View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by International Journal of Advances in Pharmaceutics

International Journal of Advances in Pharmaceutics
ISSN: 2320–4923; DOI: 10.7439/ijap
Volume 4 Issue 5 [2015]
Journal home page: http://ssjournals.com/index.php/ijap

Research Article

Preparation of sodium alginate nanoparticles by desolvation technique using iso propyl alcohol as desolvating agent

A. Krishna Sailaja* and P. Swathi

RBVRR Women's College Of Pharmacy, Affiliated to Osmania University,Department Of Pharmaceutics, Barkatpura, Hyderabad,India

***Correspondence Info:**
A. Krishna Sailaja,
RBVRR Women's College of Pharmacy, Affiliated to Osmania University, Department of Pharmaceutics, Barkatpura, Hyderabad, India
Email: shailaja1234@rediffmail.com

Keywords:
Ibuprofen,
Nanoparticles,
Desolvation technique,
Sodium Alginate,
Isopropyl Alcohol,
Particle size analyser, Zeta Sizer

Abstract

Objective: The present study was undertaken to prepare and characterize drug loaded Sodium Alginate nanoparticles using Desolvation technique. The model drug chosen was Ibuprofen.

Method: These nanoparticles were prepared by two methods i.e; Continuous addition method and intermittent addition methods in which the addition of desolvating agent was done at the rate of 1ml per minute and 1 ml per 5 minutes respectively. The optimized formulation developed using these two methods were characterized for particle size and stability.

Results: The best method among two was determined by comparing the particle size and stability of the formulation using Particle Size analyzer and Zeta Sizer. Mean particle diameter and Zeta Potential was found to be 289.8 nm, -44.5 mV and 289.2 nm, -49.9 mV for the particles prepared by Continuous addition method and Intermittent addition method respectively.

Conclusion: Intermittent addition method was concluded as the best method for the preparation of Ibuprofen loaded sodium alginate nanoparticles because of their smaller particle size and greater stability.

1. Introduction

Throughout the world, continuous efforts are in progress for developing improved, optimized and advanced drug delivery system. In the recent years, for the formulation of efficacious drugs, there has been tremendous growth of research in the field of nanoscience and nanotechnology. The reason why these nanoparticles are attractive for the medical applications is based on the unique features like higher surface to mass ratio which provides tremendous driving force for diffusion, their quantum properties and larger surface area promoting their ability to bind, adsorb and carry drugs. Improved stability of therapeutic agents against various stress conditions can be achieved using biodegradable Nanoparticles, mainly those prepared using biodegradable polymers. These are of nanoscale (subnano sized) colloidal particulates whose size ranges from 10-100 nm. The properties of materials change as their size approaches the nanoscale. They are composed of polymeric materials of either synthetic or natural origin. The drug can be either encapsulated in the polymeric matrix or can just be adsorbed onto the surface of the polymeric membrane. These have been widely used for their unique applications like targeting the drug to the specific site without being attacked by RES and also the release of the drug from the nanoformulation can be in a controlled and sustained manner [1].

The important characteristics of ideal drug delivery like ability to target and control the drug release can be achieved by nanoparticles. And also nanoparticles composed of biodegradable polymer possess additional advantages like improved stability of the therapeutic agents against various stress conditions. Primary goals for research of nano-bio-technologies in drug delivery include:

- More specific drug targeting and delivery,
- Reduction in toxicity while maintaining therapeutic effects,

- Greater safety and biocompatibility, and
- Faster development of new safe medicines [2].

The various biodegradable polymers used for the preparation of nanoparticles include

Natural Polymers: Chitosan, Starch, Sodium alginate, glucose, mannose, fructose, keratin sulfate etc.

Synthetic Polymers: Poly lactic acid, Poly glycolic acid, poly ethylene glycol, poly lactide coglycolide, homo and coplolyemrs of caprolactone, etc.

In today's science, research has been more focused on the nanoparticles prepared using biodegradable hydrophilic polymers such as alginate and chitosan. Sodium Alginate, a natural biodegradable polysachcharide, because of its several advantages such a high compatability, biodegradability, non-toxicity, non-immunogenicity, chelating ability and the possibility of chemical modification and encapsulation ability it has been widely used in biomedical applications[3]. The model drug selected for this work was Ibuprofen. It is a non-steroidal anti-inflammatory drug that is used to relieve symptoms of pain of arthritis. Other uses includes primary dysmenorrheal, alleviating fever and reducing inflammation, also helping in showing analgesic, anti-platelet and vasodilation effect.

Numbers of methods are available for the preparation of nanoparicles, such as amphiphilic macromolecular cross linking, polymerization and polymer precipitation methods. In amphiphic cross linking method desolvation technique is mainly applicable for the preparation of protein nanoparticles. Desolvation is a thermodynamically driven self-assembly process for polymeric materials to prepare nanoparticles. The process includes three steps: protein dissolution, protein aggregation and protein deaggregation. The aggregated nanoparicles are crosslinked by using crosslinking agents like glutaraldehyde, sodium sulphate. A desolvating agent such as acetone, isopropanol or n-butanol can be used. The addition can be optimized turbidometriclly using nephlometer. Both lipophilic and hrdrophilic drugs can be entrapped in nanoparticles using this technique [4,5].

2. Materials

Drug: Ibuprofen (Gift sample)
Polymer: Sodium Alginate (1%), SD Fine Chemicals Limited, Mumbai.
Desolvating agent: Isopropanol, SD Fine Chem Limited, Mumbai.
Cross linking agent: 25% Glutaraldehyde, SD Fine Chem Limited, Mumbai.

2.1 Methodology

Desolvation technique was adopted for the preparation of Ibuprofen loaded sodium alginate nanoparticles. The processing parameters like concentration of the drug and polymer, speed of rotation were optimized. 1 % drug-polymer solution was prepared and its pH was adjusted to 4. The desolvating agent used was Isopropanol. The addition of desolvating agent to the drug-polymer solution was done by two methods, i.e.; continuous and intermittent, in which the solvent was added at the rate of 1ml/min and 1ml/ 5 min respectively. The appearance of turbidity in the solution was considered as the end point. Then, 3-4 drops of 25% glutaraldehyde was added. For complete cross linking, the stirring was continued for 12 hours. The solvent and water were removed from the resultant solution by means of rotary evaporator. The obtained free flowing powder was then characterized for particle size distribution to ensure that they were within nanosize range. Further, it was evaluated for following parameters like zeta potential, entrapment efficiency and invitro drug release [6,7].

