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FORMULATION AND EVALUATION OF AMOXICILLIN NANOSUSPENSION

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ABSTRACT

Nanotechnology is one of the growing fields in medicine. “Nano” stands for the particle size ranging from 1-1000µm. Nanosuspensions are the sophisticated technology in the field of nanoscience. These are simple to prepare and are more advantageous than other approaches. Other techniques like microemulsion, solid dispersions and inclusion complexes using cyclodextrin even though showed increased solubility, but not applicable for drugs which are insoluble in both aqueous and organic media. The objective of this study was to formulate nanosuspensions to resolve solubility issue of amoxicillin which is widely used as antibiotic, which will improve antibiotic therapy and to make the dosage form more cost-effective. These focuses on, method of preparation, physical characteristics and evaluation of nanosuspensions. The nanoprecipitation method presents numerous advantages, in that it is a straight forward technique, rapid and easy to perform. Polymeric nanosuspensions were prepared by nanoprecipation method by using biodegradable polymer PVP-K 30 loaded with Amoxicillin in the ratio of 1:1, 1:2, 1:3, 1:4 and 1:5 respectively and the formulation was evaluated for drug excipients compatibility study, drug content, particle size analysis and zeta potential. Nanoparticulate drug delivery have advantages over conventional dosage forms which include improved efficacy, reduced toxicity, enhanced biodistribution and improved patient compliance.

KEYWORDS: Amoxicillin**,** Nanosuspension, Nanoprecipitation**,** Particle Size, Solubility, Zeta Potential.

INTRODUCTION

Nanosuspensions are colloidal dispersions of solid drug particles in a liquid phase with average particle sizes below 1 µm stabilized by the use of surfactants. Nanosuspension technology can be used to improve the stability as well as the bioavailability of poorly soluble drugs. Nanosuspensions are biphasic systems consisting of pure drug particles dispersed in an aqueous vehicle, stabilized by surfactants. Techniques such as wet

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milling, high-pressure homogenization, emulsification-solvent evaporation and supercritical fluid have been used in the preparation of nanosuspension.(III) Nanosuspension engineering processes currently used are precipitation, high pressure homogenization and pearl milling either in water or in mixtures of water and water miscible liquids or non-aqueous media. In nanoprecipitation the drug is dissolved in organic phase, the ratio of drug to polymer is taken as 1:1,1:2,1:3,1:4,1:5. The mixture of polymer and water is used aqueous phase. The drug is added by using the syringe with needle. Then organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type of stabilizer, concentrations of stabilizer, and homogenizer speed. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed.

The super saturation is further attained by evaporation of drug solvent. This yields to the precipitation of the drug. For large-scale production of nanosuspensions, media milling and high-pressure homogenization technology have been successfully used. More than 40percent of the drugs coming from High-through output screening are poorly soluble in water. One of the critical problems associated with poorly soluble drugs is too low bioavailability and or erratic absorption. Nanotechnology can be used to resolve the problems associated with these conventional approaches for solubility and bioavailability enhancement. (VII)

MATERIALS AND METHOD

CHEMICALS: Amoxicillin (Drug), Polyvinyl pyrrolidone (Polymer), Tween80 (Surfactant) Bezoalkonium chloride (Preservative), Ethanol (Solvent) are obtained from Government College of Pharmacy, Aurangabad, Maharashtra, India.

METHODS:

1. Calibration Curve –

100 mg amoxicillin was weighed accurately and dissolved in 100 ml of distilled water in volumetric flask. Flask was shaken for 5 min to dissolve drug properly, flask was labeled as Stock solution was further diluted into 100 ml distilled water. Maximum wavelength was determined by scanning on UV –Visible spectrometer .Further dilution were prepared by1 ml stock solution in 100ml, 2 ml stock solution in 100 ml and so on. These results are surmise in tables.

2. Melting Point –

Melting point was measured with use of Thieles tube apparatus by using paraffin oil, thermometer and placed in thielse tube containing parrafin oil, he tube is heated by using burner . The range of temperature when drug just start melting and till it completely melts was noted.

3. FTIR Spectroscopy Analysis-

Fourier –transform infrared (FT –IR) spectra of moisture free powdered sample of amoxicillin ,PVP, Tween 8 and physical mixture were obtained using a spectrophotometer (FTIR –Shimadzu ,India)

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4. Differential Scanning Colorimetry (DSC) Analysis-

DSC scans of pure drug sample and polymer were recorded using DSC-Shimadzu 60 with TDA trend line software. All sample were weighed (8-

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10mg) and heated as a scanning rate of 10^0c/min under dry nitrogen flow (100ml/min) between 50 and 300^0c. Aluminum pans and lids were used for all sample. Pure water and indium were used to calibrate the DSC temperature scale and enthalpy response.

