nanoparticles and thus loss of drug from nanoparticles occurred. So as compared to optimized nanoparticles drug content % there was found to be reduced drug content in all dried nanoparticles. Similar observations were seen to W.L.J.Hinrichs *et al.*2006 and Nae-Oh Chung *et al*.2012.[20]

3.6.2 Moisture content (%w/w) and contact angle (°)

From the observations in the figure 6, 7 and figure 8 it was clear that, for all adsorb dried nanoparticles comparatively higher moisture content were observed. For HPMC dried 4.58 ± 1.0 % w/w, PLGA dried 5.2 ± 1.0% w/w, Eudragit®S-100 dried 4.2 ± 0.82 % w/w and contact angle 35.1 ± 2.4°, 43.26 ± 0.62 °, 43.2 ± 0.92 ° respectively. As adsorption drying was a manual process due to sorption and desorption of moisture comparatively higher moisture content were observed for adsorb dried products. Observations and graph suggested that, freeze dried nanoparticles D / H with 5 % w/v mannitol have smaller contact angle 29.0 ± 1.3° and moisture content 3.5± 0.3%w/w, vacuum dried nanoparticles D / P with 1.5%w/v lactose have smaller contact angle 20.1 ± 1.0° and moisture content 2.0 ± 0.12%w/w, freeze dried nanoparticles 2D/E with mannitol 5% w/v have moisture content 2.1 ± 0.12 %w/w and contact angle 30.0 ± 1.13° is shown in the figure 6, 7 and figure 8 respectively below.

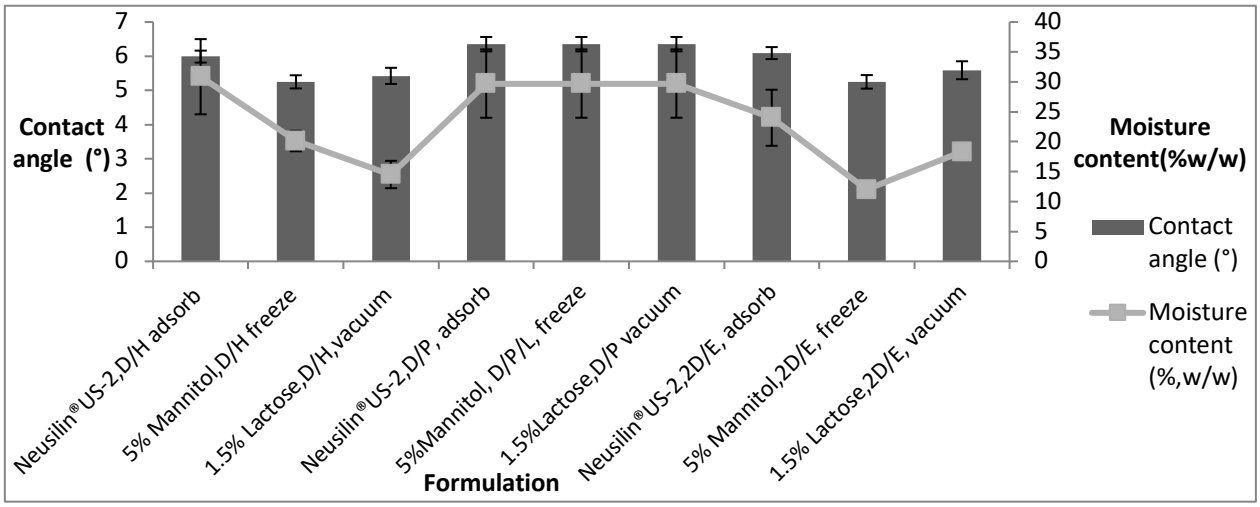

Figure 6: Formulations against moisture content and contact angle

Vacuum dried D/H nanoparticles with 1.5 %w/v lactose have found contact angle 32.0 ± 1.5º and smaller moisture content 2.0 ± 0.15 %w/w. Contact angle value ≤ 90º have higher wettening property, moisture content ≤ 2.0 w/w is necessary noted in Remington *et al.*1995, all dried products here have shown relatively wettening property were found.