2.2 Characterization Of The Nanoparticles [8,9]

Drug Content and Drug Entrapment Efficiency: [10,11]

Drug content was determined as follows

50 mg of the prepared drug loaded sodium alginate nanoparticles both by continuous and intermittent addition method, were dissolved in 50 ml of methanol and kept for stirring at 600 rpm for 3 hours respectively. The total amount of the drug in the nanoparticles was determined spectrophotometrically at 221 nm.

Entrapmetn efficiency and Loading capacity were determined as follows

50 mg of the prepared drug loaded sodium alginate nanoparticles both by continuous and intermittent addition method, were dissolved in 50 ml of 7.2 pH phosphate buffer and was kept for ultracentrifugation for 40 minutes respectively. Entrapment efficiency and loading Capacity of the nanoparticles were determined using the formula:

$$\text{Entrapment Efficiency} = \frac{\text{Total amount of the drug entrapped}}{\text{Total amount of the drug initially taken}} \times 100$$

$$\text{Loading Capacity} = \frac{\text{Total amount of the drug entrapped}}{\text{Total weight of the nanoparticles taken}} \times 100$$

Particle Size Analysis and Zeta Potential Measurement [12,13]

The average particle size and size distribution of Ibuprofen loaded Sodium alginate NP's were determined by dynamic light scattering (DLS), using Horiba Zetasizer.

The Zeta potential (Surface Charge) which indicates the stability of the NP's can be defined as electrokinetic potential that is determined by electrophoretic mobility. Samples were prepared by diluting with water and corresponding zeta pontential were measured using Horiba Zeta Sizer.

Determining the size and morphology of the nanoparticles [14]

Scanning Electron Microscopy is used to determine the shape, size and surface morphology of the nanoparticles. Nanoparticulate suspension is made to obtain Photomicrographs of the drug loaded sodium alginate nanoparticles using this SEM.

Fourier Transforms Infrared Spectroscopy [15]

FT-IR spectra were recorded on FT-IR spectrophotometer for the prepared drug loaded alginate nanoparticles. Any interactions between drug and the polymer occurred can be detected by these spectra.

***In Vitro* Drug Release Studies [16]**

The *invitro* drug release studies were carried out using Arbitary Shaker. The prepared nanoparticles were placed in conical flask and was dispersed using 50 ml of 7.4 pH buffer. The entire system was kept at 37 ± 0.5 ºC with the continuous stirring at 100 rpm. The samples were withdrawn at predetermined intervals and replaced by fresh medium simultaneously. The amount of drug released at specific time interval was determined with UV spectrophotometrically at 221 nm.

3. Results and discussions [17, 18]

The yield obtained for both the products i.e; drug loaded sodium alginate nanoparticles prepared by continuous addition method and intermittent addition methods were optimum. They were evaluated for above mentioned characters and the results obtained were as follows:

3.1 Drug Content of the formulations

The drug content for both the formulations was evaluated. It was observed that the NPs prepared by continuous addition method showed a higher drug content value i.e; 93.32%. NPs that prepared by intermittent addition method showed drug content value of 88.32%.

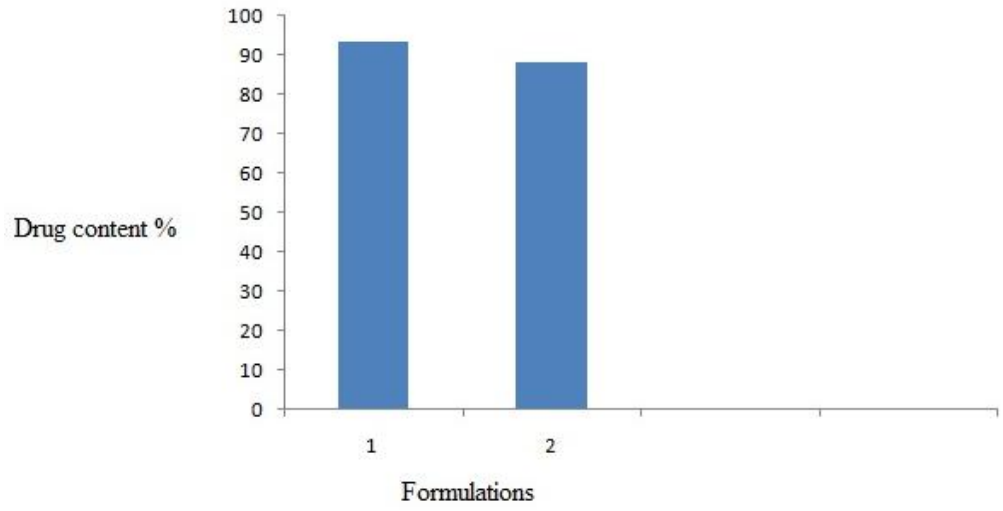


Fig.1: Drug Content of NPs prepared by Continuous addition of Isopropanol as desolvating agent

3.2 Entrapment Efficiency of the formulations

Entrapment efficiency and loading capacity were found to be more by Intermittent addition method i.e.; 76.84%

than continuous addition method i.e; 74.99%.

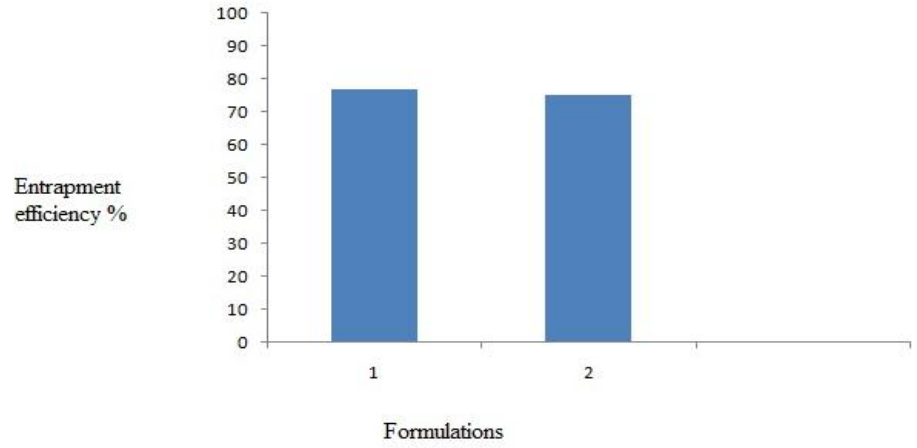


Fig.2 Entrapment Efficiency of NPs prepared by Continuous addition of Isopropanol as desolvating agent

3.3 Average particle size

The size distribution of the prepared nanoparticles along the mean diameter was measured using particle size analyser. The average particle size of the prepared drug loaded alginate nanoparticles were recorded as 289.9 nm and 289.2 nm for continuous addition and intermittent addition method respectively.