Preparation of Amoxicillin Nanosuspension by nanoprecipitation -
Nanosuspensions were prepared by the solvent evaporation technique. It content aqueous phase and organic phase, the aqueous phase containing different amount of PVPK-30 and Tween80 maintained at room temperature as ratio 1:1, 1:2, 1:3, 1:4, 1:5 (Table-2). The organic phase contain amoxicillin (drug) dissolved in an ethanol at room temperature. This was poured into aqueous phase subsequently stirred on mechanical stirrer for 4000 rpm (Remi, India) for 1 hour. Then allow the volatile solvent to evaporate.
Addition of organic solvent by mean of a syringe positioned with the needle directly into surfactant containing water. Organic solvent were left to evaporate off under a slow mechanical stirrer of the nanosuspension at room temperature for 8 h.

Table:-1 Composition of Amoxicillin Suspension
Table:-2 Composition of Various Nanosuspensions Formulation

Evaluation Parameter

1. Particle Size Analysis-
Particle size and particle size distribution was determined by photon correlation spectroscopy(PCS) using a zeta sizer(z-average, measuring range:20-1000nm)

2. Viscosity determination-
Viscosity were determined by Brookfield viscometer. The suspension is poured into a beaker without bubble then measures the reading and calculate viscosity.

RESULT AND DISCUSSION:

The sample of amoxicillin was procured for study was identified and estimated for its purity. The sample of amoxicillin was identified by melting point, FTIR, Differential Scanning Colorimetry.

1. Construction of Calibration Curve using UV spectrometer-

The UV spectrometer method was selected for the estimation of amoxicillin, showing absorbance at ʎ max 343.43nm (figure.1.1)

The standard curve of amoxicillin was constructed in distilled water using UV-visible spectrometer. Excellent linearity, precision and reproducibility were obtained in the range 2-10µg/ml. Standard calibration curve was plotted (Table 3, figure 1.1)as follow

2. Melting Point –

The melting point were determined by Thieles tube apparatus by using paraffin oil, thermometer. The melting point was found to be 141-145^0c.

3. FTIR

FTIR has been used to assess the interaction between excipients and the drug molecule in the solid state. The FTIR spectra were taken by pure drug, PVP, Tween80, reconstituted nanosuspension. The FTIR spectra of all sample show in figure--.Row amoxicillin and precipitated nanoparticle exhibited same FTIR spectra,

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the amoxicillin show peak at 3468 for N-H and the range is from 3300-3500, and the nanosuspension show the peck at 3543 as show in fig, which demonstrate that the chemical structure of the drug is not changed before and after the precipitation process.

4. DSC-

The physical state of row amoxicillin and reconstituted nanoparticle of nanosuspension was examined by DSC and there is thermo grams are shown in fig Row amoxicillin exhibited a melting point with fusion enthalpy where as DSC scan of PVP, a broad endotherm ranging from 207-234was observed due to presence of residual solvent.

5. Particle Size Analysis –

The mean particle size and particle size distribution affect the saturation solubility, dissolution rate, physical stability, even In vivo behavior of nanosuspension. The polydispersive index in range of 0.1-0.22 indicate a fairly narrow size distribution can be determined by photon correlation spectroscopy.

CONCLUSION

Nanoprecipitation technique was employed to producing nanopartiles of amoxicillin, a poorly water soluble drug, for the improvement of solubility. In this process, the particle size of amoxicillin can be obtained in the micro and nanosize ranges, by adjusting the operation parameter, such as polymer concentration, and organic to aqueous solvent ratio. The best nanosuspension of particle size of 261 nm can be obtained by 1:1 ratio of drug to polymer using a solvent evaporation technique at laboratory scale. Nanosuspension can thus be simple and effective approach to produce submicron particles of poorly water soluble drug.

FUTURE PROSPECT

NANOSUSPENSION- A PROMISINGTOOL FOR DRUG DELIVERY SYSTEM

Nanosuspension consists of the poorly water soluble compound without any matrix material suspended in dispersion. One of the major problem associated with them as the amoxicillin is not stable in aqueous medium as it causes degradation of drug. To solve these problem the reconstitution of the powder will be done as it given with the aqueous vehicle.

ACKNOWLEDGEMENT

The authors are highly thankful to Government College of Pharmacy, Aurangabad, for providing all the facilities during research work.