3.6.3 Carr's index (%) and angle of repose (º)

From the observations and the figure no.7 it was clear that, for neusilin®US-2 adsorb dried HPMCK100M encapsulated retinol acetate nanoparticles D/H (1/1 w/w) have angle of repose 43.12 ± 2º and Carr's index 38.11 ± 1.22%. For adsorb dried PLGA50/50 encapsulated retinol acetate nanoparticles D/P (1/1w/w) angle of repose 43.8 ± 2.1º and Carr's index 40.23 ± 1.34%. For adsorb dried polymer encapsulated Eudragit®S-100 encapsulated retinol acetate nanoparticles D/E (2/1w/w) nanoparticles have Carr's index 41.41 ± 1.34 % and angle of repose was 44.42 ± 2.3º was obtained as adsorption drying was a manual process due to sorption and desorption of moisture higher values of Carr's index and angle of repose were observed. Observations and graph suggested that, vacuum dried nanoparticles D/H with 1.5 % w/v lactose have smaller angle of repose 24.2 ± 1.0º and Carr's index 22.15 ± 1.19 %, vacuum dried nanoparticles D/P with 1.5% w/v lactose have smaller contact angle 21.2 ± 1º and Carr's index 21.52 ±1.1%, freeze dried nanoparticles (2D /E) with mannitol 5% w/v have angle of repose 22.32 ± 1.1º, Carr's index 21.72 ±1.1%. Angle of repose (θ) < 20 have excellent flow property and Carr's index (%) 5-15 excellent as suggested by Remington *et al.*1995.

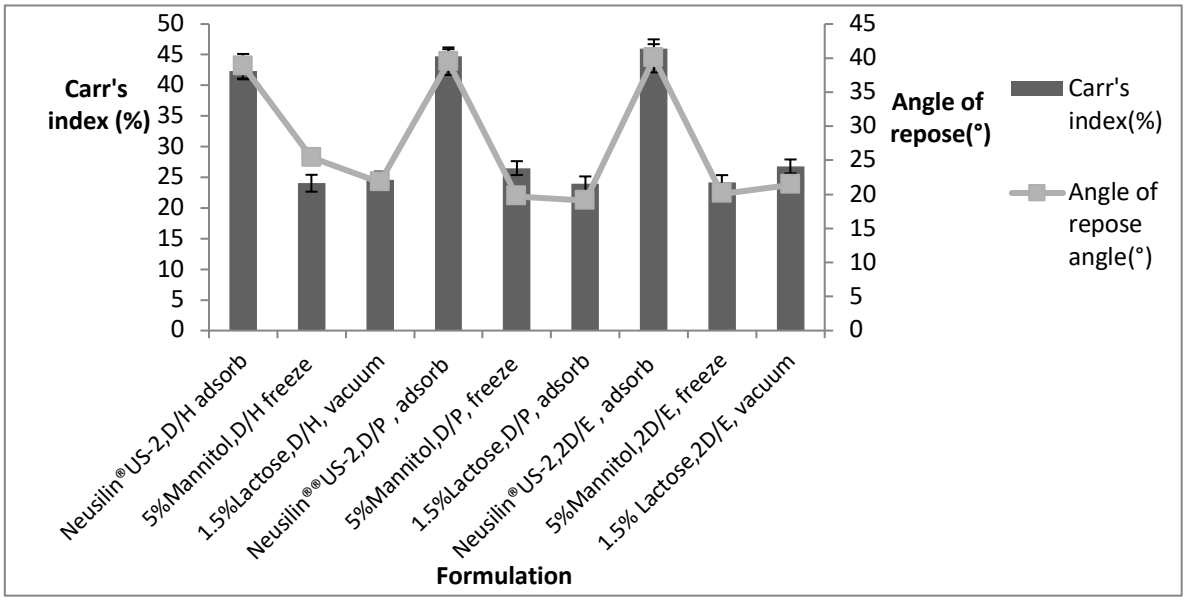

Figure 7: Formulations Carr's index (%) and angle of repose (°)

3.6.4 FTIR (Fourier Transform Infra-Red) spectroscopy studies

Helped in understanding internalization of drug by polymers. Observations led us to believe

that there were an interaction of drug and excipients, mainly diffusion, encapsulation and conjugation, adsorption, hydrogen bonding formations, broadening of bond lengths. These interactions were responsible for changes in the functional group positions with increased starching are encircled in all dried and optimized nanoparticles FTIR spectras figure 8, 9,
10. Optimized nanoparticles and dried nanoparticles had shown selective groups of all
excipients used.