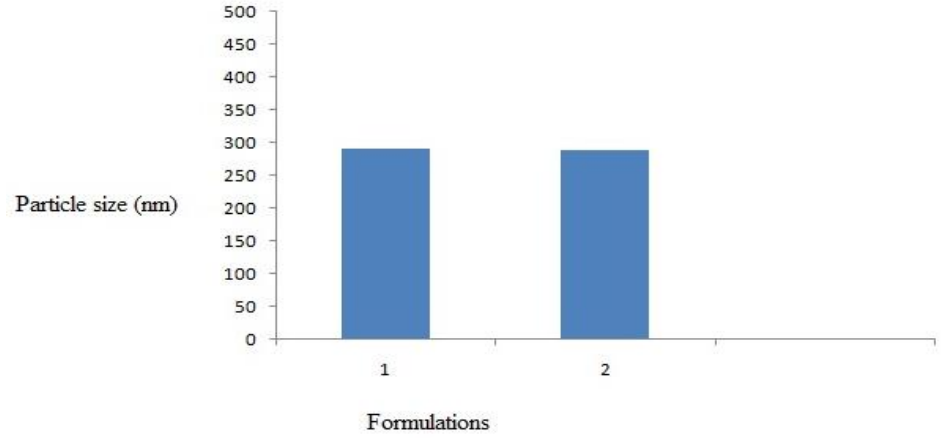


Fig.3 Mean particle diameter of NPs prepared by Continuous and Intermittent addition method.

3.4 Zeta Potential

Zeta Potential of the prepared drug loaded alginate nanoparticles measured using zeta meter indicated that the formulations showed optimum stability. NPs prepared from intermittent method showed higher stability, bearing a value of -49.9 mV.

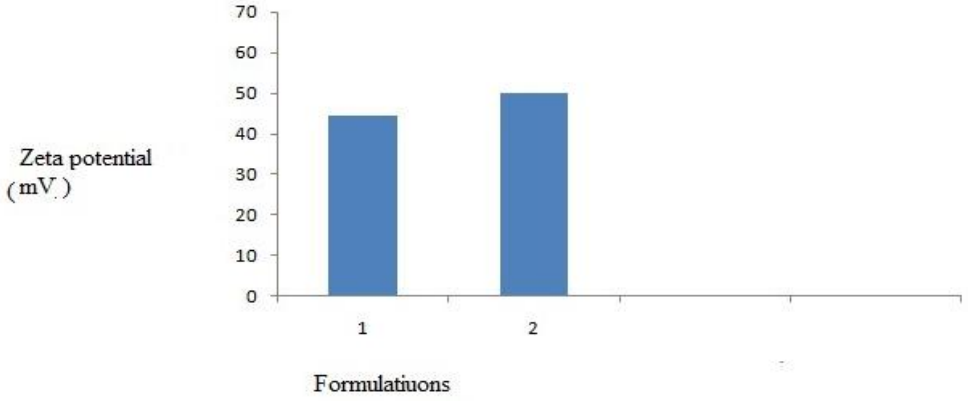


Fig.4 Zeta Potential of NPs prepared by Continuous addition and Intermittent addition method.

2014.03.27 14:15:19

201403271357000.nsz
Measurement Results

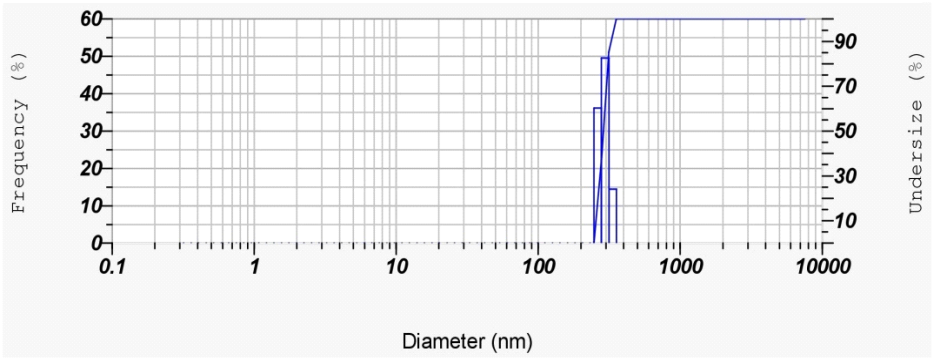
Date	:	27 March 2014 13:57:55
Measurement Type	:	Particle Size
Sample Name	:	Ibu-sod alg-IPA con
Scattering Angle	:	90
Temperature of the holder	:	25.0 deg. C
T% before meas.	:	34534
Viscosity of the dispersion medium	:	0.894 mPa.s
Form Of Distribution	:	|Standard|
Representation of result	:	Scattering Light Intensity
Count rate	:	1022 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	289.8 nm	24.2 nm	288.8 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	289.8 nm	24.2 nm	288.8 nm

Cumulant Operations

Z-Average : 289.8 nm



No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation
1	0.34	0.000	0.000	29	10.34	0.000	0.000	57	315.27	49.500	85.594
2	0.38	0.000	0.000	30	11.68	0.000	0.000	58	356.20	14.406	100.000
3	0.43	0.000	0.000	31	13.20	0.000	0.000	59	402.44	0.000	100.000
4	0.49	0.000	0.000	32	14.91	0.000	0.000	60	454.69	0.000	100.000
5	0.55	0.000	0.000	33	16.84	0.000	0.000	61	513.71	0.000	100.000
6	0.62	0.000	0.000	34	19.03	0.000	0.000	62	580.41	0.000	100.000
7	0.70	0.000	0.000	35	21.50	0.000	0.000	63	655.76	0.000	100.000
8	0.80	0.000	0.000	36	24.29	0.000	0.000	64	740.89	0.000	100.000
9	0.90	0.000	0.000	37	27.45	0.000	0.000	65	837.07	0.000	100.000
10	1.02	0.000	0.000	38	31.01	0.000	0.000	66	945.74	0.000	100.000
11	1.15	0.000	0.000	39	35.03	0.000	0.000	67	1068.52	0.000	100.000
12	1.30	0.000	0.000	40	39.58	0.000	0.000	68	1207.24	0.000	100.000
13	1.47	0.000	0.000	41	44.72	0.000	0.000	69	1363.97	0.000	100.000
14	1.66	0.000	0.000	42	50.53	0.000	0.000	70	1541.04	0.000	100.000
15	1.87	0.000	0.000	43	57.09	0.000	0.000	71	1741.10	0.000	100.000
16	2.11	0.000	0.000	44	64.50	0.000	0.000	72	1967.14	0.000	100.000
17	2.39	0.000	0.000	45	72.87	0.000	0.000	73	2222.51	0.000	100.000
18	2.70	0.000	0.000	46	82.33	0.000	0.000	74	2511.05	0.000	100.000
19	3.05	0.000	0.000	47	93.02	0.000	0.000	75	2837.04	0.000	100.000
20	3.45	0.000	0.000	48	105.10	0.000	0.000	76	3205.35	0.000	100.000
21	3.89	0.000	0.000	49	118.74	0.000	0.000	77	3621.48	0.000	100.000
22	4.40	0.000	0.000	50	134.16	0.000	0.000	78	4091.63	0.000	100.000
23	4.97	0.000	0.000	51	151.57	0.000	0.000	79	4622.81	0.000	100.000
24	5.61	0.000	0.000	52	171.25	0.000	0.000	80	5222.96	0.000	100.000
25	6.34	0.000	0.000	53	193.48	0.000	0.000	81	5901.02	0.000	100.000
26	7.17	0.000	0.000	54	218.60	0.000	0.000	82	6667.10	0.000	100.000
27	8.10	0.000	0.000	55	246.98	0.000	0.000	83	7532.65	0.000	100.000
28	9.15	0.000	0.000	56	279.04	36.093	36.093	84	8510.56	0.000	100.000

