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IN VITRO DISSOLUTION STUDIES OF ASPIRIN LOADED GELATINE NANOPARTICLES BY DESOLVATION TECHNIQUE USING ACETONE AS DESOLVATING AGENT

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ABSTRACT

In this present study Aspirin loaded gelatine nanoparticles were prepared by desolvation technique using acetone as desolvating agent. In the treatment of Ankylosing spondylitis and arthritis the dose of aspirin required is 3 g/ day in divided doses. As there are more chances of missing the dose of drug it is better to formulate sustained release dosage forms for better administration. Two methodologies were followed in the addition of desolvating agent to the aqueous solution of gelatine. First one was the continuous addition method and second one was the Intermittent addition method. Comparative study was performed to determine the best method for the preparation of gelatine nanoparticles. Two formulations were prepared by continuous and intermittent addition of acetone as desolvating agent to the aqueous solution of gelatine. Comparative study was made between these two Formulations for particle size, Mean particle diameter, Product yield, Drug content, Electrophoretic mobility, Zetapotential, Entrapment efficiency, Loading capacity. In vitro drug release studies were performed to determine the sustained release effect of these two gelatine Formulations. On comparison Intermittent addition method was showing Promising results. The particle size was found to be 725.3 nm. Drug content (90 %), was found to be satisfactory. The Formulation was found to be more stable with Electrophoretic mobility, Zetapotential value of -1.645, -21.3 respectively. In a time period of 8.5 hrs, 60.9 % of the drug has been released from this Formulations. On comparison the drug release was slightly more in the Formulation 2 prepared by intermittent addition method. From the results it was concluded that Intermittent addition method can be considered to be the best method for the preparation of gelatine nanoparticles.

Keywords: *Desolvation Technique, Invitro Drug Release, Polymeric Nanoparticles, Gelatine, Acetone*

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. (Robinson, 2005). 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 (Vyas, 2002).

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 (Rahimnejad, 2006).

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

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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: (Uhrich *et al.,* 1999).
- More specific drug targeting and delivery,
- Reduction in toxicity while maintaining therapeutic effects,
- Greater safety and biocompatibility, and
- Faster development of new safe medicines.

The various biodegradable polymers used for the preparation of nanoparticles include

Natural Polymers: Chitosan, Starch, Sodium alginate, glucose, mannose, fructose, keratin sulfate etc (Takeuchi, *et al.,* 2002).

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. 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 (Mehravar, 2009).

Number 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 (Patil, 2008). 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.

MATERIALS AND METHODS
Materials
Gelatine is commercially supplied from Sigma Aldrich (St. Louis MO, USA). Analytical Grade high purity acetone is supplied from Fisher Scientifics. Glutaraldehyde was supplied from Sigma Aldrich.

Methodology: Preparation of Gelatin Nanoparticles by Continuous and Intermittent Addition of Acetone as Desolvating Agent
Desolvation technique was adopted for the preparation of aspirin loaded gelatine 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 2.5. The desolvating agent used was acetone (Zhao, 2010).

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 (Sivabalan, 2010). The appearance of turbidity in the solution was considered as the end point. Then, 3-4 drops of 25% glutaraldehyde were added (Lehr, 1994).

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 nano size range. Further, it was evaluated for following parameters like zeta potential, entrapment efficiency and invitro drug release (Das *et al.,* 2005).