Thus the FTIR spectras of dried nanoparticles has shown that various drying methods successfully preserve the structure and composition of compounds. Formation of hydrogen bonding was lead to shifting of wave number of groups. Selective FTIR spectras of dried products are shown below. Neusilin®US-2, lactose and mannitol has shown prominent peak of hydroxyl around wave number 3200-3500 cm.$^{-1}$ Prominent peak of neusilin®US-2 metal at 1384.93 cm^{-1} and characteristic carboxylic group at 2667.4 cm^{-1} in lactose and ester (-COO) of mannitol at 1021.8 cm^{-1}.These all positions were slightly changed during drying process. Characteristic of retinol acetate ionone ring was found at 2929.68 cm^{-1}with -COO group 1063.79 cm^{-1} with hydroxylic group 3424.36 cm^{-1} were found in optimized nanoparticles HPMCK-100 M encapsulated retinol acetate nanoparticles FTIR spectra. These groups location were changed in adsorption drying with neusilin®US-2 with characteristic metal group at 1358.31 cm^{-1} suggested adsorption of nanoparticles on neusilin®US-2. And in all freeze dried 5 %w/v mannitol and all vacuum dried sample with 1.5% w/v lactose suggested protective role of mannitol and lactose in drying processes. Slightly changes in functional group's peak positions were observed. Characteristic of retinol acetate ionone ring was found at 2924.36cm^{-1} with -COO group 1054.54 cm^{-1} with carboxylic acid group 3731.09 cm^{-1} were found in optimized nanoparticles PLGA50:50 encapsulated retinol acetate nanoparticles FTIR spectra. These groups location were changed in adsorption drying with neusilin®US-2 with characteristic metal group at 1421.2 cm^{-1} suggested adsorption of nanoparticles on neusilin®US-2 and in freeze dried 5 % w/v mannitol and vacuum dried sample with 1.5% w/v lactose suggested protective role with changes in peak positions.

Similar results were obtained for optimized Eudrgit®S-100 encapsulated retinol acetate (2D/E) nanoparticles and their components functional group peak positions of FTIR spectras .Nevertheless, drying techniques have shown to exert negligible effect on the chemical profile of dried nanoparticles as illustrated by the FTIR spectras and the drug content analysis results data. Cryoprotectant like mannitol, lactose or adsorbent like neusilin®US-2 formed protective capping layer around the nanoparticles which was sustained by hydrogen bonds formation in drying processes. This formation of a hydrosoluble matrix in drying processes, where the particles were embedded facilitates the reconstitution of the dispersions. These observations suggested that various drying methods preserve the structure composition, integrity and location of functional groups with slight changes in the positions. This had solved the issues of instabilities of nanoparticles dispersions, similar experiment observations were noted by Lee M. *et al.*2009 [11] and Wasim *et al.*2006[5] in drying processes.