1 / 1

Fig.5 Mean Particle Diameter measurement for Sodium Alginate NPs prepared by Continuous addition method

2014.03.27 15:21:20

Measurement Results

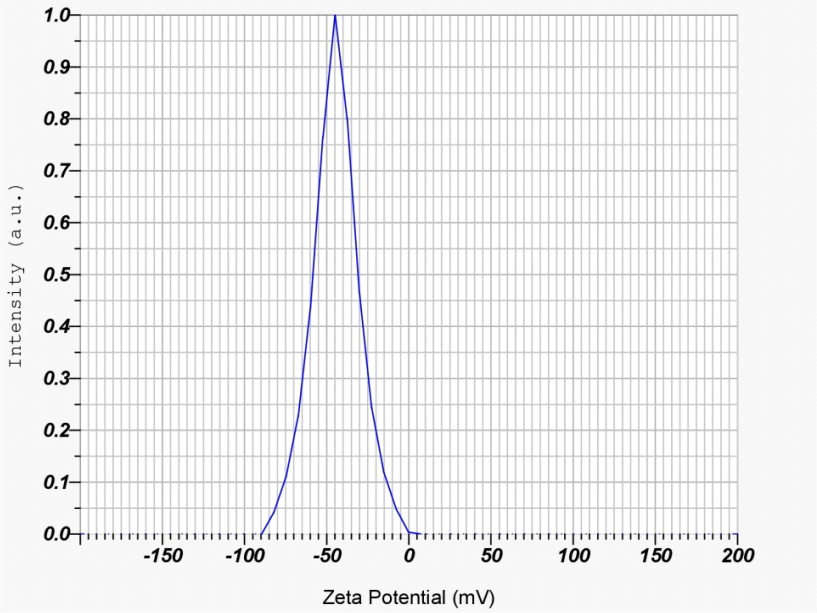
Measurement Results

Date	:	27 March 2014 15:20:09
Measurement Type	:	Zeta Potential
Sample Name	:	Ibu-sod alg-IPA Con
Temperature of the holder	:	25.0 deg. C
Viscosity of the dispersion medium	:	0.894 mPa.s
Conductivity	:	0.114 mS/cm
Electrode Voltage	:	3.4 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-44.5 mV	-0.000345 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	-44.5 mV
Electrophoretic Mobility mean	:	-0.000345 cm^2/Vs



1 / 1

Fig.6 Zeta Potential measurement for Sodium Alginate NPs prepared by Continuous addition method

2014.03.27 14:45:31

201403271421003.nsz
Measurement Results

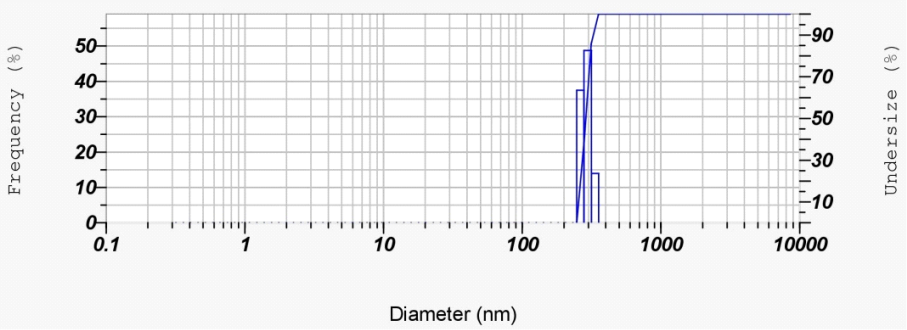
Date	:	27 March 2014 14:21:35
Measurement Type	:	Particle Size
Sample Name	:	Ibu-sod alg-IPA Int
Scattering Angle	:	90
Temperature of the holder	:	25.0 deg. C
T% before meas.	:	32449
Viscosity of the dispersion medium	:	0.894 mPa.s
Form Of Distribution	:	|Standard|
Representation of result	:	Scattering Light Intensity
Count rate	:	4215 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	289.2 nm	24.2 nm	288.0 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	289.2 nm	24.2 nm	288.0 nm