TABLE AND FIGURE

Table:-1 Composition of Various Nanosuspension

Formulation code	Drug mg	Polymer (mg)	Surfactant	Preservative	Solvent(ml) Ethanol	Solvent(ml) water
F1	200	100	2	0.04	2	20
F2	200	200	2	0.04	2	20

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F3	200	300	2	0.04	2	20
F4	200	400	2	0.04	2	20
F5	200	500	2	0.04	2	20

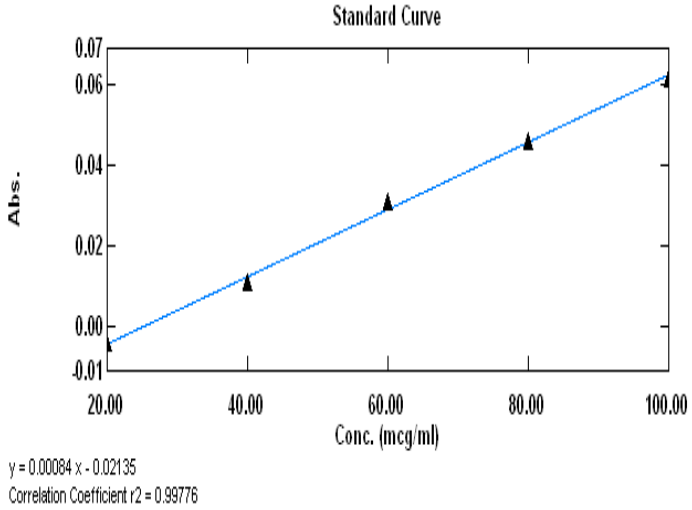
Table:-2 Composition of Amoxicillin Suspension

Sr. No.	Ingredient	Quantity	Use
1	Amoxicillin	200mg	Antibiotic
2	Polyvinyl pyrrolide	200mg	Polymer
3	Ethanol	2ml	Solvent
4	Tween-80	0.04g	Surfactant
5	Bezoalkonium chloride	0.04%	Preservative
6	Pineapple essence	0.1ml	Flavoring agent
7	Saccharine	0.1ml	Sweetening agent
8	Purified water	20ml	Vehicle

