DETAILS OF THE MANUSCRIPT

Preparation and characterization of nanoparticulate drug delivery system for Naproxen Sodium using acetonitrile as desolvating Agent

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DECLARATION

Ethics approval and consent for participation:-Not applicable

Consent for publication:-Not applicable

Availability of data and material:-Data is available

Competing Interest:- Not interested

Funding:-Not applicable

Author's contribution:-All authors have read and approved the manuscript**.**

AKS developed the concept and reviewed the manuscript

HS executed the work

Acknowledgements:-Both authors thank the management, RBVRR Women's college of pharmacy for supporting the work.

Total no Figures 8
Total no of Tables 1
Total no of words 3484

ABSTRACT: Naproxen sodium is proficient of providing benefit to patients suffering from: rheumatoid arthritis, osteoarthritis, juvenile arthritis and ankylosing spondylitis.

In this work naproxen nanoparticles were prepared using acetonitrile as desolvating agent. For this continuous addition and Intermittent addition methods were adapted The obtained formulations were studied for characterization and evaluation parameters. Among all six formulations F1 formulation prepared by using acetonitrile as desolvating agent at 700 rpm was showing promising results with drug content, entrapment efficiency, loading capacity, particle size, zeta potential and in vitro drug release as 90.06%, 73.18%, 16.1%, 462.1nm, -21.7mv and drug release of 91.32% were able to sustain the drug release for 12 hours following zero order release rate constant with non -fickian diffusion mechanism.Naproxen nano particles were prepared successfully by Intermittent addition method using acetonitrile as desolvating agent.In summary the study successfully prepared naproxen nanoparticles using the Intermittent addition method with acetonitrile as the desolvating agent.The F1 formulation displayed promising characteristics and drug release behaviour, which suggests its potential as an effective treatment optionfor patients suffering from various forms of arthritis. However, further studies and clinical trials would be required to validate the efficacy and safety of these nanoparticles in real-world settings before they can be considered for clinical use.

KEYWORDS: Desolvation technique, Drug release, Particle size, Zeta potential,

INTRODUCTION

Nanoparticles may be described as particulate dispersions with a size in the range of 10-100nm.[1,2] In the preparation of nanoparticles the drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix Nanoparticles display new or improved properties based on specific characteristics such as size, distribution and morphology[3,4].

Naproxen is a member of the 2-arylpropionic acid family of NSAIDS having analgesic, anti-inflammatory and antipyretic activity.Naproxen sodium is capable for providing benefit to patients experiencingfrom: rheumatoid arthritis, osteoarthritis, juvenile arthritis, tendonitis, bursitis and acute gout. Naproxen is available in the form of tablets, capsules, delayed release

tablets, extended release tablets and capsules. Naproxen principally acts by inhibiting cox1 and cox2 receptors[5, 6].

On oral administration Naproxen causes gastrointestinal bleeding and peptic ulcers by blocking Cox1 enzyme. It increases blood pressure and cardiovascular problems by blocking Cox2 enzyme.It acts by blocking Cox1 and Cox2 enzymes it causes cardiac problems[7].

Consequently there is a need to develop novel drug delivery system for naproxen to limit the adverse effects and to reduce the frequency of administration.[21] In order to avoid Naproxen side effects attempts have been made to prepare controlled release naproxen nanoparticle drug delivery system.[21] The naproxen Sodium Salt is explicitly shown in the treatment of different kinds of acute and very high intensity pain since it induces a rapid and sustained remission.[23]likewise, it is conceivable to get a good analgesic effect with few administrations, because of naproxen's specific pharmacokinetics[7,8].
Bovine Serum Albumin (BSA) is a macromolecular carrier and is widely used to prepare nanoparticles, due to its biodegradability, non toxicity and non immunogenicity.[9] BSA can be considered as an attractive polymer that can be used as a good carrier for drugs[9].

The goal of the study was to prepare Naproxen sodium loaded BSA nanoparticles by desolvation technique. Acetonitrile was selected as desolvating agent The impact of addition of desolvating agent on the particle size was considered.