Cumulant Operations

Z-Average : 289.2 nm



No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation
1	0.34	0.000	0.000	29	10.34	0.000	0.000	57	315.27	48.680	86.095
2	0.38	0.000	0.000	30	11.68	0.000	0.000	58	356.20	13.905	100.000
3	0.43	0.000	0.000	31	13.20	0.000	0.000	59	402.44	0.000	100.000
4	0.49	0.000	0.000	32	14.91	0.000	0.000	60	454.69	0.000	100.000
5	0.55	0.000	0.000	33	16.84	0.000	0.000	61	513.71	0.000	100.000
6	0.62	0.000	0.000	34	19.03	0.000	0.000	62	580.41	0.000	100.000
7	0.70	0.000	0.000	35	21.50	0.000	0.000	63	655.76	0.000	100.000
8	0.80	0.000	0.000	36	24.29	0.000	0.000	64	740.89	0.000	100.000
9	0.90	0.000	0.000	37	27.45	0.000	0.000	65	837.07	0.000	100.000
10	1.02	0.000	0.000	38	31.01	0.000	0.000	66	945.74	0.000	100.000
11	1.15	0.000	0.000	39	35.03	0.000	0.000	67	1068.52	0.000	100.000
12	1.30	0.000	0.000	40	39.58	0.000	0.000	68	1207.24	0.000	100.000
13	1.47	0.000	0.000	41	44.72	0.000	0.000	69	1363.97	0.000	100.000
14	1.66	0.000	0.000	42	50.53	0.000	0.000	70	1541.04	0.000	100.000
15	1.87	0.000	0.000	43	57.09	0.000	0.000	71	1741.10	0.000	100.000
16	2.11	0.000	0.000	44	64.50	0.000	0.000	72	1967.14	0.000	100.000
17	2.39	0.000	0.000	45	72.87	0.000	0.000	73	2222.51	0.000	100.000
18	2.70	0.000	0.000	46	82.33	0.000	0.000	74	2511.05	0.000	100.000
19	3.05	0.000	0.000	47	93.02	0.000	0.000	75	2837.04	0.000	100.000
20	3.45	0.000	0.000	48	105.10	0.000	0.000	76	3205.35	0.000	100.000
21	3.89	0.000	0.000	49	118.74	0.000	0.000	77	3621.48	0.000	100.000
22	4.40	0.000	0.000	50	134.16	0.000	0.000	78	4091.63	0.000	100.000
23	4.97	0.000	0.000	51	151.57	0.000	0.000	79	4622.81	0.000	100.000
24	5.61	0.000	0.000	52	171.25	0.000	0.000	80	5222.96	0.000	100.000
25	6.34	0.000	0.000	53	193.48	0.000	0.000	81	5901.02	0.000	100.000
26	7.17	0.000	0.000	54	218.60	0.000	0.000	82	6667.10	0.000	100.000
27	8.10	0.000	0.000	55	246.98	0.000	0.000	83	7532.65	0.000	100.000
28	9.15	0.000	0.000	56	279.04	37.416	37.416	84	8510.56	0.000	100.000

1 / 1

Fig.7 Mean Particle Diameter measurement for Sodium Alginate NPs prepared by Intermittent addition method.

2014.03.27 14:56:16

Measurement Results

Ibu-sod alg-IPA Int.nzt

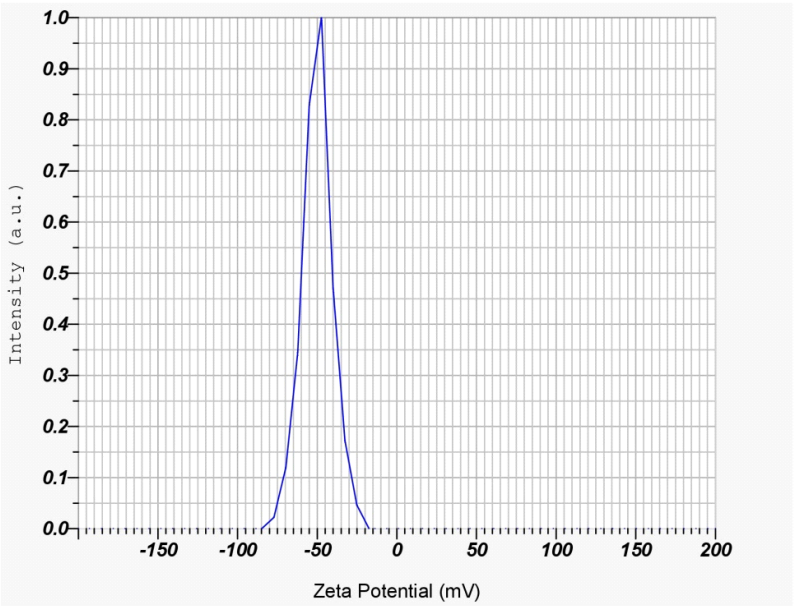
Measurement Results

Date	:	27 March 2014 14:55:03
Measurement Type	:	Zeta Potential
Sample Name	:	Ibu-sod alg-IPA Int
Temperature of the holder	:	25.0 deg. C
Viscosity of the dispersion medium	:	0.894 mPa.s
Conductivity	:	0.161 mS/cm
Electrode Voltage	:	3.4 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-49.9 mV	-0.000387 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	-49.9 mV
Electrophoretic Mobility mean	:	-0.000387 cm^2/Vs



1 / 1

Fig.8 Zeta Potential measurement for Sodium Alginate NPs prepared by Intermittent addition method

3.5 Size and Morphology of the nanoparticles

The morphological characteristics of the prepared NP's were examined using SEM. The images revealed that the particles were having smooth texture and were spherical in shape.