Table 3- Preparation of Calibration Curve

Sr. No.	Conc.	Abs.
1	20	0.01
2	40	0.02
3	60	0.04
4	80	0.06

Fig 1.1- Construction of Calibration Curve



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2. FTIR

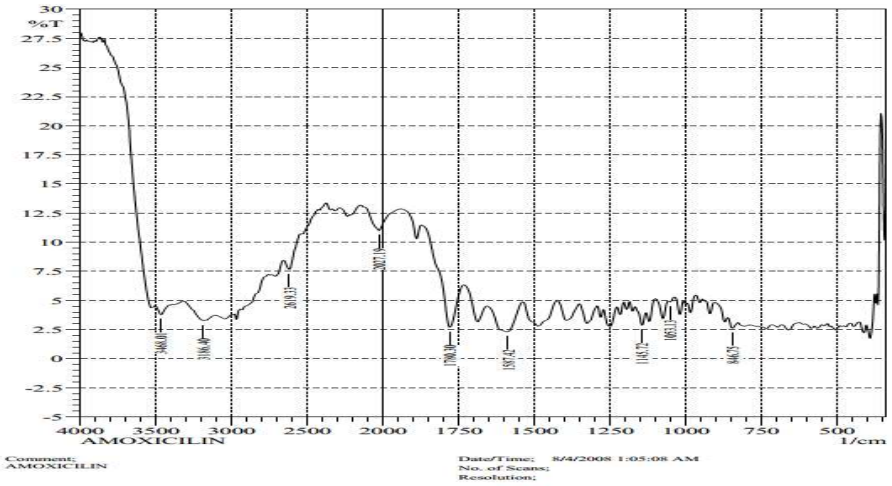

Fig – 1.2 Amoxicillin

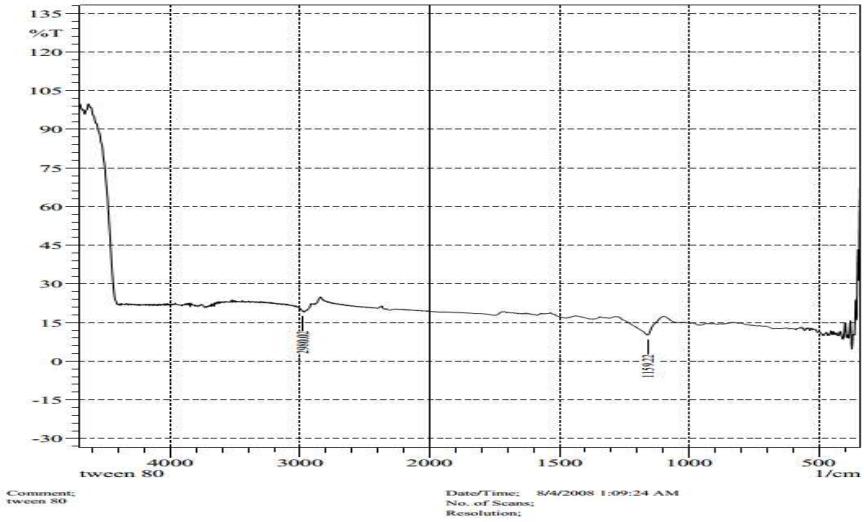

Fig 1.3 - Tween80

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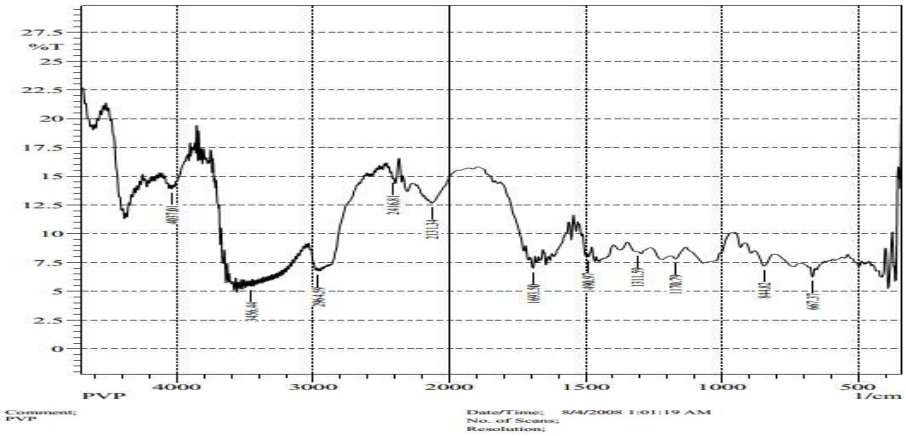