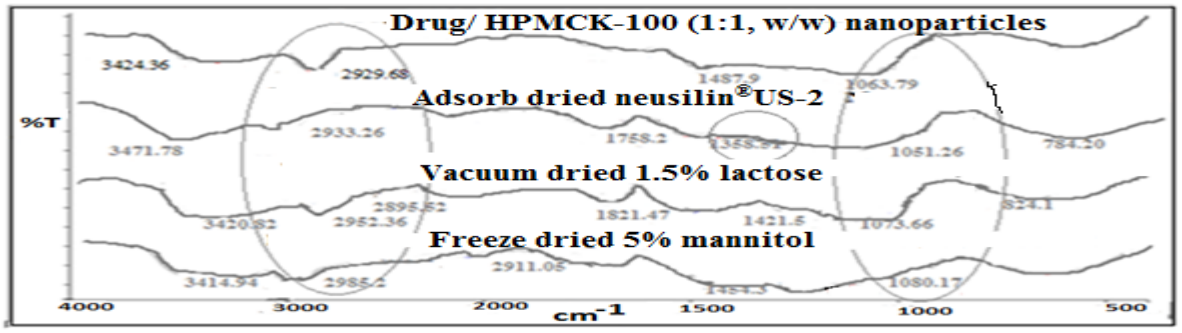

Figure 8: FTIR spectras of optimized and dried nanoparticles (D/H)

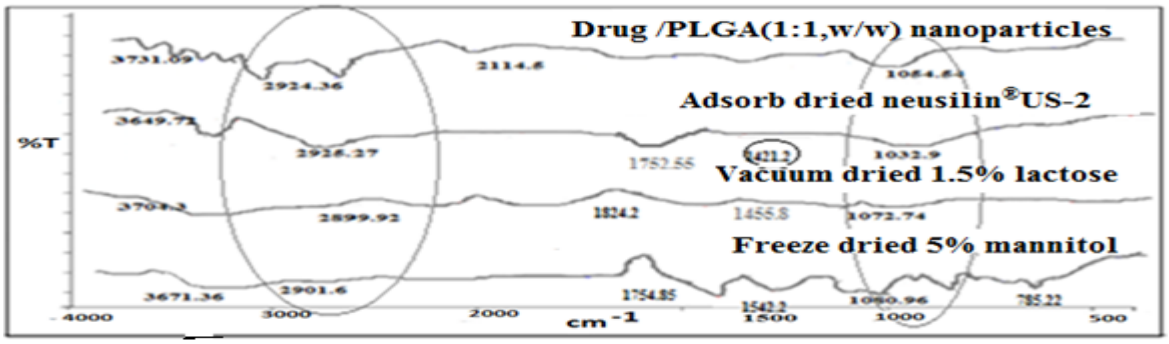

Figure 9: FTIR spectras of optimized with dried nanoparticles (D/P)

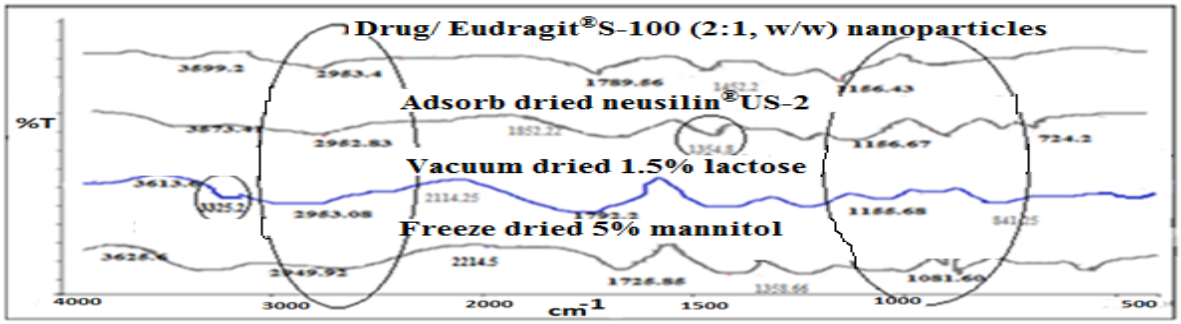

Figure 10: IR spectras of optimized and dried nanoparticles (2D/E)

3.6.5 Differential scanning calorimetry (DSC) studies

There were found slightly changed in melting points of composition constituent excipients incorporated in drying processes. DSC thermograms of various dried products with cryoprotectnts have shown, melting point of neusilin®US-2 around 240ºC, lactose around 202.8°C, mannitol around 165°C. Melting point endotherms are encircled in all dried nanoparticles thermograms in the figure 11, 12, figure 13. These components melting point with glass transition temperature had shown broaden range peaks for all dried nanoparticles. DSC thermoanalysis studies had also shown that, there were found to be diffusion of major drying cryoprotectants with polymer encapsulated nanoparticles. DSC thermograms have suggested absence of any interaction between drug and selected excipients in drying processes. Melting point depression of the excipients incorporated might be due to the increased lattice defects resulting from various changes in the processes. Other reasons may be nanoparticulate size of formulation with high specific surface area and presence of cryoprotectants / adsorbents in dried products. Obtained all nanoparticles powder were amorphous in nature, results were complied with Wasim *et al.*2006 experiments results.