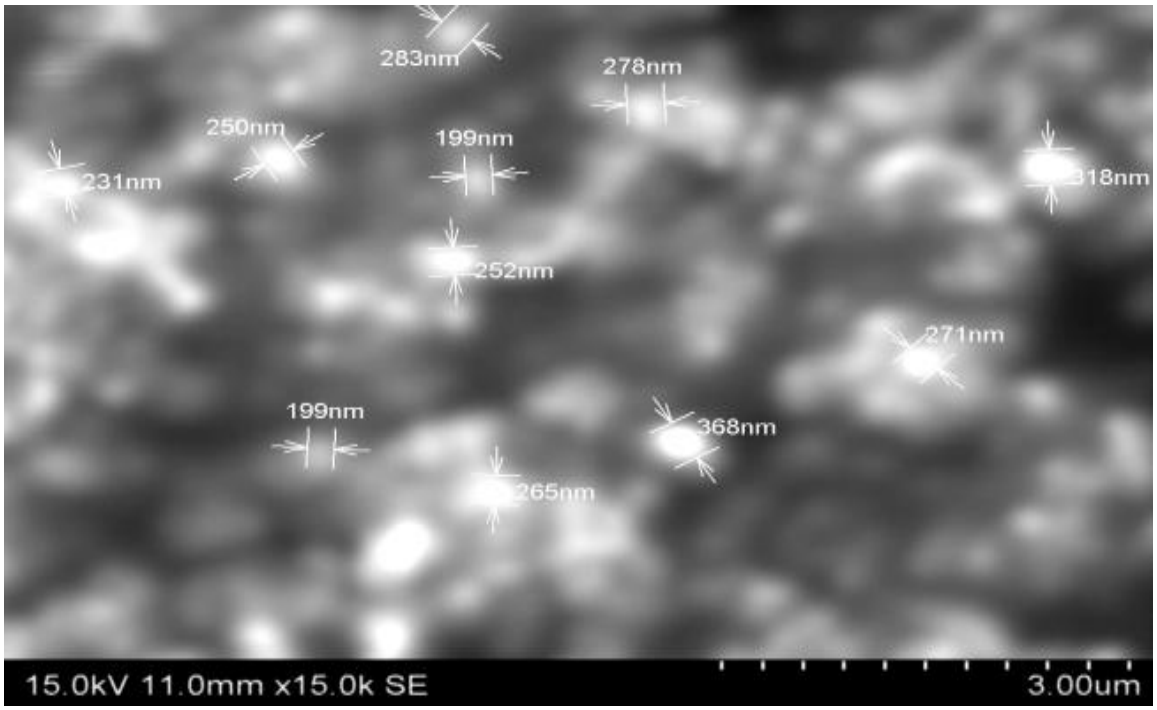


Fig.9 SEM of Drug loaded Alginate Nanoparticles By Continuous Addition method

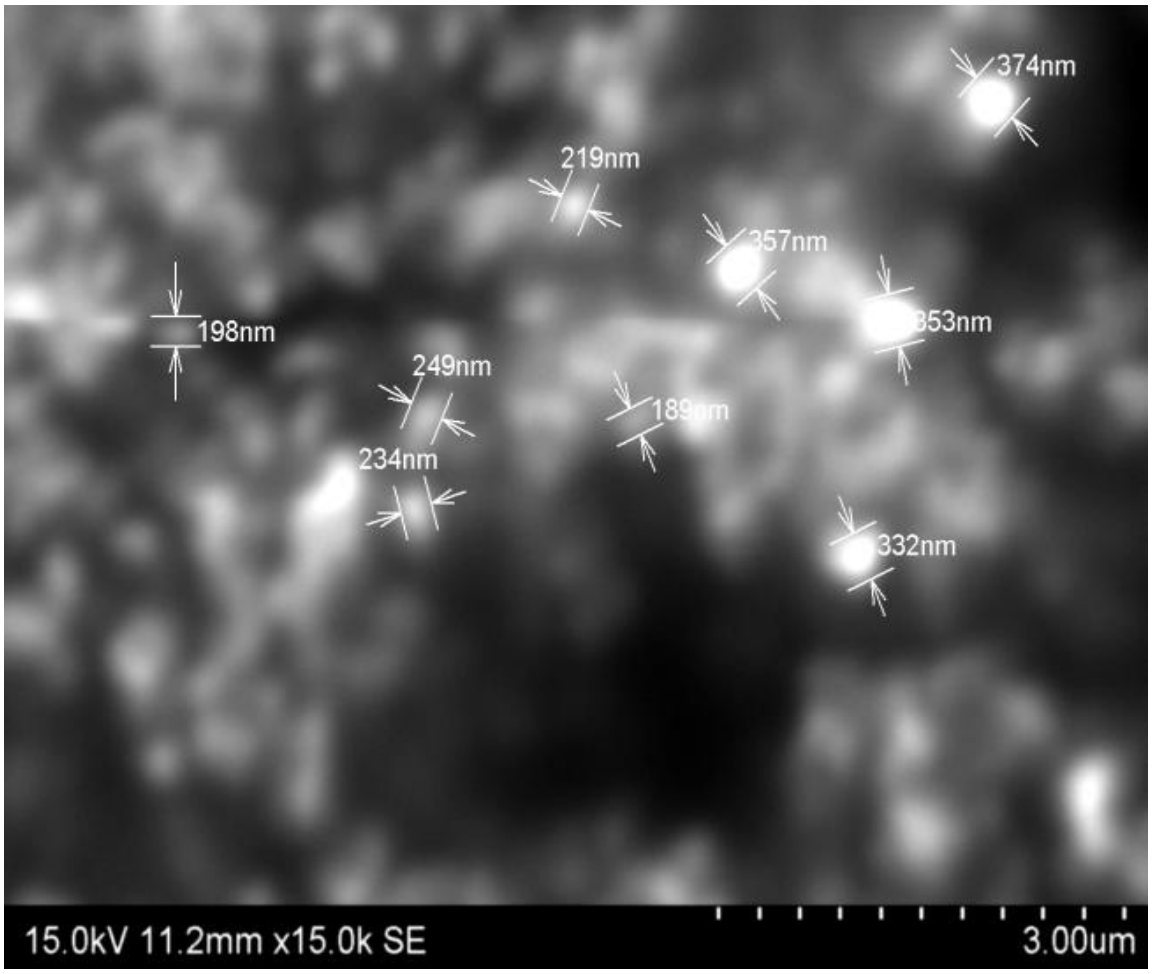


Fig.10 SEM of Drug loaded Alginate Nanoparticles By Intermittent Addition Method

FT-IR Spectra: From the FT-IR results obtained for sodium alginate nanoparticles prepared by continuous and intermittent method, it was confirmed that, there were no undesired interactions between the model drug chosen and the polymer.

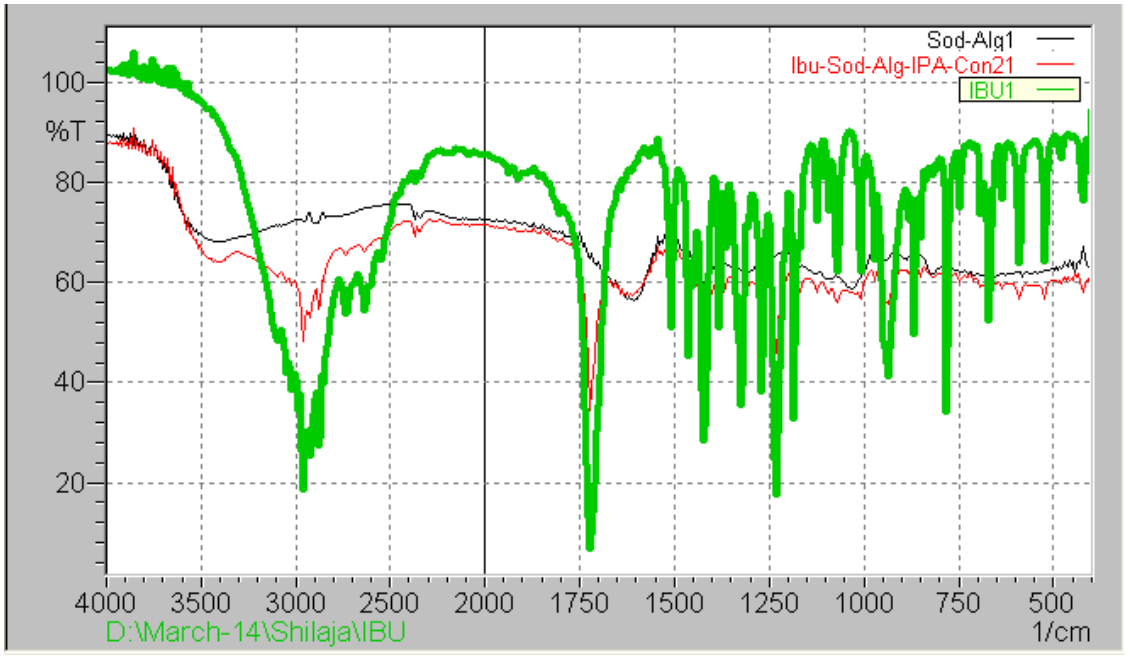


Fig.11 FT-IR of drug loaded alginate NPs (Continuous Addition Method)