Fig -1.4 PVP

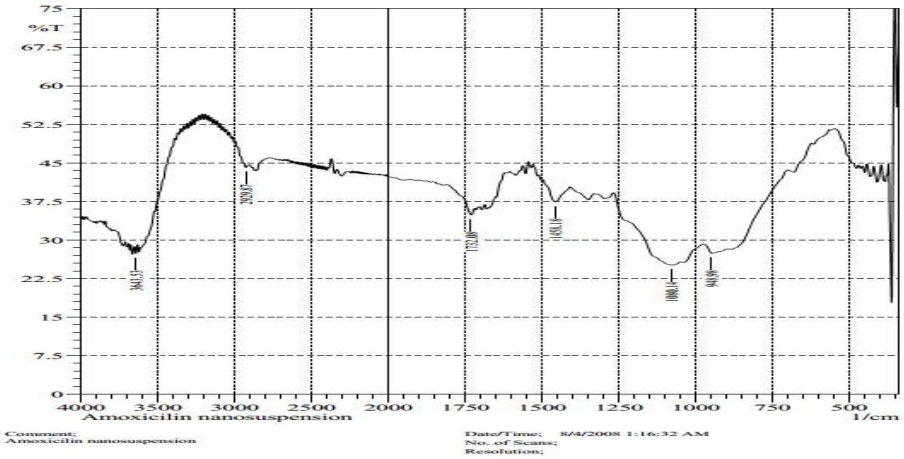

Fig – 1.5 Amoxicillin Nanosuspension

3. DCS Result

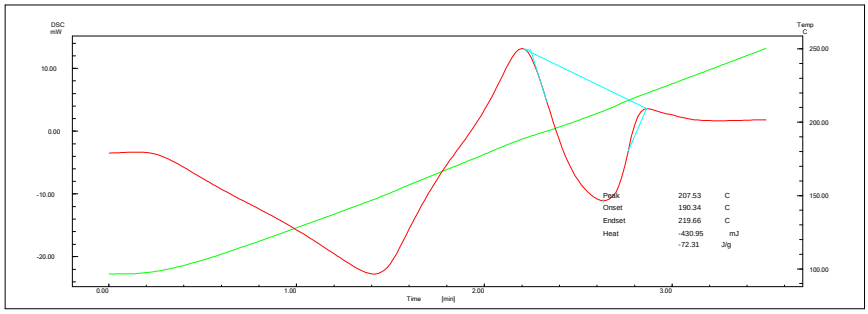

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Fig 1.6 - Amoxicillin

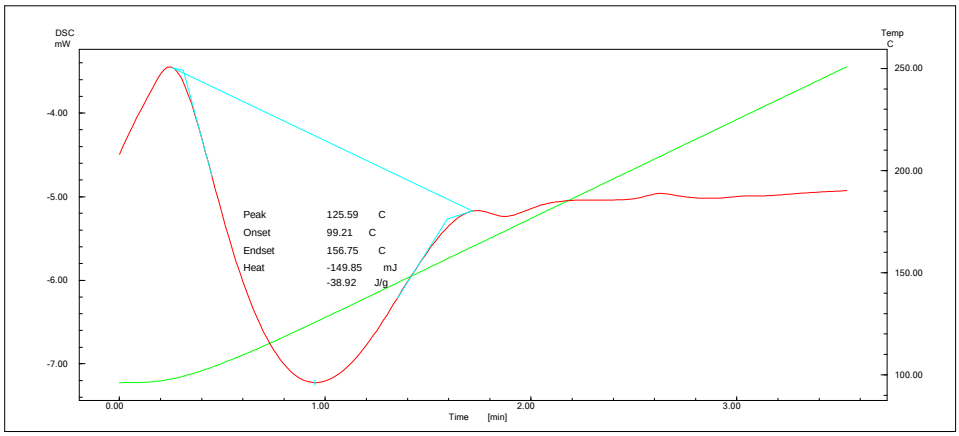

Fig 1.7 - PVP

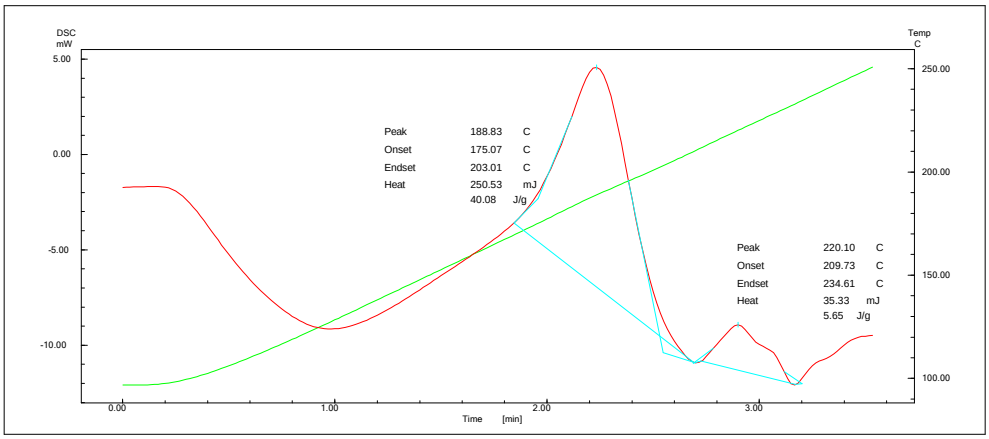

Fig – 1.8 Amoxicillin + PVP

4. PARTICLE SIZE DETERMINATION

Formulation	Particle Size (nm)
F1	833
F2	261
F3	682.3
F4	401.7
F5	1245.7

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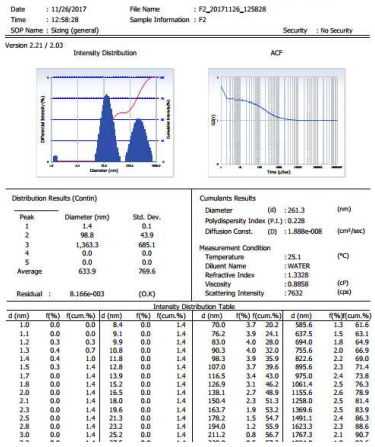
Fig- 1.9 Particle Size Formulation F2



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SOP Name : Sizing (general) Security : No Security
Version 2.21 / 2.03

Intensity Distribution ACF

Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	1.4	0.1
2	98.8	43.9
3	1,363.3	685.1
4	0.0	0.0
5	0.0	0.0
Average	633.9	769.6

Residual : 8.166e-003 (O.K)

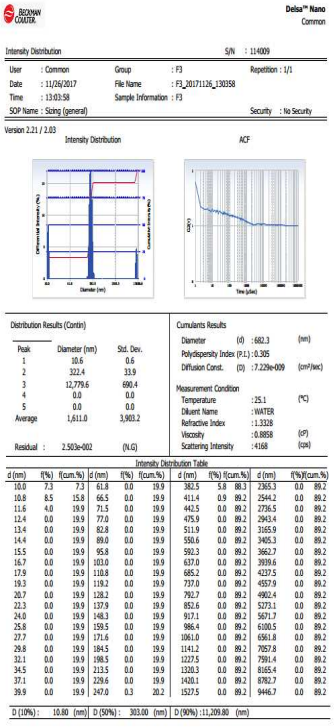
Cumulants Results

Diameter	(d)	:261.3	(nm)
Polydispersity Index (P.I.)		: 0.228	
Diffusion Const.	(D)	: 1.888e-008	(cm²/sec)
Measurement Condition			
Temperature		:25.1	(°C)
Diluent Name		: WATER	
Refractive Index		: 1.3328	
Viscosity		: 0.8858	(cP)
Scattering Intensity		: 7632	(cps)