In case of optimized D/H dried nanoparticles; broader melting point peak of drug around 52-56ºC with endothermic melting point peak of excipient like stabilizers in drug were also reduced to around 210ºC. In this, due to merging of cryoprotectant or adsorbents melting point peak of drug was reduced to around 52-55ºC and excipients polymers like HPMC K-100M melting point was around (actual 56ºC) 50-54º C with as shown in the figure 11. Similarly for PLGA50/50 200-204 ºC (actual 204 ºC), Eudragit®S-100 around 155-160ºC (actual 165ºC) peak were observed for adsorption, freeze, vacuum drying products. These were resulted in lowering and merging of endothermic melting point peaks of adsorbent or cryoprotectans; as shown in the thermograms. No new endothermic or exothermic peaks were observed in the nanoparticles dried products, indicating no incompatibilities between drug and excipients were observed in drying processes. Total enthalpy changes for all dried nanoparticulate products endotherms during melting were observed smaller as compared to excipients melting separately as seen in all the DSC thermograms. Similar results were obtained to Shyam *et al.*2004.

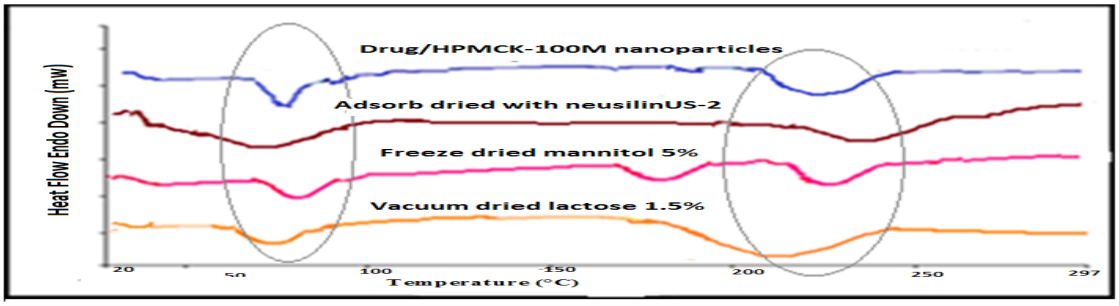

Figure 11: DSC thermograms of optimized and dried nanoparticles (D/H)

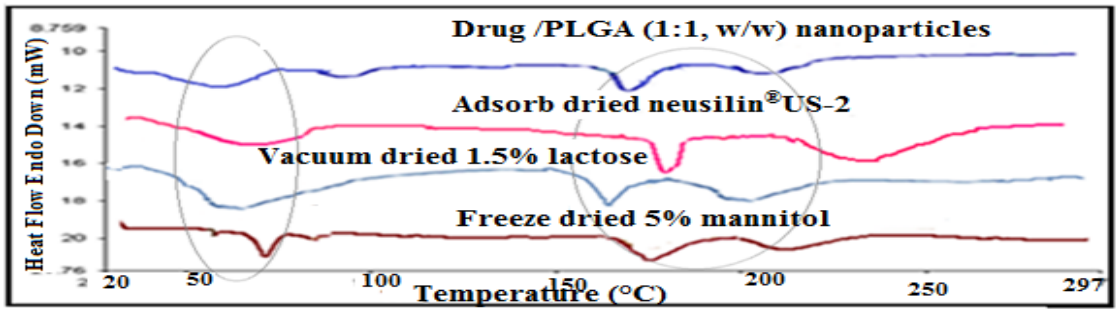

Figure 12: DSC thermograms of optimized and dried nanoparticles (D/P)