Depending upon the technique for preparation, nanoparticles, nanospheres or nanocapsules can be achieved.[10]

DESOLVATION METHOD

Desolvation is simply the thermodynamically determined self-assembly process for polymeric materials. The addition of desolvating agents like acetonitrile, acetone, sodium sulphate, and ethanol coacervates the polymeric materials. The polymeric molecules form particles of different sizes depending on the parameters like pH, ionic strength, cross linking agent concentration, agitation speed, quantity of desolvating agent added[11].

It contains three steps. They are:

1. Protein dissolution.
2. Protein aggregation.
3. Protein deaggergation.

With applicable levels of desolvation and resolvation, appropriate size of the particles is maintained. The aggregated particles were cross linked using glutaraldehyde.
The amphiphilic macromolecular cross linking method (Desolvation) is commonly utilized for naturally occurring polymers.

MATERIALS AND METHODS

Materials:- Naproxen was purchased from Hetero Chemicals Pvt. Ltd. Hyderabad. Bovine Serum Albumin was purchased from Hi-Media Laboratories Pvt Ltd., Mumbai. Glutraldehyde Solution 25% obtained from Sd Fine-Chem. Limited, Mumbai.

Methodology:-Naproxen sodium nanoparticles were prepared by desolvation technique, BSA polymer was designated for this method, and acetonitrile was used as a desolvating agent.
Naproxen sodium loaded BSA nanoparticles were prepared at various drug and polymer concentrations i.e.(1:1, 1:2 and 1:3) and two techniques i.e., Continuous and intermittent addition methods were utilized for the preparation of polymeric nanoparticles.
For preparing nanoparticles continuous addition method and Intermittent addition methods were adapted[12]

- **Continuous addition method:** In this method, the desolvating agent was added at the rate of 1 mL/min until the turbidity appears[13]

- **Intermittent addition method:** here the rate of addition is 1 ml per every 5 minutes

PROCEDURE

Aqueous drug polymer dispersion was prepared and pH was adjusted to 7 (away from the iso-electric point).[14] The desolvating agent was added under continuous mechanical stirring at 700 rpm. In continuous addition method the desolvating agent was added at a rate of 1 ml per minute. In intermittent addition method the desolvating agent was added at a rate of 1 ml per 5 minutes.

The development of insoluble precipitate was seems the end point of the reaction. A crosslinking agent (Gluteraldehyde 25%) was added and stirring was carried out for next 8 hours. [7,21]The solvent was removed by vaccum rotary evaporator at a vacuum pressure of 760 mmHg. Free flowing nanoparticles were acquired. A complete of six formulations were prepared by continuous and intermittent addition of acetonitrile as desolvating agent.[15]

EVALUATION STUDIES OF NANOPARTICLES

The obtained formulations of technique are evaluated for the subsequent parameters:

Determination of Drug Content

Free drug of the formulations was first determined in the supernatant by selecting a solvent during which solely the free drug gets dissolved and not the other ingredients.[12] To determine the drug content, 50 mg drug equivalent to formulation was weighed precisely and transferred into100 ml beaker containing 50 ml of methanol. The solution was stirred at 700 rpm for 3 hrs by utilizing magnetic stirrer. The resultant solution was filtered and the quantity of the drug in the filtrate was estimated once appropriate dilution by UV spectrophotometer at 271 nm.[16]

Entrapment Efficiency

Entrapment efficiencydemonstrates the quantity of the drug encapsulated within the formulation.[16].

Percentage entrapment efficiency is also calculated from the subsequent formula:

Entrapment efficiency:

$$= \frac{\text{Amount of drug encapsulated in the formulation}}{\text{Total amount of drug in the formulation}} \times 100$$

Loading Capacity

It indicates the capability of the polymer to load a drug.

Loading capacity may be calculated from the subsequent formula:

Loading capacity:

$$= \frac{\text{Total amount of the drug- amount of free drug concentration}}{\text{Nanoparticles weight}} \times 100$$

In Vitro Drug Release Study

Orbitary shaker method was adapted for conducting invitro dissolution studies. 50 mg of each precisely weighed formulation was transferred into 250 ml conical flask containing 50 ml pH 7.4 phosphate buffer.[17] They were kept in an orbitary shaker at 100 rpm maintained at 37°C. Aliquotsof 2 ml buffer was withdrawn at predefined time intervals and also the medium was replaced with same volumeof buffer.[18] This study was administrated for 12 hours, and also the quantity of drug release was estimated by determining the absorbance at 271 nm using Elico UV spectrophotometer[17].