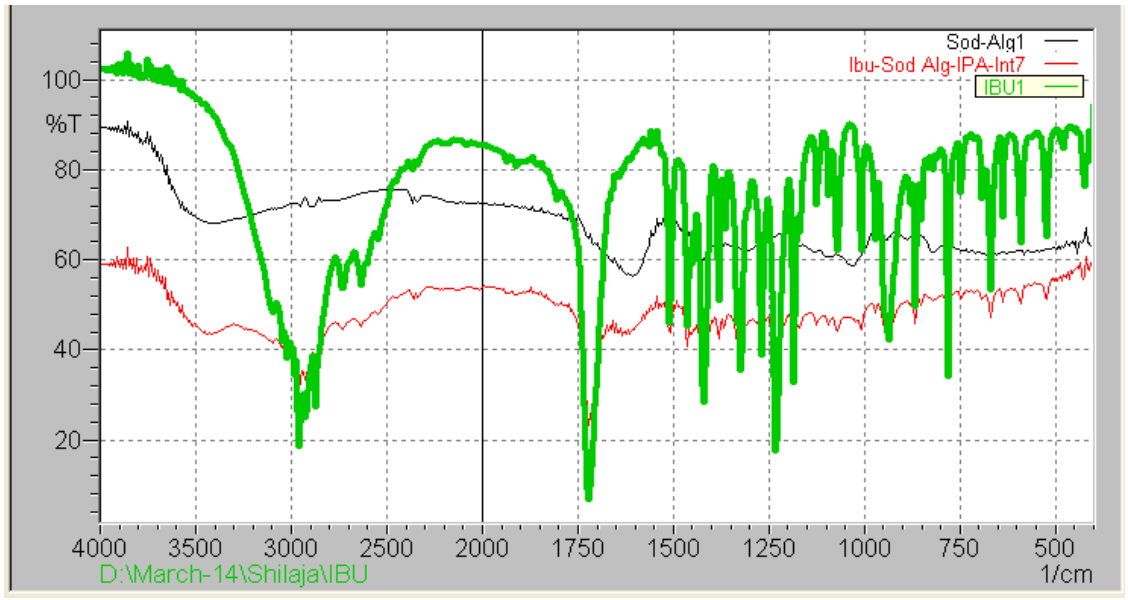


Fig.12 FT-IR of drug loaded alginate NPs(Intermittent addition method)

3.6 *In Vitro* Drug Release Studies

The *in vitro* release data of the two formulations were observed. The drug from the NPs prepared from continuous addition method showed drug release of 93% than from intermittent addition method that showed release of 98.53 % in 12 hours. From the various plots mentioned, it can be concluded that the drug release from the nanoparticles obeyed zero order kinetics following fickian diffusion mechanism.

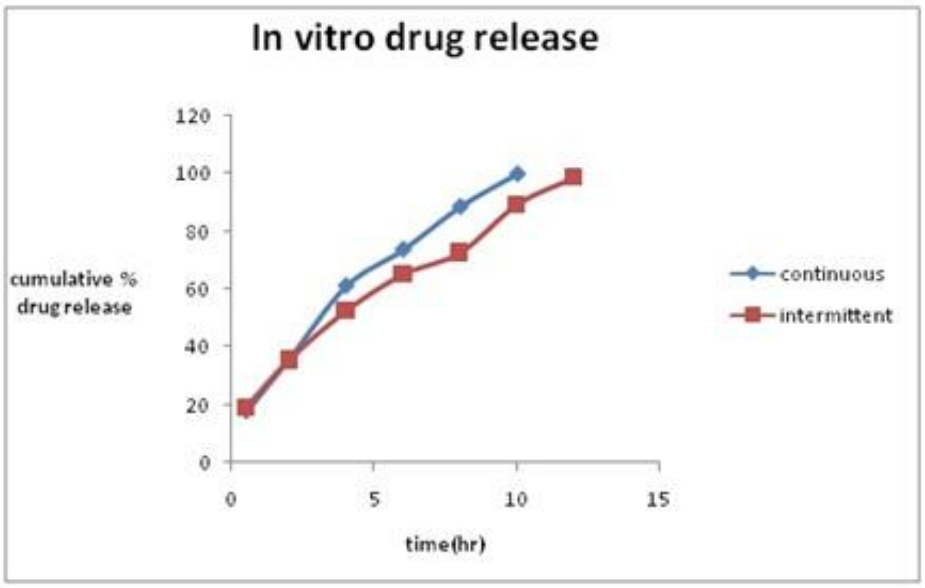


Fig.13 *In vitro* drug release pattern of Ibuprofen loaded sodium alginate nanoparticles by Desolvation technique

Table.1 Parameters determined from the Invitro Release Studies performed on Drug loaded alginate NPs

Formulation	Zero Order Plot	First order Plot	Peppas Plot(n)	Higuch Plot(R^2)
Continuous method	0.968	0.875	0.25	0.925
Intermittent method	0.977	0.935	0.235	0.981

4. Discussion

Ibuprofen loaded sodium alginate nanoparticles were prepared by desolvation technique using isopropanol as desolvating agent [19,20]. Two methods i.e; Continuous and intermittent addition methods were employed for the preparation of polymeric nanoparticles. Comparative study was performed to determine the effect of continuous and intermittent addition methods on particle size, average particle diameter, entrapment efficiency, loading capacity, zeta potential.

On comparision, intermittent addition method was resulting particles in nanosize. May be intermittent method give more time for the desolvation process to occur, more stable nanoparticles were obtained with intermiitent addition method. Zeta potential represents index for particle stability.

The amount of drug entrapped and drug loading capacity of the polymer were found to be more for intermittent addition method than continuous addition method. It may be because of the higher drug content and mazximum product yield for the nanoparticles prepared by intermiitent addition method than continuous addition method.