Intensity Distribution Table

d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)
1.0	0.0	0.0	8.4	0.0	1.4	70.0	3.7	20.2	585.6	1.3	61.6
1.1	0.0	0.0	9.1	0.0	1.4	76.2	3.9	24.1	637.5	1.5	63.1
1.2	0.3	0.3	9.9	0.0	1.4	83.0	4.0	28.0	694.0	1.8	64.9
1.3	0.4	0.7	10.8	0.0	1.4	90.3	4.0	32.0	755.6	2.0	66.9
1.4	0.4	1.0	11.8	0.0	1.4	98.3	3.9	35.9	822.6	2.2	69.0
1.5	0.3	1.4	12.8	0.0	1.4	107.0	3.7	39.6	895.6	2.3	71.4
1.7	0.0	1.4	13.9	0.0	1.4	116.5	3.4	43.0	975.0	2.4	73.8
1.8	0.0	1.4	15.2	0.0	1.4	126.9	3.1	46.2	1061.4	2.5	76.3
2.0	0.0	1.4	16.5	0.0	1.4	138.1	2.7	48.9	1155.6	2.6	78.9
2.1	0.0	1.4	18.0	0.0	1.4	150.4	2.3	51.3	1258.0	2.5	81.4
2.3	0.0	1.4	19.6	0.0	1.4	163.7	1.9	53.2	1369.6	2.5	83.9
2.5	0.0	1.4	21.3	0.0	1.4	178.2	1.5	54.7	1491.1	2.4	86.3
2.8	0.0	1.4	23.2	0.0	1.4	194.0	1.2	55.9	1623.3	2.3	88.6
3.0	0.0	1.4	25.2	0.0	1.4	211.2	0.8	56.7	1767.3	2.1	90.7

Fig- 2 Particle Size Formulation F3



Delsa™ Nano Common

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Time : 13:03:58 Sample Information : F3
SOP Name : Sizing (general) Security : No Security
Version 2.21 / 2.03

Intensity Distribution ACF

Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	10.6	0.6
2	322.4	33.9
3	12,779.6	690.4
4	0.0	0.0
5	0.0	0.0
Average	1,611.0	3,903.2

Residual : 2.503e-002 (N.G)

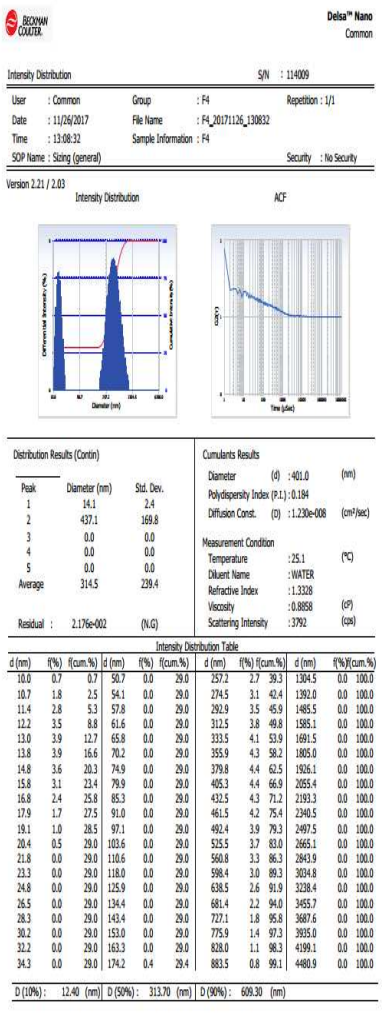
Cumulants Results

Diameter	(d)	: 682.3	(nm)
Polydispersity Index (P.I.)		: 0.305	
Diffusion Const.	(D)	: 7.229e-009	(cm²/sec)
Measurement Condition			
Temperature		:25.1	(°C)
Diluent Name		: WATER	
Refractive Index		: 1.3328	
Viscosity		: 0.8858	(cP)
Scattering Intensity		: 4168	(cps)