CHARATERIZATION OF NANOPARTICLES

The researchers prepared and characterized Naproxen sodium loaded BSA (bovine serum albumin) nanoparticles using the desolvation technique. The following methods were employed for characterization

Drug-excipient interactions: To investigate drug-excipient interactions, the prepared nanoparticles of Naproxen sodium and BSA were mixed separately with IR grade KBr (potassium bromide) and compressed into pellets using 8000 metric tons of pressure in a hydraulic press. The resulting pellets were scanned using Fourier Transform Infrared (FTIR) spectroscopy over a wave number range of 4000 to 400 cm−1 in an FTIR instrument. This technique helps to identify any potential interactions between the drug and the excipient.[18]

Determining the size and morphology of the nanoparticles:

Scanning electron microscopy (SEM) was used to determine the shape, size and surface morphology of the Naproxen polymeric nanoparticles.[21] The processinvolved dispersing the prepared amorphous nanoparticles in deionised water and subjecting them to sonication for 30 minutes to ensure proper dispersion.[19] A circular metal plate was taken and a carbon double tape (1 mm×1 mm) was stuck onto it.A drop of the resultant nanoparticle dispersion was placed on to

the tape and allowed to dry for a while. The sample was then scanned under SEM to visualize the morphology of the nanoparticles.

Particle size analysis and zeta potential measurement:

Particle size and zeta potential measurement: The particle size and zeta potential of the nanoparticles were determined using a Zetasizer. Zeta potential is a measure of the surface charge of the nanoparticles, which plays a crucial role in stability and interaction with the surrounding medium.

The results of the evaluation and characterization of the nanoparticles include information on particle size, drug content, entrapment efficiency, loading capacity, surface morphology, and zeta potential. These results provide valuable insights into the properties and performance of the Naproxen sodium loaded BSA nanoparticles prepared by desolvation technique.

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RESULTS AND DISCUSSION

Evaluation and characterization of Naproxen sodium loaded BSA nanoparticles by Desolvation technique

The obtained nanoparticles were evaluated for particle size, drug content, entrapment efficiency and loading capacity

Evaluation and characterization of Naproxen Nanoparticles

Scanning Electron Microscopy: It is a powerful imaging technique used to observe the surface morphology of various materials at high magnification.Scanning Electron Microscopy (SEM) is. In this context, SEM was employed to examine the surface morphology of bovine serum albumin

nanoparticles prepared using the desolvation technique.The desolvation technique is a method used to produce nanoparticles from a protein solution. In this process, a protein solution, in this case, bovine serum albumin, is mixed with a non-solvent (desolvating agent) that causes the protein to precipitate and form nanoparticles. To characterize the nanoparticles, the optimized formulations were examined using the Hitachi S-3700N SEM instrument, located in Mumbai. The S-3700N is a specific model of the SEM made by Hitachi, a leading manufacturer of scientific and industrial equipment**.**During SEM imaging, a focused beam of electrons is scanned over the surface of the nanoparticles. When the electrons interact with the sample's surface, various signals are generated, such as secondary electrons and backscattered electrons. These signals are detected and used to create high-resolution images of the sample's surface, providing information about its topography and texture**.**By utilizing SEM, researchers can visualize the morphology of the bovine serum albumin nanoparticles, allowing them to assess the particle size, shape, and distribution. This information is crucial for understanding the quality and characteristics of the prepared nanoparticles, which is essential for their application in various fields, such as drug delivery, biotechnology, or material science. The bovine serum albumin nanoparticles prepared by desolvation technique. The optimized formulations were characterized for surface morphology using Scanning electron microscopy (S-3700N, Hitachi, Mumbai)[20].

The SEM images are illustrated in Figure-1.

Figure 1:- SEM Image of the nanoformulation

2. Fourier transforms infrared spectroscopy (FTIR):

Drug and polymer compatibility study was performed using IR Spectrophotometer.