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RESULTS AND DISCUSSION
Results
Particle size of the both the formulations was measured by scanning electron microscopy.
Scanning electron microscopy (SEM)
The sample for the SEM analysis was prepared by sprinkling the nanoparticles on one side of double adhesive stub. The nanoparticles were viewed at an accelerating voltage of 15-20 kv. Particle size was found to be in nanorange. SEM Image was shown in Figure 1(Weber *et al.,* 2000).
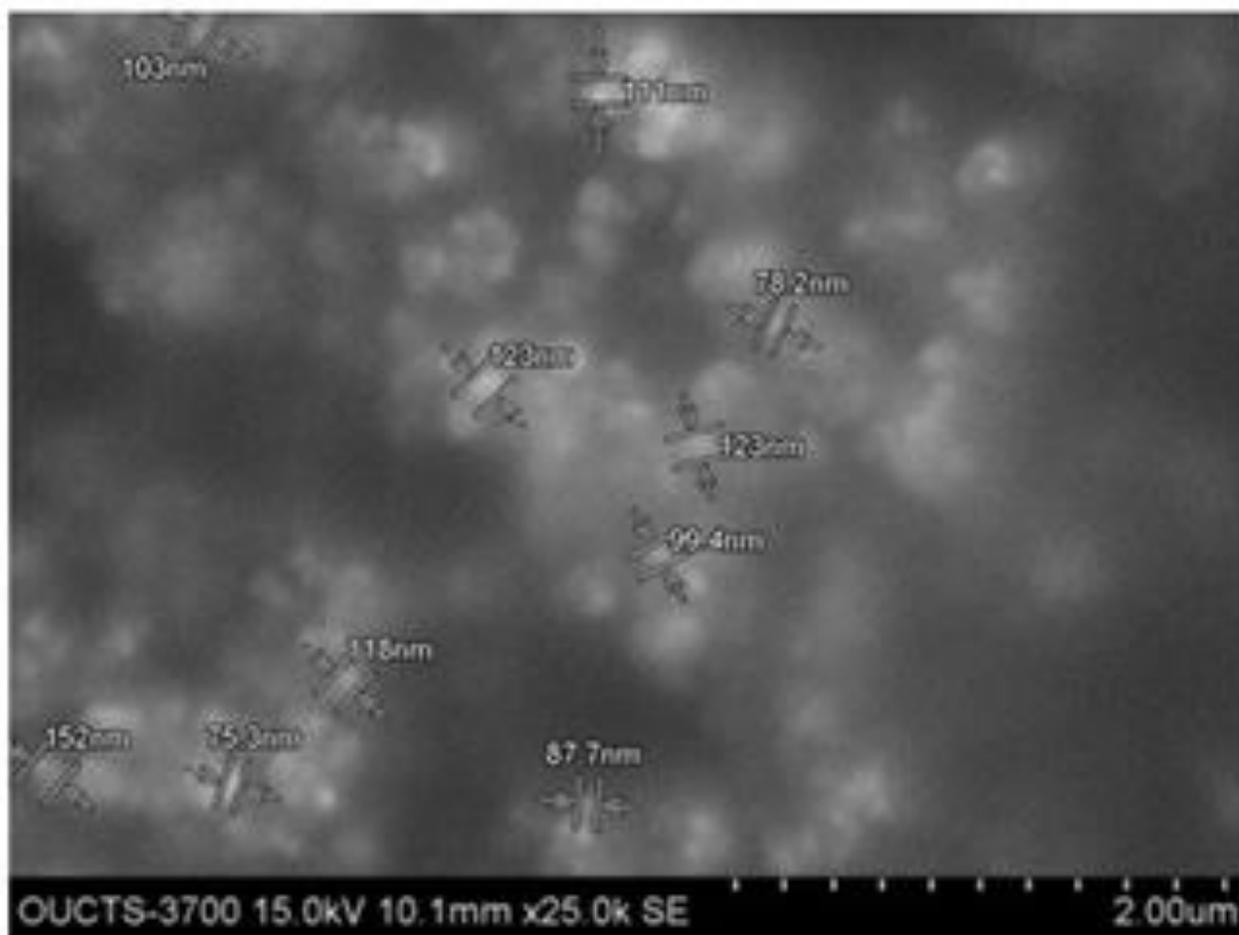
Figure 1: SEM Images of Aspirin Loaded Gelatin Nanoparticles Prepared by Intermittent Addition of Acetone as Desolvating Agent

Particle Size Analysis
Mean diameter of the particle was determined by Photon Correlation Spectroscopy (PCS) with a Malvern Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK). Measurements were realized in triplicate at a 90º angle at 25ºC under suitable dilution conditions. Particle size distribution was expressed as mean diameter (nm) ± standard deviation and polydispersity index. The Mean particle diameters of gelatine nanoparticles prepared by continuous and intermittent addition method were found to be 923.5 nm and 725.3 nm respectively. From the results it was found that intermittent addition method was resulting particles in the nanorange when compared to continuous addition method. The particle size distribution was shown in Fig 2. Comparison of mean particle diameters of the formulations was shown in Figure 3.

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Size Distribution Report by Intensity
v2.0

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Sample Details

Sample Name: SME1 1

SOP Name: SME1 SIZE.sop

General Notes:

|	
File Name: a2.dts	Dispersant Name: Water
Record Number: 1	Dispersant RI: 1.330
Material RI: 1.50	Viscosity (cP): 0.8864
Material Absorbtion: 0.10	Measurement Date and Time: Thursday, September 23, 201.

System

|	
Temperature (°C): 25.0	Duration Used (s): 80
Count Rate (kcps): 168.5	Measurement Position (mm): 4.65
Cell Description: Disposable sizing cuvette	Attenuator: 10

Results

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 725.3	Peak 1:	571.7	68.8	185.4
Pdl: 0.636	Peak 2:	2350	18.0	669.3
Intercept: 0.962	Peak 3:	4791	13.3	728.9
Result quality :	**Refer to quality report**			
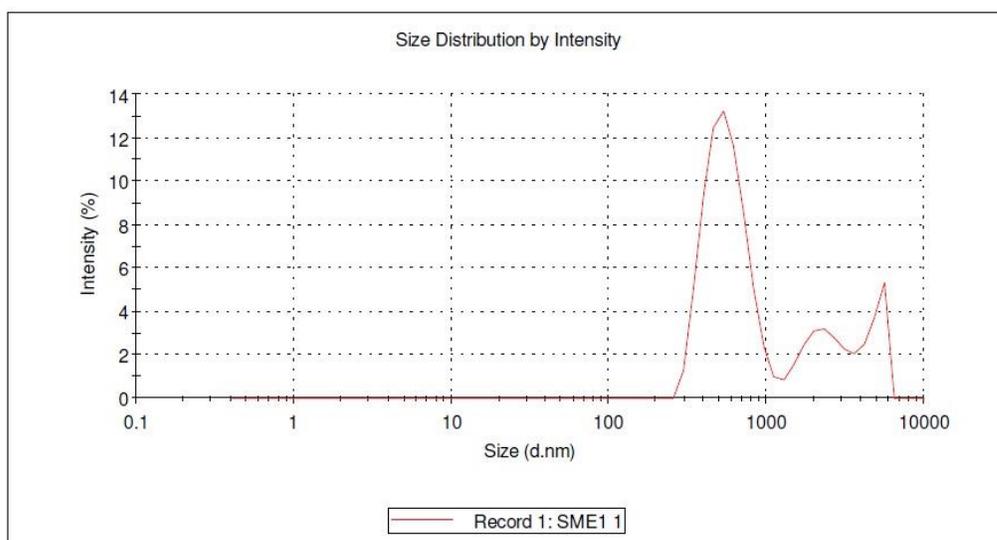
Figure 2: Particle Size Distribution Report of Gelatine Nanoparticles Prepared by Intermittent Addition of Acetone as Desolvating Agent