Intensity Distribution Table

d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)
10.0	7.3	7.3	61.8	0.0	19.9	382.5	5.8	88.3	2365.3	0.0	89.2
10.8	8.5	15.8	66.5	0.0	19.9	411.4	0.9	89.2	2544.2	0.0	89.2
11.6	4.0	19.9	71.5	0.0	19.9	442.5	0.0	89.2	2736.5	0.0	89.2
12.4	0.0	19.9	77.0	0.0	19.9	475.9	0.0	89.2	2943.4	0.0	89.2
13.4	0.0	19.9	82.8	0.0	19.9	511.9	0.0	89.2	3165.9	0.0	89.2
14.4	0.0	19.9	89.0	0.0	19.9	550.6	0.0	89.2	3405.3	0.0	89.2
15.5	0.0	19.9	95.8	0.0	19.9	592.3	0.0	89.2	3662.7	0.0	89.2
16.7	0.0	19.9	103.0	0.0	19.9	637.0	0.0	89.2	3939.6	0.0	89.2
17.9	0.0	19.9	110.8	0.0	19.9	685.2	0.0	89.2	4237.5	0.0	89.2
19.3	0.0	19.9	119.2	0.0	19.9	737.0	0.0	89.2	4557.9	0.0	89.2
20.7	0.0	19.9	128.2	0.0	19.9	792.7	0.0	89.2	4902.4	0.0	89.2
22.3	0.0	19.9	137.9	0.0	19.9	852.6	0.0	89.2	5273.1	0.0	89.2
24.0	0.0	19.9	148.3	0.0	19.9	917.1	0.0	89.2	5671.7	0.0	89.2
25.8	0.0	19.9	159.5	0.0	19.9	986.4	0.0	89.2	6100.5	0.0	89.2
27.7	0.0	19.9	171.6	0.0	19.9	1061.0	0.0	89.2	6561.8	0.0	89.2
29.8	0.0	19.9	184.5	0.0	19.9	1141.2	0.0	89.2	7057.8	0.0	89.2
32.1	0.0	19.9	198.5	0.0	19.9	1227.5	0.0	89.2	7591.4	0.0	89.2
34.5	0.0	19.9	213.5	0.0	19.9	1320.3	0.0	89.2	8165.4	0.0	89.2
37.1	0.0	19.9	229.6	0.0	19.9	1420.1	0.0	89.2	8782.7	0.0	89.2
39.9	0.0	19.9	247.0	0.3	20.2	1527.5	0.0	89.2	9446.7	0.0	89.2

D (10%) : 10.80 (nm) D (50%) : 303.00 (nm) D (90%) : 11,209.80 (nm)



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Time : 13:08:32 Sample Information : F4
SOP Name : Sizing (general) Security : No Security
Version 2.21 / 2.03

Intensity Distribution ACF

Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	14.1	2.4
2	437.1	169.8
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	314.5	239.4

Residual : 2.176e-002 (N.G)

Cumulants Results

Diameter	(d)	: 401.0	(nm)
Polydispersity Index (P.I.)		: 0.184	
Diffusion Const.	(D)	: 1.230e-008	(cm²/sec)
Measurement Condition			
Temperature		:25.1	(°C)
Diluent Name		: WATER	
Refractive Index		: 1.3328	
Viscosity		: 0.8858	(cP)
Scattering Intensity		: 3792	(cps)

Intensity Distribution Table

d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)
10.0	0.7	0.7	50.7	0.0	29.0	257.2	2.7	39.3	1304.5	0.0	100.0
10.7	1.8	2.5	54.1	0.0	29.0	274.5	3.1	42.4	1392.0	0.0	100.0
11.4	2.8	5.3	57.8	0.0	29.0	292.9	3.5	45.9	1485.5	0.0	100.0
12.2	3.5	8.8	61.6	0.0	29.0	312.5	3.8	49.8	1585.1	0.0	100.0
13.0	3.9	12.7	65.8	0.0	29.0	333.5	4.1	53.9	1691.5	0.0	100.0
13.8	3.9	16.6	70.2	0.0	29.0	355.9	4.3	58.2	1805.0	0.0	100.0
14.8	3.6	20.3	74.9	0.0	29.0	379.8	4.4	62.5	1926.1	0.0	100.0
15.8	3.1	23.4	79.9	0.0	29.0	405.3	4.4	66.9	2055.4	0.0	100.0
16.8	2.4	25.8	85.3	0.0	29.0	432.5	4.3	71.2	2193.3	0.0	100.0
17.9	1.7	27.5	91.0	0.0	29.0	461.5	4.2	75.4	2340.5	0.0	100.0
19.1	1.0	28.5	97.1	0.0	29.0	492.4	3.9	79.3	2497.5	0.0	100.0
20.4	0.5	29.0	103.6	0.0	29.0	525.5	3.7	83.0	2665.1	0.0	100.0
21.8	0.0	29.0	110.6	0.0	29.0	560.8	3.3	86.3	2843.9	0.0	100.0
23.3	0.0	29.0	118.0	0.0	29.0	598.4	3.0	89.3	3034.8	0.0	100.0
24.8	0.0	29.0	125.9	0.0	29.0	638.5	2.6	91.9	3238.4	0.0	100.0
26.5	0.0	29.0	134.4	0.0	29.0	681.4	2.2	94.0	3455.7	0.0	100.0
28.3	0.0	29.0	143.4	0.0	29.0	727.1	1.8	95.8	3687.6	0.0	100.0
30.2	0.0	29.0	153.0	0.0	29.0	775.9	1.4	97.3	3935.0	0.0	100.0
32.2	0.0	29.0	163.3	0.0	29.0	828.0	1.1	98.3	4199.1	0.0	100.0
34.3	0.0	29.0	174.2	0.4	29.4	883.5	0.8	99.1	4480.9	0.0	100.0