In the FTIR spectrum C-H stretching vibrations at 3153- 3059 cm-1, C- O stretching vibrations at 1303-1000 cm-1, aromatic C=C stretching vibration at 1631 cm-1and CH3 bending vibration at 1363 cm-1, indicating the significant peaks of Naproxen sodium. Thus no drug-polymer interactions observed as shown in figure -2.

Figure-2: FTIR spectra of Naproxen loaded BSA nanoparticles using desolvation technique

3. Mean Particle Size: The mean particle diameter of the optimized formulations of bovine serum albumin nanoparticles was evaluated using a Malvern Zetasizer. The Zetasizer is a widely used instrument for measuring particle size in the nano range. All the formulations were found to have particle sizes within the nano range, which is desirable for many applications. Among all the formulations, F1 formulation exhibited the smallest particle size, measuring approximately 462.1 nanometers (nm). This indicates that F1 had the smallest average diameter compared to the other formulations, making it potentially more suitable for certain applications that require smaller particle sizes.

4.Zeta Potential: Zeta potential is a measure of the electrostatic charge on the surface of nanoparticles and is a critical factor in determining their stability. The zeta potential of the optimized formulations of bovine serum albumin nanoparticles was characterized using the Zetasizer at a temperature of 25°C, with double distilled water used as the dispersion medium. Among all the formulations, F1 showed a zeta potential value of -21.7 millivolts (mV). The negative zeta potential indicates that the surface of F1 nanoparticles is negatively charged. This value is significant because a higher absolute value of zeta potential typically indicates better stability. Thus, F1 demonstrated better stability compared to the other formulations, possibly due to its relatively higher negative charge.

5 Product Yield: Product yield, also known as percentage yield, is a measure of the efficiency of the nanoparticle preparation process. It is calculated by comparing the actual amount of nanoparticles obtained to the theoretical amount that could be obtained based on the starting materials.

By weighing the dried nanoparticles and using the formula above, researchers can determine the percentage yield of the prepared nanoparticles. A higher yield indicates a more efficient preparation process and a lower amount of

The yields of prepared nanoparticles were calculated using the following formula:

Product yield =

Amount of nanoparticles obtained (g)

---------- X100

Theoretical amount (g)

Product yields of six BSA formulations were compared. They were found to be 85.76%, 78.18%, 52.61%, 60.58%, 79.11%, 89.72% as shown in Figure 3

Figure-3: Comparison of product yields of BSA nanoparticles prepared by continuous and intermittent additions of acetonitrile as desolvating agent.

5. Drug content: The drug content of all the six formulations were compared. They were found to be 90.06%, 83.46%, 93.37%, 95.34%, 83.80%, 98.39%.were shown in Figure 4.

Figure-4: Comparison of Drug content of BSA nanoparticles prepared by continuous and intermittent additions of acetonitrile as desolvating agent.

6. Entrapment efficiency: Entrapment efficiencies of all six formulations were compared. They were found to be 73.18%, 46.93%, 79.14%, 73.43%, 87.27%,20.46% as shown in figure 5

Figure-5: Comparison of Entrapment efficiency of BSA nanoparticles prepared by continuous and intermittent additions of acetonitrile as desolvating agent.

7. Loading capacity: It indicates the capacity of the polymer to load a drug. Loading capacities were found to be 16.1 %, 15.3%, 12.9%, 14.1%, 14.4%, 16.1% as shown in figure 6.

Figure-6: Comparison of loading capacity of BSA nanoparticles

8. In-vitro drug release studies: The drug release studies were conducted by means of orbitary shaker for a time period of 12 hours. From the drug release studies as depicted in figure- 7.They were found to be 91.32%, 80.50%, 73.97%, 88.20, 71.45%, 94.11% within a period of 12 hours.

Figure-7: Comparison of Invitro drug release profiles

9. Kinetics of drug release:

Different plots (zero order, first order, Higuchi and Korsmeyer-Peppas plots) were drawn for the optimized formulation (F1), in order to show the release kinetics of the drug. According to the

kinetic plots, the optimized formulation F1 was following the zero order release with non fickian diffusion mechanism as shown in figure 8 and table 1.

Figure-8: Zero order release data for optimized formulation F1.

Table 1: Kinetic release data for optimized formulation F1.