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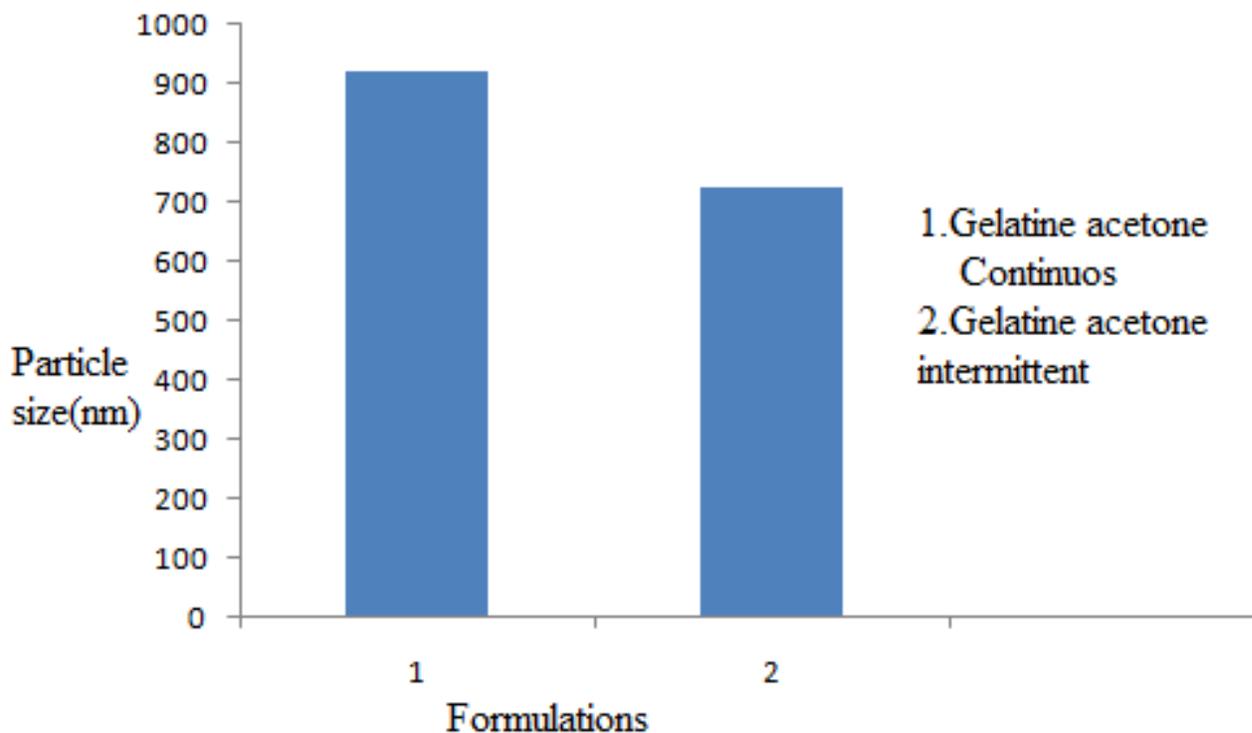
Figure 3: Comparison of Particle Sizes of Gelatin Nanoparticles Prepared by Continuous And Intermittent Addition of Acetone as Desolvating Agent

Zeta Potential Measurement

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

Zeta potential is a measure of the charge of the particle, as such the larger the absolute value of the zetapotential the larger the amount of charge of the surface. In a sense, the zeta potential represents an index for particle stability.

For the case of charged particles, as the zeta potential increases, the repulsive interactions will be larger leading to the formation of more stable particles with a more uniform size distribution. A physically stable nanosuspension solely stabilized by electrostatic repulsion will have a minimum zeta potential of $\pm$ 20 mV. This stability is important in preventing aggregation.

Electrophoretic mobility values and zetapotential values of gelatine nanoparticles prepared by continuous and intermittent addition method were found to be -1.335, -17 and -1.732,-22.169 respectively. On comparison electrophoretic mobility value and zetapotential value of the nanoparticles prepared by intermittent addition method was found to be higher than continuous addition method. This was mainly because of the particle size. Formulation having particles in nanorange will be exhibiting greater stability.

The zeta potential report of Formulation 2 was shown in Figure 4. Comparison of zeta potential values of F1 and F2 was shown in Figure 5.