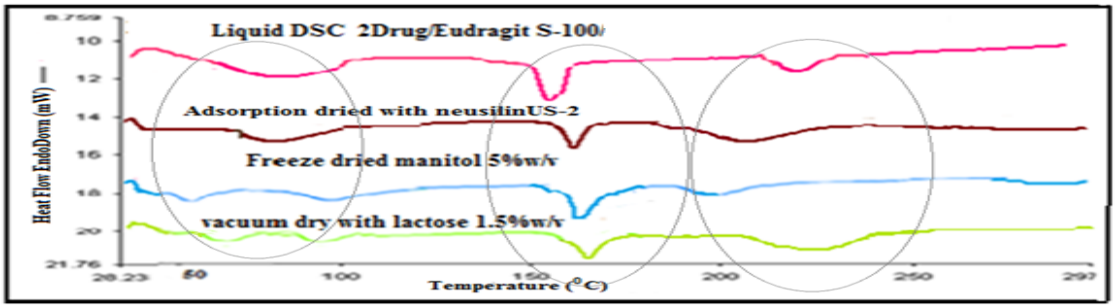

Figure 13: DSC thermograms of optimized and dried nanoparticles (2D/E)

3.6.5 X-Ray Diffraction studies

Characteristic crystallinity diffraction sharp pattern peaks of drug and excipients were found reduced, showing amorphous state of all nanoparticles obtained. These are encircled in all XRD figure 14, 15 figure 16. Main reason to get amorphous state during SFT processing and drying was highly ordered crystallinity structure of excipients changed due to various changes in the process conditions. During drying processes; increased number of imperfections in the crystal lattice such as point defects (e.g. vacancies, impurity defects etc), line defects (e.g. edge dislocation) and plane defects (e.g. grain boundaries) due to changes in the conditions. Similar observations were also noted by Brittain *et al.*1999[21] and Zhiyi L. *et al.*2009.[22]

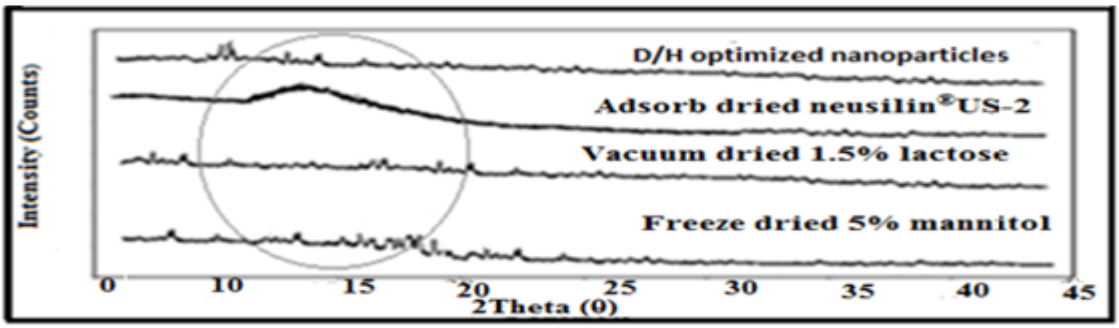

Figure 14: XRD patterns of optimized and dried nanoparticles (D/H)

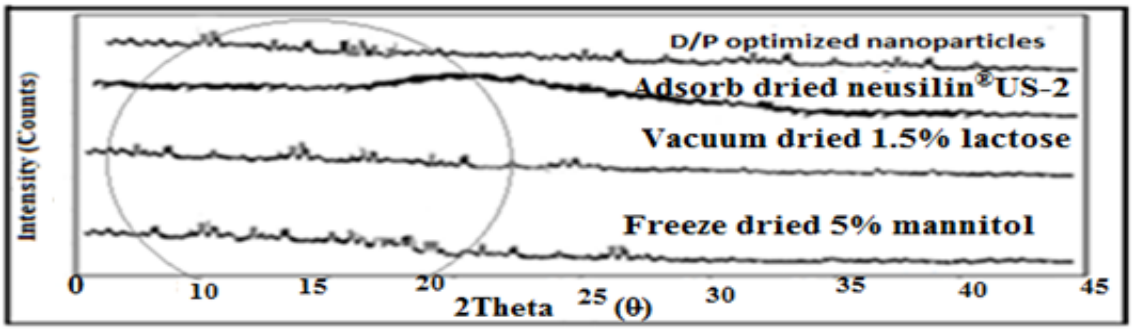

Figure 15: XRD patterns of optimized and dried nanoparticles (D/P)