Formulation	Zero order (R^2)	First order (R^2)	Higuchi plot (R^2)	Peppas plot (n)
F1	**0.886**	**0.786**	**0.832**	**0.693**

DISCUSSION

The study aimed to determine the effect of continuous addition method and intermittent addition method on various parameters during the preparation of Naproxen sodium nanoparticles using the desolvation technique with acetonitrile as the desolvating agent. Serum Albumin (BSA) was used as the natural polymer for this study. The researchers found that pH played a crucial role in controlling the coagulation of BSA molecules during the desolvation process. At a pH close to the iso-electric point (pI) of BSA (around 4.7), the coagulation among BSA molecules increased due to enhanced protein-protein reactions. However, at pH 7, BSA molecules possessed a negative charge, leading to reduced coagulation and the formation of smaller particles.

In their experimentation, the researchers identified the best formulation (F1) with a 1:1 ratio, which exhibited the smallest mean particle size diameter (462.1 nm) and a zeta potential of -21.7 mv. The optimized formulation showed a percentage yield of 85.76%, drug content of 90.06%, entrapment efficiency of 73.18%, and loading capacity of 16.1%. The drug release from the best formulation was sustained up to 12 hours with 91.32% of the drug being released. The drug release kinetics of the optimized formulation followed zero-order release, indicating a sustained and controlled release pattern. The R2 value of 0.886 indicated that the drug release mechanism was non-fickian mechanism.

CONCLUSIONS

The researchers concluded that both continuous addition and intermittent addition methods were effective for the preparation of Naproxen nanoparticles using the desolvation technique. The results obtained in their study aligned with existing literature available on the desolvation technique.In summary, the study successfully prepared and evaluated Naproxen nanoparticles using Bovine Serum Albumin and acetonitrile as the desolvating agent.

Acknowledgments: The authors sincerely thank Dr. M. Sumakanth, The Principal, RBVRR Women's college of Pharmacy for providing access for library and databases. We also thank Mrs. Suvarna and Mrs. Sumalatha, for providing technical assistance.

REFERENCES

1. Weber C, Coester C, Kreuter J, Langer K. Desolvation process and surface characterisation of protein nanoparticles. *International Journal of Pharmaceutics*. 2000;194(1): 91-102.
2. Hemant K.S. Yadav.Different techniques for preparation of polymeric nanoparticles- A Review. *Asian Journal of Pharmaceutical and Clinical Research*. 2012; Vol 5: Suppl 3.
3. A.K. Sailaja, P. Amareshwar, and P. Chakravarty. Different techniques used for the preparation of nanoparticles usingnatural polymers and their applications. *Internationaljournal of pharmacy and pharmaceutical sciences*. 2011;3(2): 45A-50A.
4. K. Langer, S. Balthasar, V. Vogel, et al.Optimization of the preparation process for human serum albumin (HSA)Nanoparticles. *Int J Pharm*, 2003;257(1-2): 169-180.
5. Rahimnejad M., M. Jahanshahi*, G. D. Najafpour. Production of biological nanoparticles from bovin serum albumin for drug delivery. *African Journal of Biotechnology*, 2006; Vol. 5 (20): pp. 1918-1923.
6. Weber C, Coester C, Kreuter J, Langer K. Desovation process and surface characterization of protein nanoparticles. *Int J Pharm*. 2000;194(1): 91-102.
7. Kreuter, J.O. Historical Perspectives Nanoparticles —a historical perspective. International Journal of Pharmaceutics.2007;331: p. 1-10.