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Zeta Potential Report
v2.2

Malverr

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Sample Details

Sample Name:	Small sample bottle with red cap 5
SOP Name:	mansettings.nano
General Notes:	

File Name:	Sample 14 Small sample bottl...	**Dispersant Name:**	water 25
Record Number:	5	**Dispersant RI:**	1.330
Date and Time:	Tuesday, October 04, 2011 3:52:...	**Viscosity (cP):**	0.9000
		Dispersant Dielectric Constant:	78.5

System

|			
Temperature (°C):	25.0	**Zeta Runs:**	10
Count Rate (kcps):	163.2	**Measurement Position (mm):**	2.00
Cell Description:	Clear disposable zeta cell	**Attenuator:**	10

Results

|			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-21.3	**Peak 1:**	-23.0	85.7	7.13
Zeta Deviation (mV):	8.58	**Peak 2:**	-6.97	14.3	2.85
Conductivity (mS/cm):	0.498	**Peak 3:**	0.00	0.0	0.00
Result quality :	Good				
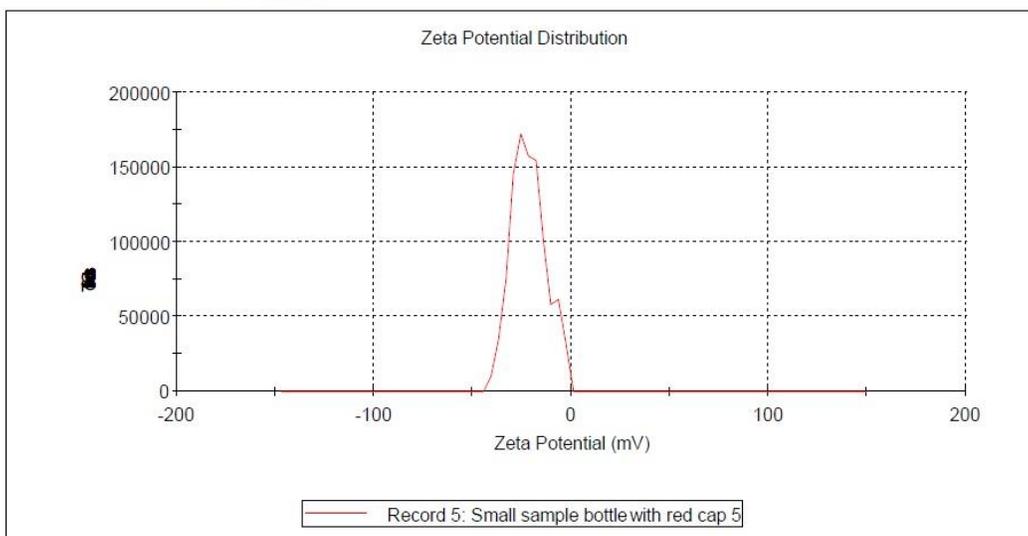
Figure 4: Zetapotential Report of Gelatine Nanoparticles Prepared by Intermittent Addition of Acetone as Desolvating Agent
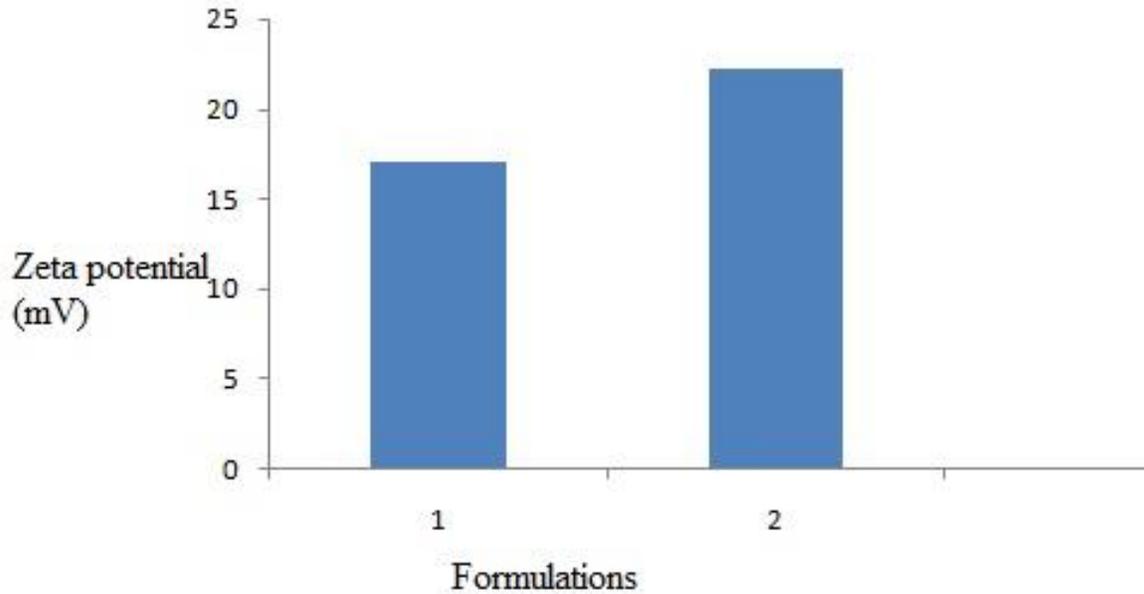
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Figure 5: Comparison of Zetapotential of Gelatine Nanoparticles Prepared by Continuous Addition Intermittent Addition of Gelatin as Desolvating Agent
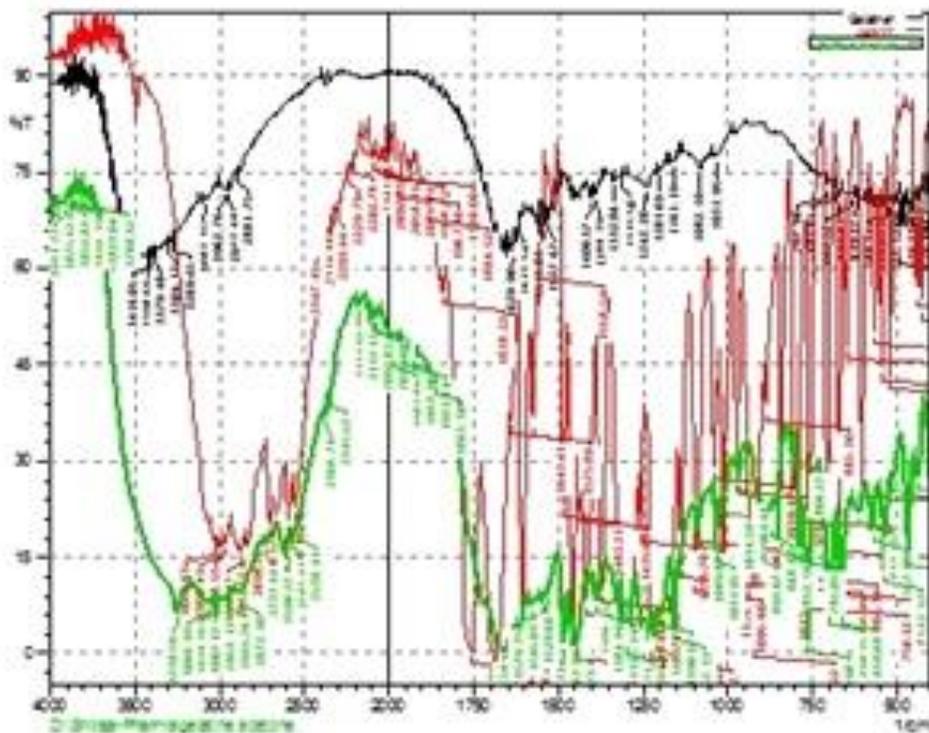
Figure 6: FTIR Spectra

