Formulation, development and evaluation of injectable formulation of Aspirin

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Abstract

The objective of this study was to develop and manufacture a stable parenteral formulation for Aspirin, a non steroidal anti-inflammatory agent. The solubility and stability of the drug was determined. Solubility studies suggested that Aspirin exhibited poor aqueous solubility but showed appreciable solubility in non-aqueous solvents. Based on the preformulation studies, a lyophilized parenteral formulation containing 25 mg/mL of Aspirin was prepared in a solvent system containing of 80% v/v water and 20% v/v polyethylene glycol-400 (PEG-400). Rubber closures, filter membranes, and liquid transfer tubing were selected on the basis of compatibility studies. The formulation was subjected to accelerated stability studies. After reconstitution with sterile water for injection, Aspirin injection was stable for a period of 8 hr at 2°C to 8°C. Accelerated stability studies suggested that the lyophilized product should be kept at controlled room temperature for long-term storage. The proposed non-aqueous solvent concentration used, are known to safe hence, toxicities/safety related issues may not raise. The proposed techniques would be economical, convenient and safe. Thus, the study opens the chances of preparing lyophilized formulation of poorly-water soluble drugs.

Introduction

The term *parenteral* has its derivation from the Greek words *para* and *enteron*, meaning beside the intestine, and denotes route other than the oral route. Through parenteral route, drugs are administered by injection under or through one or more layers of skin or mucous membrane into body tissues and many times directly into blood.[1] Parenteral medications are a vital component of the modern therapeutic armamentarium. They offer a number of advantages over other dosage forms. The immediate availability of the drug to the system and successful administration of drugs sensitive to the digestive system are the most significant gains. It also enhance the bioavailability.[2,3] Parenteral delivery systems must be syringeable and injectable to allow for the simple and reproducible administration of a drug product.[4] Maintenance of stability is a next major problem while formulating injection.[5,6] It is commonly recognized in the pharmaceutical industry that on average more than 40% of newly discovered drug candidates are poorly water-soluble.[7] Aspirin (ASP), a non steroidal anti-inflammatory agent is selected as a model drug which is BCS class IV drug (low soluble and low permeable). The oral bioavailability of ASP is only 68%. Thus, one-third of an ingested dose does not reach the peripheral circulation. Also potency of ASP is limited due to its rapid first pass metabolism (ASP hydrolyses in both the gut wall and liver) and its propensity for gastric irritation at larger doses. Intravenous administration overcomes the above problem associated with the ASP therapy.[8] Hence parenteral formulation of ASP is selected. Due to hydrolyses of ASP in aqueous solution, stability is major problem for ASP Injection. Lyophilization process is used when the drug is not stable in aqueous solution.[9] Lyophilization is defined as a process in which the substance is frozen and then the quantity of the solvent is reduced by sublimation (primary drying) and then by desorption (secondary drying).[10] The objective of the present research is to develop safe and stable injectable formulation of ASP. Analytical methods were developed and the solubility of ASP in different solvents were assessed. A liquid formulation was developed and subjected to lyophilization. Compatibility studies with Rubber stopper, SS-316 vessel and silicon tubing as well as accelerated stability studies were conducted.

Materials and Methods

Materials

ASP was obtained from Saint Fons Cedex (France). Polyethylene glycol 400 (PEG-400) was obtained from Shell Chemicals (Germany) and Mannitol was obtained from Roquette (France). Sodium Hydroxide Pellets and Sodium chloride were obtained from Merck (India) and Sujata Chemicals (India) respectively. DMSO and DMA were obtained from Finar Chemicals (India). Ethanol, Propylene Glycol and Glycerin were obtained from S D Fine Chemicals (India). The USP Type I glass vials and the slotted rubber stoppers were obtained from Tube Glass (India) and Adit (India) respectively. Aluminium flip off seal obtained from Autofit (India). Nylon 66 membrane filters (0.2 μ and 2 μ) were obtained from Pall (India).

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Key words: Aspirin, parenteral, lyophilized, accelerated stability studies, sterile water for injection.

Conflict of interests: the authors declare no potential conflict of interests.

Received for publication: 17 October 2012.
Revision received: 25 January 2013.
Accepted for publication: 25 January 2013.

Drugs and Therapy Studies 2013; 3:e2
doi:10.4081/dts.2013.e2

Preformulation studies

Analytical method development: using UV spectrophotometer

A stability-indicating UV spectrophotometric method was developed to analyze ASP in samples. A solution of ASP was prepared in methanol: water (1:9) and UV spectrum was taken using UV spectrophotometer. The UV maxima of ASP was found to be 222 nm in methanol: water (1:9). All analysis was done in triplicate, and the mean was used to determine the concentration of ASP in the formulation.