8. Renutiruwa. A review on nanoparticles, *Indian Journal of Pharmaceutical and Biological Research*,2015; 4(2):27-31.
9. A.Krishna sailaja, P.Amareshwar. "Preparation of chitosan coated nanoparticles by emulsion polymerization technique". *Asian journal al of pharmaceutical and clinical research*.2011 vol 4 supple 1, pp 73-74.
10. Zhang, H.M. and Lou, K.; Cao, J.; Wang. Interaction of a hydrophobic-functionalized PAMAM dendrimer with bovine serum albumin: Thermodynamic and structural changes. *Langmuir*, 2014; 30: pp. 5536–5544.
11. Naheed Begum and Krishna A. Sailaja. Effect of formulation variables on the Preparation of Ibuprofen loaded polymeric nanoparticles. *Pharmaceutical Nanotechnology*. 2015; pp.111-121.
12. Abbaraju Krishna Sailaja and Marneni Shreya. Preparation and Characterization of Naproxen Loaded Niosomes by Ether Injection Method, '*N***ano** *Biomedicine and Engineering'***,** 2018;10(2): pp.174-180.
13. A.K. Sailaja, M. Nandini, Effect of various desolvating agents in the formulation of naproxen loaded BSA nanoparticles. *Journal of Bionanoscience*, 2017; 11(6): 497-503.
14. G. Archana, Formulation and evaluation of naproxen sodium loaded bovin serum albumin nanoparticles by desolvation technique. *Asian Journal of Pharmaceutical Technology & Innovation*, 2015; 3(14): 23-33.
15. A.Krishna sailaja and P.Shashikala An overall review on Liposomal drugdelivery systems. 2014. *Indian journal of Novel drug delivery*. 2014;6(2):112-119
16. A.Krishna sailaja. A Comparative Study of Aspirin Loaded Bovine Serum Albumin Nanoparticles Prepared by Desolvation Technique Using Various Desolvating Agents. *Nanobiomedicine and engineering*. 2017; 9(2): 143-151
17. Derkach S.R., Voron'ko N.G., Kuchina Y.A., Kolotova D.S. Modified Fish Gelatin as an Alternative to Mammalian Gelatin in Modern Food Technologies. *Polymers.* 2020;12:3051. doi: 10.3390/polym12123051.
18. Kianfar E. Protein nanoparticles in drug delivery: Animal protein, plant proteins and protein cages, albumin nanoparticles. *J. Nanobiotechnol.* 2021;19:159. doi: 10.1186/s12951-021-00896-3.

19. Clark C.C., Aleman J., Mutkus L., Skardal A. A mechanically robust thixotropic collagen and hyaluronic acid bioink supplemented with gelatin nanoparticles. *Bioprinting.* 2019;16:e00058. doi: 10.1016/j.bprint.2019.e00058.
20. Shamarekh K.S., Gad H.A., Soliman M.E., Sammour O.A. Towards the production of monodisperse gelatin nanoparticles by modified one step desolvation technique. *J. Pharm. Invest.* 2020;50:189–200. doi: 10.1007/s40005-019-00455-x.
21. Khramtsov P., Kalashnikova T., Bochkova M., Kropaneva M., Timganova V., Zamorina S., Rayev M. Measuring the concentration of protein nanoparticles synthesized by desolvation method: Comparison of Bradford assay, BCA assay, hydrolysis/UV spectroscopy and gravimetric analysis. *Int. J. Pharm.* 2021;15:120422.