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

Compatibility studies were performed using IR spectrophotometer. The IR spectrum of pure drug and formulations were studied. Spectra was shown in Figure 6. The characteristic absorption peaks of Aspirin were obtained at wave numbers 2870cm^{-1}, 2546 cm^{-1}, 1700 cm^{-1}, 3109.35cm^{-1} , 1300 cm^{-1}, 1085 cm^{-1} (K Br disk). The characteristic absorption peaks of gelatine were obtained at wave numbers 3306cm^{-1}, 2937.68cm^{-1}, 1527cm^{-1}, 1629cm^{-1}, 1082 cm^{-1}. The peaks obtained in the spectra's of each formulation correlates with the peaks of drug spectrum. This indicates that the drug was compatible with the formulation components.

The obtained formulations were evaluated for Product yield, Drug content, Entrapment efficiency, Loading capacity and drug release.

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

$$\textbf{Percent Yield} = \frac{\text{The amount of nanoparticles obtained (g)}}{\text{The theoretical amount (g)}} \times 100$$

Product yields of gelatine nanoparticles prepared by continuous and Intermittent addition method were found to be 45 % and 50 % respectively. From the figure number 7 it was found that product yield of nanoparticles prepared by intermittent addition method was more when compared to continuous addition method.
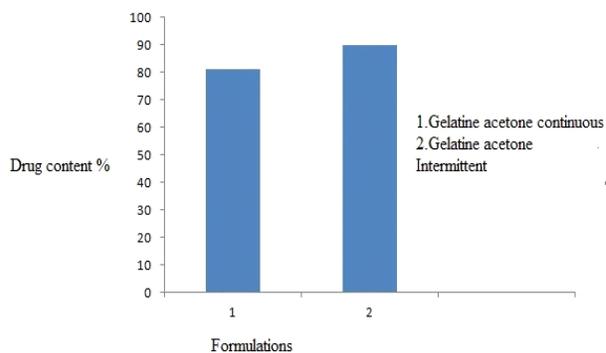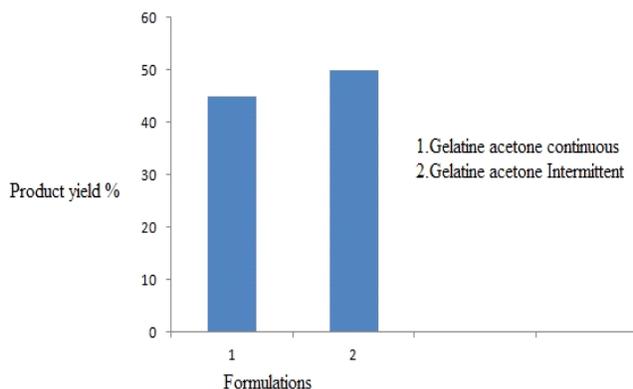
Figure 7: Comparison of Product Yield of Gelatine Nanoparticles Prepared by Continuous and Intermittent Addition of Acetone as Desolvating Agent