D (10%) : 12.40 (nm) D (50%) : 313.70 (nm) D (90%) : 609.30 (nm)

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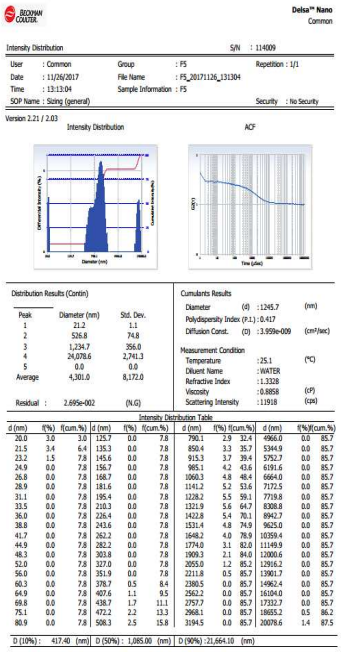
Fig- 2.2 Particle Size Formulation F5



Delsa™ Nano
Common

Intensity Distribution S/N : 114009

User : Common Group : F5 Repetition : 1/1
Date : 11/26/2017 File Name : F5_20171126_131304
Time : 13:13:04 Sample Information : F5
SOP Name : Sizing (general) Security : No Security

Version 2.21 / 2.03

Intensity Distribution ACF

Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	21.2	1.1
2	526.8	74.8
3	1,234.7	356.0
4	24,078.6	2,741.3
5	0.0	0.0
Average	4,301.0	8,172.0
Residual :	2.695e-002	(N.G)

Cumulants Results

Diameter	(d)	:1245.7	(nm)
Polydispersity Index (P.I.)		: 0.417	
Diffusion Const.	(D)	:3.959e-009	(cm²/sec)

Measurement Condition

Temperature	: 25.1	(℃)
Diluent Name	: WATER	
Refractive Index	: 1.3328	
Viscosity	: 0.8858	(cP)
Scattering Intensity	: 11918	(cps)

Intensity Distribution Table

d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)
20.0	3.0	3.0	125.7	0.0	7.8	790.1	2.9	32.4	4966.0	0.0	85.7
21.5	3.4	6.4	135.3	0.0	7.8	850.4	3.3	35.7	5344.9	0.0	85.7
23.2	1.5	7.8	145.6	0.0	7.8	915.3	3.7	39.4	5752.7	0.0	85.7
24.9	0.0	7.8	156.7	0.0	7.8	985.1	4.2	43.6	6191.6	0.0	85.7
26.8	0.0	7.8	168.7	0.0	7.8	1060.3	4.8	48.4	6664.0	0.0	85.7
28.9	0.0	7.8	181.6	0.0	7.8	1141.2	5.2	53.6	7172.5	0.0	85.7
31.1	0.0	7.8	195.4	0.0	7.8	1228.2	5.5	59.1	7719.8	0.0	85.7
33.5	0.0	7.8	210.3	0.0	7.8	1321.9	5.6	64.7	8308.8	0.0	85.7
36.0	0.0	7.8	226.4	0.0	7.8	1422.8	5.4	70.1	8942.7	0.0	85.7
38.8	0.0	7.8	243.6	0.0	7.8	1531.4	4.8	74.9	9625.0	0.0	85.7
41.7	0.0	7.8	262.2	0.0	7.8	1648.2	4.0	78.9	10359.4	0.0	85.7
44.9	0.0	7.8	282.2	0.0	7.8	1774.0	3.1	82.0	11149.9	0.0	85.7
48.3	0.0	7.8	303.8	0.0	7.8	1909.3	2.1	84.0	12000.6	0.0	85.7
52.0	0.0	7.8	327.0	0.0	7.8	2055.0	1.2	85.2	12916.2	0.0	85.7
56.0	0.0	7.8	351.9	0.0	7.8	2211.8	0.5	85.7	13901.7	0.0	85.7
60.3	0.0	7.8	378.7	0.5	8.4	2380.5	0.0	85.7	14962.4	0.0	85.7
64.9	0.0	7.8	407.6	1.1	9.5	2562.2	0.0	85.7	16104.0	0.0	85.7
69.8	0.0	7.8	438.7	1.7	11.1	2757.7	0.0	85.7	17332.7	0.0	85.7
75.1	0.0	7.8	472.2	2.2	13.3	2968.1	0.0	85.7	18655.2	0.5	86.2
80.9	0.0	7.8	508.3	2.5	15.8	3194.5	0.0	85.7	20078.6	1.4	87.5

D (10%) : 417.40 (nm) D (50%) : 1,085.00 (nm) D (90%) : 21,664.10 (nm)

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