Solubility studies in different solvents

The solubility of ASP was determined by mixing an excess quantity of drug with approximately 2 mL of the solvent which was taken in a screw-capped bottle. The bottles were rotated on a Glass-Col (Terre Haute, IN) laboratory rotator at 30 rpm for 24 hours at room temperature. Preliminary studies indicated that this time period was adequate to obtain equilibrium solubility. After the particles had settled, the supernatant was carefully withdrawn and filtered through a 0.22-μm filter and analyzed by UV.[11-13]

Physical compatibility study

Compatibility studies were carried out for appropriate selection of excipients. Studies were carried out by mixing the drug with various excipients in required proportion in glass vials. Vials were closed with rubber stopper and kept at three conditions, namely 40ºC/75% RH; 25ºC/60% RH; and Photo stability for 4 weeks. Physical observations of the blend were

done during the study at regular intervals.

Compatibility of ASP and mannitol is also confirmed by Differential scanning calorimetry. DSC scans of powdered sample of ASP, mannitol and mixture of ASP with mannitol were carried out. DSC analyses were recorded using DSC - Shimadzu 60 with TDA trend line software. The pans were positioned on sample pan holder of a DSC 60. The thermal traces were obtained by heating from 50ºC to 300ºC at heating rate of 10ºC. Thermograms were obtained by the DSC 60 thermal analyzer program and recorded chart speed of 1 inch/min. Differential scanning calorimetry enables the quantitative detection of all processes in which energy is required or produced (*i.e.*, endothermic or exothermic phase transformations).

Formulation development

Preparation of Aspirin solution

For the preparation of the solution of ASP, a weighed amount of ASP was gradually added to mentioned volume of solvent (Table 1) in a glass beaker on magnetic stirrer. Besides this, mannitol solution in sterile water for Injection was prepared in another glass beaker with gentle stirring. Both solutions were mixed with gentle stirring. Desired pH was adjusted with sufficient quantity of 10% w/v sodium hydroxide or 10% v/v hydrochloric acid solution. Finally volume was made up with sterile water for injection.

Compatibility of rubber closures with Aspirin injection

The formulation was filled in 10-mL glass vials and stoppered. The vials were placed upright and inverted in the stability chambers maintained at 25°C with 60% RH, and 2°-8°C (refrigeration) for 4 hours. The vials were inverted to obtain maximum exposure of the rubber closure to the formulation.[14,15] The content of the ASP in vials were analyzed.

Effect of silicon tubing on Aspirin injection

In pharmaceutical manufacturing, silicone tubing is used in transfer of solution and as such must not interact with the drug product.[14,16] The effect of silicon tubing on ASP formulation was tested by immersing the tubing in the drug solution for 4 hours at room temperature. The solution was analyzed to determine any loss of drug via adsorption.

Effect of SS-316 vessel on Aspirin injection

SS-316 vessel is used as storage tank for prepared solution and as such must not interact with the drug product.[16] The effect of SS-316 vessel on ASP formulation was tested by keeping the drug solution in SS-316 vessel for 4 hours at room temperature. The solution was analyzed for loss of drug content.

Optimization of lyophilization cycle time

Studies were performed on selected formulation at different time interval. Lyophilization cycle time was optimized from moisture content, cake property and clarity of solution after reconstitution.

Characterization of the developed lyophilized formulation

Powder X-ray diffractometry of lyophilized sample and drug were obtained at room temper-

Table 1. Composition of trial batches (batch size of each is 50 mL).

Ingredients	Batch code					
	T1	T2	T3	T4	T5	T6
ASP (in g)	1.25	1.25	1.25	1.25	1.25	1.25
Mannitol (% w/v)	5	5	5	5	5	5
Glycerin (in mL) (15% V/V)	7.5	-	-	-	-	-
Ethanol (in mL) (20% V/V)	-	10	-	-	-	-
Propylene glycol (in mL) (20% V/V)	-	-	10	-	-	-
DMA (in mL) (20% V/V)	-	-	-	10	-	-
PEG 400 (in mL) (20% V/V)	-	-	-	-	10	-
DMSO (in mL) (20% V/V)	-	-	-	-	-	10
HCl/NaOH	Quantity sufficient for pH 6.0-6.4 adjustment					
SWFI	Up to 50 mL					

Table 2. Solubility of Aspirin in different solvents.

Sr. N.	Solvent	Solubility (mg/mL)
01	Water at 37°C	10
02	PEG 400	254
03	Propylene glycol	160
04	Ethanol	200-400
05	Chloroform	25-60
06	Dimethyl sulphoxide	40
07	Dimethyl formamide	30
08	Dimethyl Isosorbide	280

Table 3. Observation of compatibility study of drug and excipients initially and after 4 weeks.