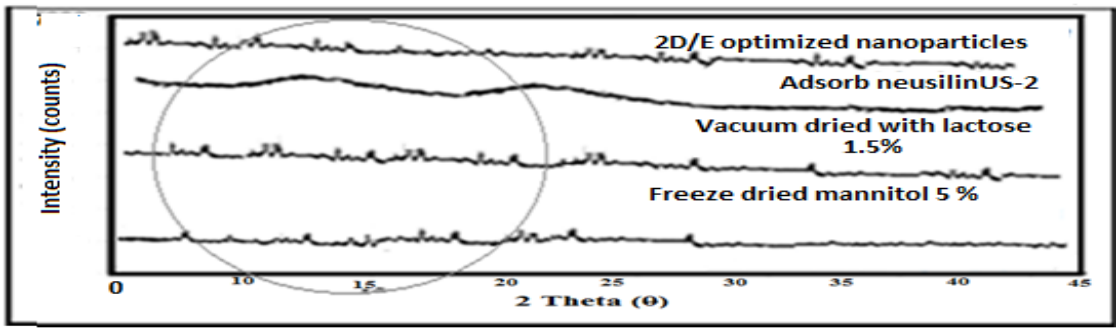

Figure 16: XRD patterns of optimized and dried nanoparticles (2D/E)

4.0 Conclusions

For all polymer encapsulated drug nanodispersions to achieve stability and therapeutic effectiveness during storage drying methods such as adsorption drying, vacuum drying and freeze drying were successfully used to convert them to an oral solid dosage form. When the dried form was reconstituted in an aqueous system, redispersed powder achieved its original particle size with slight increase. These studies revealed that the drying techniques have considerable effect on the morphological attributes of the nanoparticles as observed in the XRD pattern studies and SEM images of dried products. Consequently, these morphological changes contributed to the drastic change in the particle size of the redispersed formulation. All dried nanoparticles were found amorphous in nature, proved by appearance, XRD pattern studies and DSC thermograms studies. Nevertheless, drying techniques had shown to exert negligible effect on the chemical profile of the developed nanoparticles as illustrated by the FTIR spectra and the drug content analysis results. Flow properties of the processed powders were studied as final dosage form was oral capsule.

These studies demonstrated the potential of vacuum oven drying with 1.5 % w/v lactose as cryoprotectant for rendering the dispersion into free flowing powder for HPMCK-100M encapsulated retinol acetate nanoparticles and for PLGA50:50 encapsulated retinol acetate nanoparticles. Reason for this was enhanced drying rate with good quality product. And for Eudragit®S-100 encapsulated retinol acetate nanoparticles freeze dried nanoparticles with 5 % w/v mannitol as cryoprotectant were found to be suitable for use in final dosage form capsule, as shown by results of characterizations. Also for the nanoparticles dosage form wherein the active drug substance quantity is less, the additives are preferred during the drying process. So during drying process, screening and evaluation of different adsorbent or cryoprotectants with nanoparticles were done successfully. Drying of nanoparticles was a complex process with many parameters such as drying condition, nature of formulation; nature and concentration of adsorbent / cryoprotectant were found important factors determining overall success of drying nanoparticles. These studies described herewith given an indication that the industrially feasible, cost effective adsorption, vacuum drying and freeze drying processes can be suitably used for the drying of polymer encapsulated thermolabile retinol acetate nanoparticulate dispersions and converting into solid powder form. However, more studies are required to modulate the impact of these processes and for its easy adaptability.

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