Figure 8: Comparison of Drug Contents of Gelatine Nanoparticles Prepared by Continuous and Intermittent Addition of Acetone as Desolvating Agent

Drug Content

Drug loaded nanoparticles were weighed, then grinded to fine powder and dissolved in a solvent in which the drug is completely soluble. It was subjected to stirring around 700 rpm for 3 hrs. Amount of drug in the supernatent was determined by UV-Spectrophotometric method. Drug contents of gelatine nanoparticles prepared by continuous and intermittent addition method were found to be 81.25 % and 90 % respectively. From the results it was observed that the Drug content of nanoparticles prepared by intermittent addition method was more when compared to continuous addition method. The result was depicted in Figure 8.

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Encapsulation Efficiency (EE)

For determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation was determined (*w*) by UV-spectrophotometry. A standard calibrationcurve of concentration versus absorbance was plotted for this purpose[26]. The amount of drug in supernatant was then subtracted from the total amount of drug added during the preparation (*W*). Effectively, (*W-w*) will give the amount of drug entrapped in the pellet. Then percentage entrapment is given by

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

Loading capacity was calculated by the Following equation

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

Entrapment efficiencies and Loading capacities of gelatine nanoparticles prepared by continuous and intermittent addition method were found to be 47.8%, 29.4% and 40 %, 24% respectively. It was shown in Figure 9 and 10 respectively. From the results it was found that Entrapment efficiency and loading capacity of nanoparticles prepared by continuous addition method was more when compared to intermittent addition method. It may be because of the large diameter of the particles.
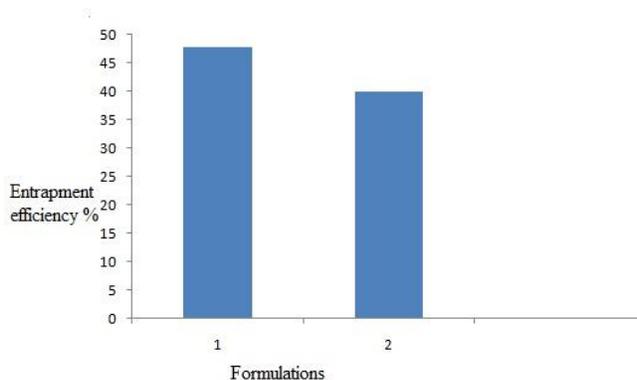
Figure 9: Comparison of Entrapment Efficiencies of Gelatine Nanoparticles Prepared by Continuous and Intermittent Addition of Acetone as Desolvating Agent
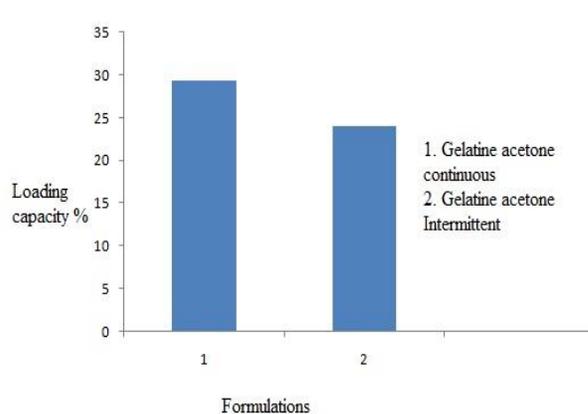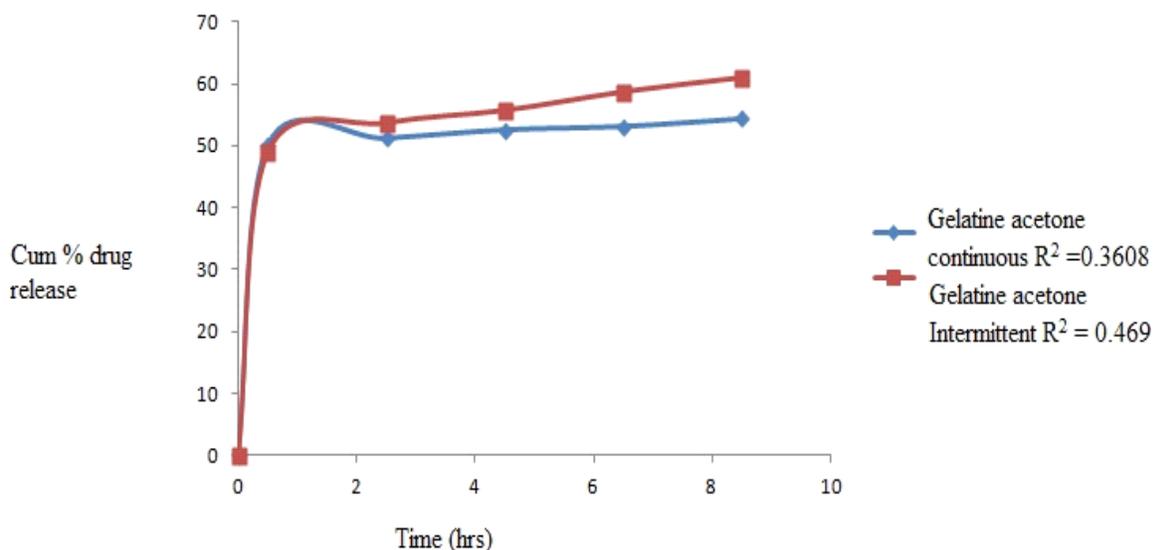
Figure 10: Comparison of Loading Capacites of Gelatine Nanoparticles Prepared by Continuous and Intermittent Addition of Acetone as Desolvating Agent