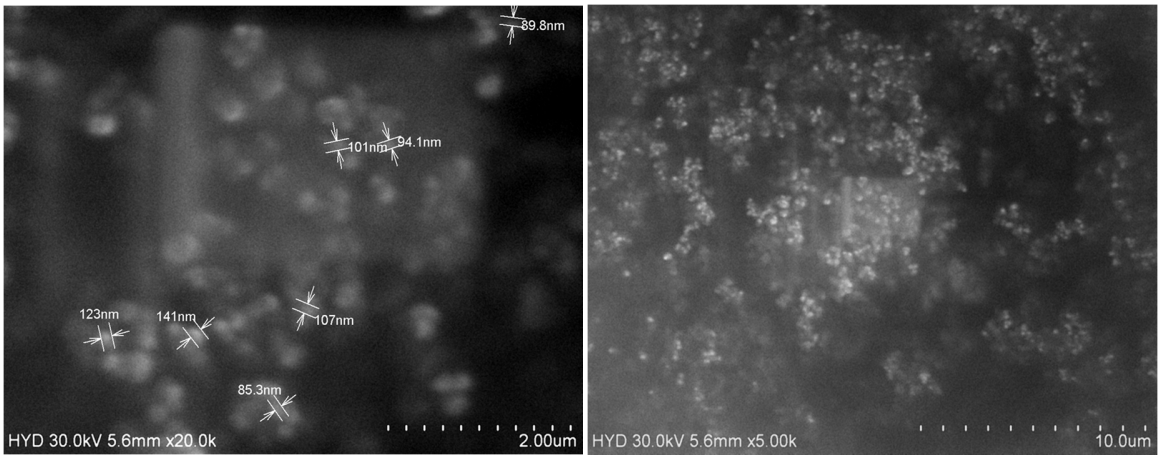

Figure 1:- SEM Image of the nanoformulation

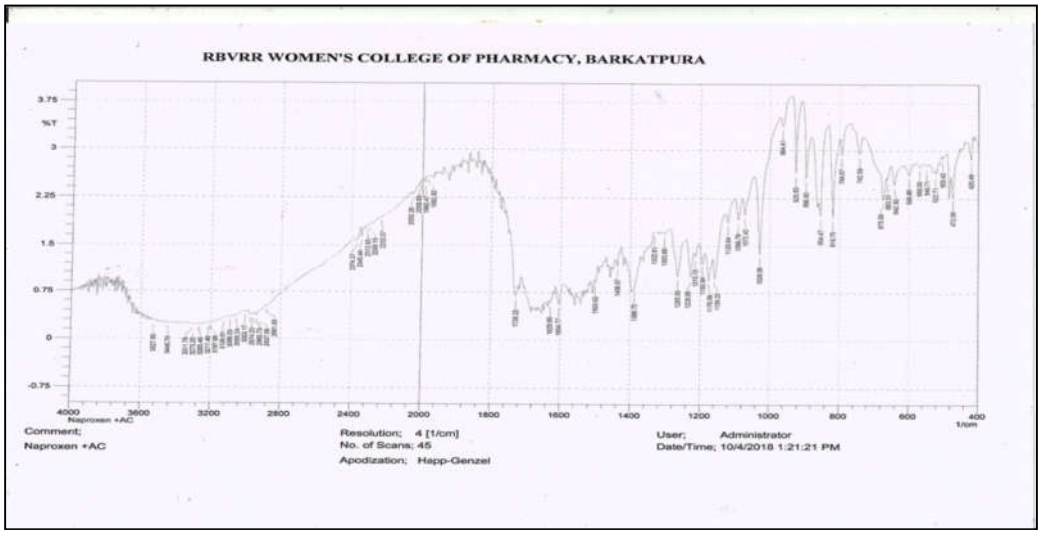

Figure-2: FTIR spectra of Naproxen loaded BSA nanoparticles using desolvation technique

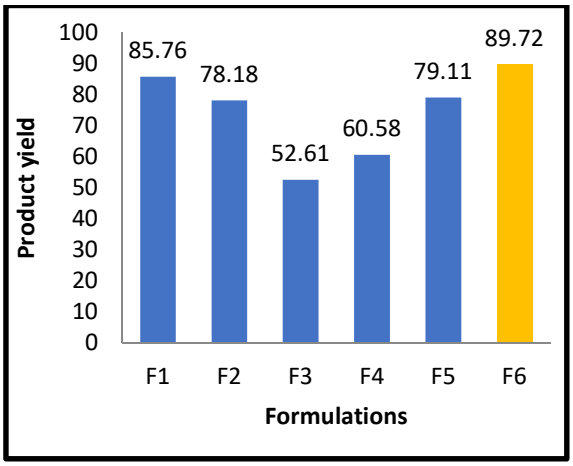

Figure-3: Comparison of product yields of BSA nanoparticles prepared by continuous and intermittent additions of acetonitrile as desolvating agent

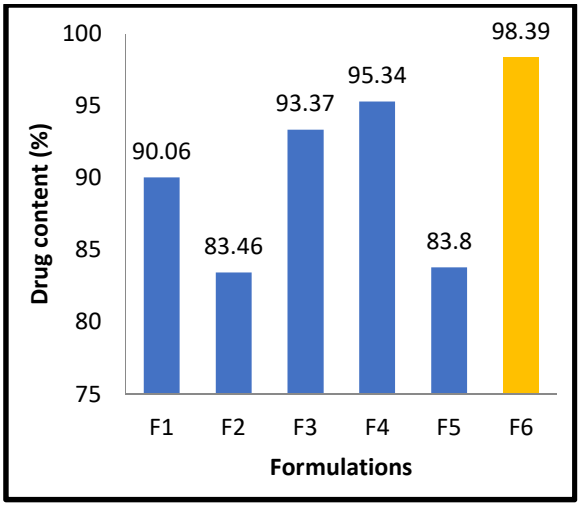

Figure-4: Comparison of Drug content of BSA nanoparticles prepared by continuous and intermittent additions of acetonitrile as desolvating agent.