Invitro drug release studies were performed for both the formulations. In intermittent addition method, the drug release was continued for a period of 6 hours. It may be because of the smaller particle size. Smaller particle size provides maximum surface area, hence, the drug release was found to maximum in a period of 6 hours. In continuous addition method, the drug release was sustained upto 8 hours. Sustained release nature was because of the large diameter of the particle.

In order to determine the order of drug release and mechanism of drug release, several plots were plotted [21,22]. From the plots, it was observed that the drug release follows zero order kinetics. The percentage of drug release is independent of drug concentration. From the Higuchi and Peppas plot, it can be concluded that mode of drug release is Fickian diffusion controlled [23,24].

5. Conclusions

From the results, it can be concluded that intermittent addition method is considered to be better than continuous addition method. So, Ibuprofen loaded sodium alginate nanoparticles can be better prepared by intermittent addition method to achieve targeted drug delivery for the treatment of Rheumatoid Arthritis and Ankylosing Spondylitis.

References

[1] Wim H De Jong, Paul Ja Borm. Drug delivery and nanoparticles : Applications and Hazards, *Int J Nanomedicine*. June 2008; 3(2): 133-149.

[2] Vyas S P, Khar R K, Targeted & Controlled Drug Delivery, Novel Carrier Systems, CBS publicarion, 2002, 331-387.

[3] Kumaresh S. Sppinath, Tejraj M.Aminabhavi, Anandrao R.Kulkarni, Walter E. Rudzinski. Biodegradable polymeric nanoparticles as drug delivery devices, *Journal of Controlled Release*. June 2000; 70(2001): 1-20.

[4] Krishna Sailaja A., Amareshwar P., Chakravarty P. Different Techniques for the preparation of Nanoparticles using natural polymers and their application, *International Journal of Pharmacy and Pharmaceutical Sciences*. 2011; 3(2).

[5] Jiang B, Hu L, Gao C, Shen J. Ibuprofen loaded nanoparticles prepared by a co-precipitation method and their release properties, *Int J Pharm*. Nov 2005; 304(1-2): 220-30.

[6] Gupta Dilip Kumar, Razdan B.K. Bajpai Meenakshi. Formulation and Evaluation of nanoparticles containing Artemisinin HCL, *International Journal of Research and Development in Pharmacy and Life Sciences*. March 2014; 3(2): 925-934.

[7] Krishna Sailaja A., P. Amareshwar. Preparation of alginate nanoparticles by desovaltion technique using acetone as desolvating agent. *Asian Journal of Pharmaceutical and Clinical Research.* 2012; 5(2).

[8] Merodio M,. Arnedo A, Renedo M.J. Irache J.M, Ganciclovir-loaded albumin nanoparticles: characterization and in vitro release properties, *European J of Pharmceutical Sciences*, 2001; 12(3): 251.

[9] Barratt G.M, Therapeutic Applications of colloidal drug carriers, *Pharm. Sci. Technol. Today* 3, 163-171.

[10] The Merck Index. An Encyclopedia of Chemicals, drug and biological, Merck Laboratories, 13th Edition. 2001. Pp139.

[11] Saeed Moradhaseli, Abbas Zare Mirakabadi, Naseer Mohammadour dounighi, Mehrasa Rahimi Borumand. Preparation and Characterization of Soium alginate Nanoparticles Containing ICD-85(Venom Derived Peptides): International *Journal of Innovation and Applied Studies*, Nov 2013, 4(3): 534-542.

[12] Storp Bv, Engel A, Boeker A, Ploeger M, Langer K. Albumin nanoparticles with predictable size by desolvation procedure: *Journal of Microencapsulation*, 2012; 29(2): 138-46.

[13] Weber C, Coester C, Kreuter J, Langer K. Desovation process and surface characterization of protein nanoparticles. *Int J Pharm*, Jan 2000; 194(1): 91-102.

[14] Muller B.G, Leuenberger H, Kissel T. Albumin nanospheres as Carriers for Passive Drug Targeting: An optimized manufacturing Technique. *Pharmaceutical Research*, 1996; 13(1): 32-37.

[15] Krishna Sailaja A., Amareshwar P. and Jayaprakash D. Preparation of Aspirin loaded BSA nanoparticles by Desolvation Technique using Sodium Sulphate as Desolvating Agent, *International Journal of Pharmacy and Integrated Life Sciences*. V1 (I3):69-75.

[16] Radhika Ramachandran and Piranamayagam Shanmughavel. Preparation and characterization of biopolymeric nanoparticles fro Drug-delivery, *Indian Journal of Biochemistry and Biophysics*. 2010; 47:56-59.

[17] Bahareh Azimi, Parviz Nourpanah, Mohammad Rabiee and Shahram Arbab. Producing Gelatin Nanoparticless as Delivery System for Bovine Serum Albumin, *Iranina Biomedical Journal*. 2014; 18(1): 34-40.

[18] Mohsen Jahanshahi and Zahra Babaei Protein nanoparticle: A unique system as drug delivery vehicles *African Journal of Biotechnology*. 2008; 7 (25): 4926-4934.

[19] Mohanraj VJ, Chen Y Nanoparticles – A Review. *Trop. J. Pharm. Res*. 2006; 5(1): 561-573

[20] Rahimnejad M., M. Jahanshahi, G. D. Najafpour. Production of biological nanoparticles from bovine serum albumin for drug delivery. *African Journal of Biotechnology*. 2006; 5 (20): 1918-1923.

[21] Amir Maghsoudi, Seyed Abbas Shojaosadati and Ebrahim Vasheghani Farahani. 5-Fluorouracil-Loaded BSA Nanoparticles: Formulation Optimization and In Vitro Release Study *AAPS PharmSciTech*, December 2008; 9 (4): 1092-1096.

[22] Coester CJ, Langer K, Van Briesen H, Kreuter J Gelatin nanoparticles by two-step desolvation-a new preparation method, surface modifications and cell uptake. *J. Microencapsul*. 2000; 17: 187-193.

[23] Dongmei Zhao, Xiuhua Zhao, Yuangang Zu, Jialei Li Preparation, characterization, and in vitro targeted delivery of folate-decorated paclitaxel-loaded bovine serum albumin nanoparticles. *International Journal of Nanomedicine* 2010; 5: 669–677.

[24] Truong-Le VL, Walsh SM, Guggino WB, August JT, Leong KW. Gene transfer by DNA-Gelatin nanospheres, *Arch. Biochem. Biophys*. 1999; 361(1): 47-56.