Drug Release Studies

Drug release studies were performed by means of orbitary shaker. Drug release from polymeric nanoparticles was determined as follows. A known amount of nanoparticles was transferred to a conical flask and 50 ml of the Phosphate buffer pH 7 was added to the tube. The temperature and rotation were adjusted to 37^0C and 90 rpm, respectively. At predetermined time of 0.5, 2, 4, 6, 8, 10, 12, and 24, 36, 48

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hours. 5ml of sample was removed and ultra centrifuged at 15, 000 × *r* for 60 minutes, and 5mL of the supernatant were replaced by fresh medium. The samples were further analyzed using UV Spectrophotometer. In gelatine nanoparticles prepared by continuous and intermittent addition of acetone as desolvating agent the drug release was slow, extended over a period of 8.5 hrs. In a time period of 8.5 hrs 54.34 % and 60.9 % of the drug has been released from these Formulations. When compared Drug release was slightly more in intermittent addition method than continuous addition method. Smaller particles have larger surface area, therefore, most of the drug associated would be at or near the particle surface, leading to fast drug release. The kinetic data was revealed in Figures 11-14.
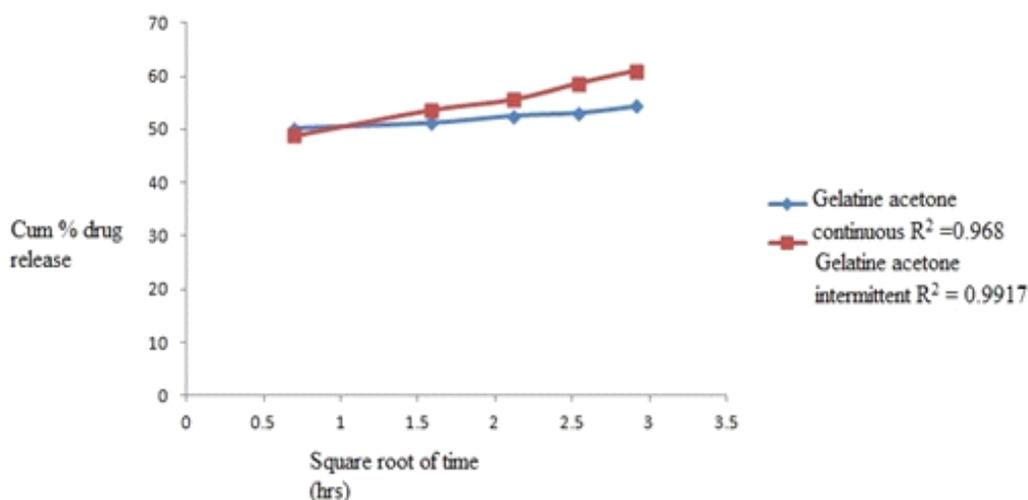