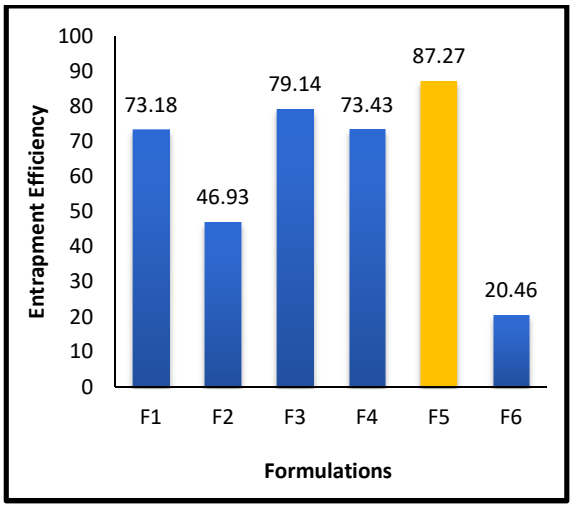

Figure-5: Comparison of Entrapment efficiency of BSA nanoparticles prepared by continuous and intermittent additions of acetonitrile as desolvating agent.

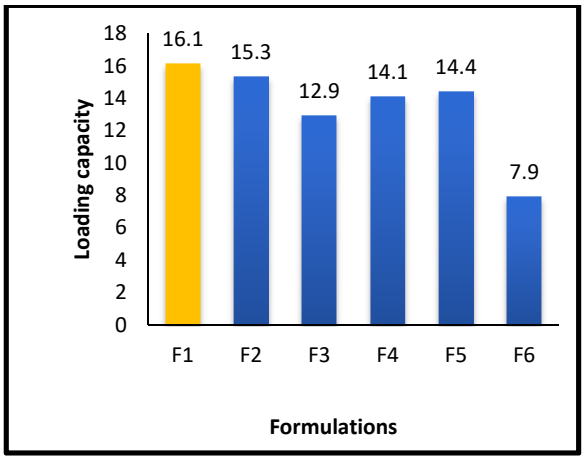

Figure-6: Comparison of loading capacity of BSA nanoparticles

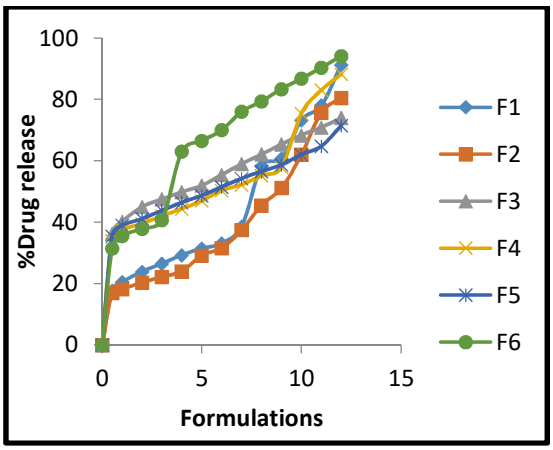

Figure-7: Comparison of Invitro drug release profiles

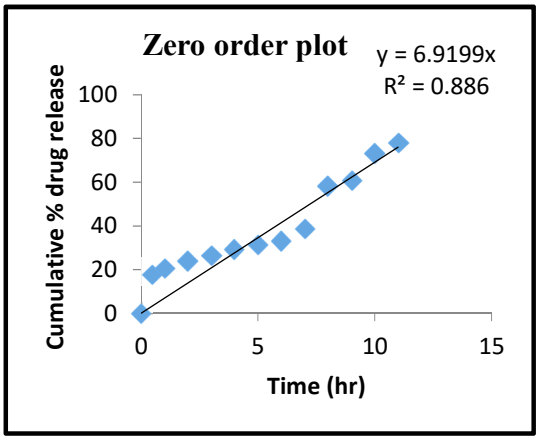

Figure-8: Zero order release data for optimized formulation F1.