Figure 11: Comparison of Invitro Drug Release Profile of Aspirin Loaded Gelatine Nanoparticles Prepared by Continuous and Intermittent Addition of Acetone as Desolvating Agent
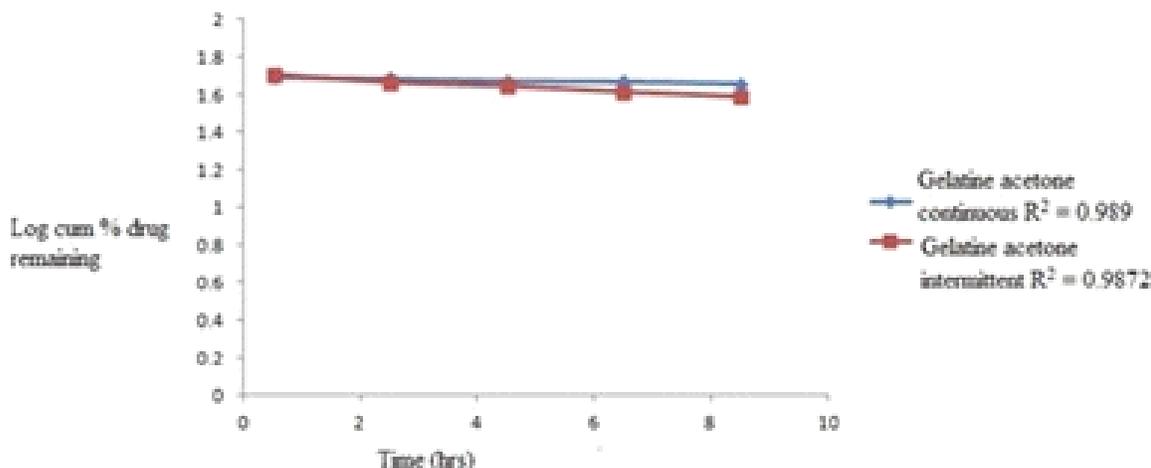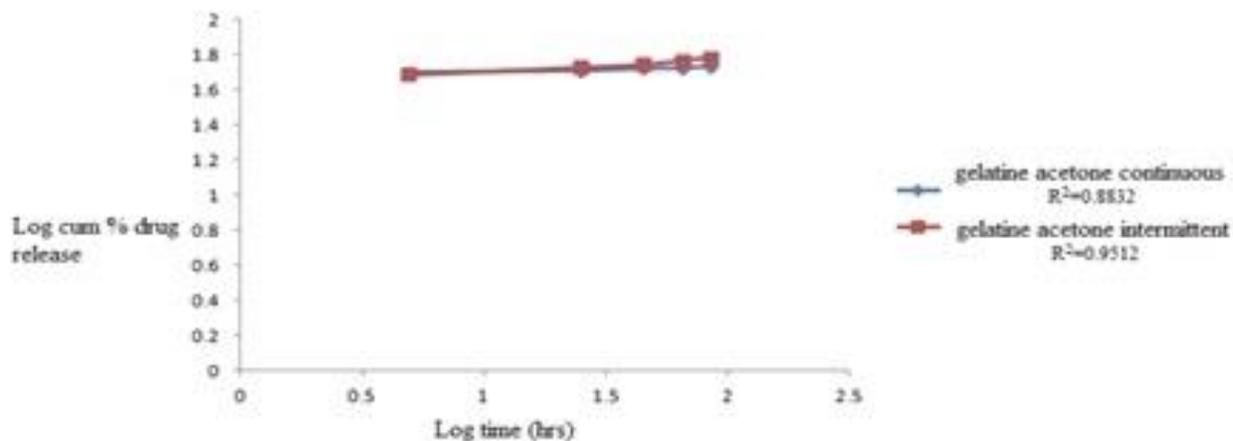

Figure 12: Comparison of First Order Release of Aspirin Loaded Gelatine Nanoparticles Prepared by Continuous and Intermittent Addition of Acetone as Desolvating Agent

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Figure 13: Comparison of Higuchis Square Root Time Dependent Plots of Aspirin Loaded Gelatine Nanoparticles Prepared by Continuous and Intermittent Addition of Acetone as Desolvating Agent

Figure 14: Comparison of Peppas Double Log Plots of Aspirin Loaded Gelatine Nanoparticles Prepared by Intermittent Addition of Acetone as Desolvating Agent

Discussion

In this study aspirin loaded gelatine nanoparticles were prepared by desolvation technique using acetone as desolvating agent. Two methods i.e. continuous and intermittent addition methods were followed for the addition of desolvating agent to aqueous solution of gelatine. A comparative stydy was performed to determine the best method for the preparation of aspirin loaded gelatine nanoparticles. The two formulations prepared by continuous and intermittent addition of acetone as desolvating agent were compared for particle size, stability, product yield, drug content and drug release studies.

When mean particle diameters of both the formulations were compared F2 prepared by intermittent addition method produced particles in nanorange. May be intermittent addition gives sufficient time for the desolvation process to occur. Entrapment Entrapment efficieny of F2 formulation was less when compared to that of F1 formulations. The lower encapsulation efficiencies obtained with the smaller

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particles could be explained by the longer surface area of smaller droplets for a given volume of organic phase. Hence, during the emulsification step, a more direct contact between internal and external phases occurred, resulting in a higher drug loss by diffusion towards the external medium.

The loading capacity of Formulation 2 was less when compared to Formulation 1. It may be because of the large diameter of the particles. Zeta potential value was high in F2 formulation indicating that the F2 formulation was highly stable. This was mainly because of the small particle size. Formulation having particles in nanorange will be exhibiting greater stability. In a sense, the zeta potential represents an index for particle stability. For the case of charged particles, as the zeta potential increases, the repulsive interactions will be larger leading to the formation of more stable particles with a more uniform size distribution. A physically stable nanosuspension solely stabilized by electrostatic repulsion will have a minimum zeta potential of $\pm$ 20 mV. This stability is important in preventing aggregation.

When compared Drug release was slightly more in intermittent addition method. Sustained release effect was more in continuous addition method. This was mainly because of particle size. Smaller particles have larger surface area, therefore, most of the drug associated would be at or near the particle surface, leading to fast drug release. While, larger particles have large cores which allow more drug to be encapsulated, particles also have greater risk of aggregation during storage and transportation of nanoparticle dispersion. The curve Fitting data revealed that the release followed First order kinetics and higuchis and Peppas plots stated Fickian diffusion controlled pattern in both the formulations.

Conclusion

From the results it can be concluded that Intermittent addition method can be considered to be the best method for the preparation of gelatine nanoparticles because of its small particle size (725.3 nm), optimum drug content (90%), good stability (-21.3 mV) and sustain release property.

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