Material	Ratio A:E	Observation	Initial and after 4 weeks		
			Light	25°C/60% RH	40°C/75% RH
ASP	-	White powder	NC	NC	NC
ASP+Mannitol	1:2	White powder	NC	NC	NC
ASP+PEG 8000	1:2	White powder	NC	NC	NC
ASP+Nacl	1:0.25	White powder	NC	NC	NC
ASP+PEG 400	1:8	Colourless, clear solution	NC	NC	NC

NC, no change; E, excipients.

ature using Xpert MPD-XRD instrument by Philips, Holland. The powder was spread on a graticule and pressed in such a way that powder did not fall on keeping the graticule in vertical position. The graticule was placed in sample holder and exposed for radiation. For FTIR studies, lyophilized formulation was mixed with KBr using mortar pestle. The IR spectrum of the same was recorded in the wave number region of 400-4000 cm^{-1} on a Shimadzu 8400-S.

Stability of reconstituted Aspirin solution

After reconstitution of lyophilized cake with 5 mL of WFI, stability of ASP was studied for 8 hrs under controlled room temperature and refrigerated conditions. The percentage of the remaining drug was determined using UV spectrophotometer.

Stability of Aspirin injection

The vials were subjected to physical and chemical stability studies at 25±2°C and 60±5% RH; 30±2°C and 65±5% RH; 40±2°C and 75±5 % RH; Refrigerator (2-8°C). Sample were analyzed after 30 and 60 days for colour, pH, clarity after reconstitution, assay.[17,18]

Results and Discussion

Preformulation studies

Analytical method development

A suitable stability-indicating analytical method development is very critical. The standard curve was generated for the entire range from 4 to 24 mcg/mL. The results of standard curve preparation are shown Figure 1.

Solubility of Aspirin

The solubility of ASP in various solvents were estimated and is given in Table 2. The solubility of ASP in water was 10 mg/mL. ASP was also soluble in below mentioned parenterally acceptable co-solvents. In PEG-400, it was soluble to the extent of 254 mg/mL.

Physical compatibility study

Various excipients were selected for the compatibility study with ASP. Studies were carried out using glass vials. Vials were closed with rubber stopper and kept at 40ºC/75% RH; 25ºC/60% RH and Photo stability for 4 weeks (Table 3).

Compatibility of ASP and mannitol is also comfirmed by Differntial scanning calorimetry. DSC scans of powdered sample of ASP, mannitol and mixture of ASP with mannitol were carried out. The thermograms of ASP, mannitol and mixture of ASP with mannitol shown in Figure 2. The melting point of ASP is about 143°C In DSC Spectra, ASP melting peak was shown 143.85ºC and in physical mixture with mannitol it was present at 143.27ºC.

Formulation development

Formulation of Aspirin

The prepared solution (Table 1) was evaluated for different parameter. For each parameter average values of 3 samples were considered. For the large scale, 10 liter batch size was considered. It takes not more than 4 hrs for

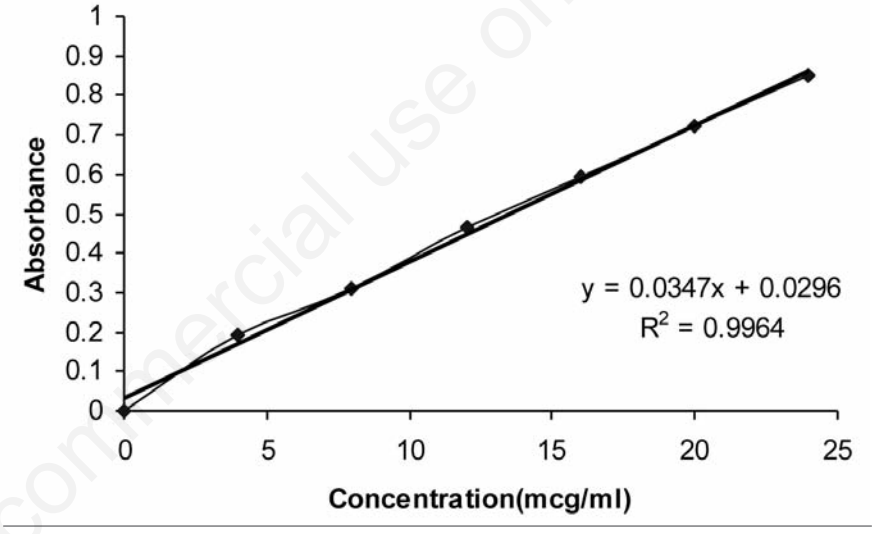

Figure 1. Calibration curve of Aspirin in methanol: water (1:9) system.

Table 4. Evaluation of prepared batches.

Batch N.	Time point	Physical appearance	Absorbance	Transmittance %	pH
T1	Initial	Clear and colourless	0.112	99.21	6.12
	4th hr	Hazy solution	*	*	*
T2	Initial	Precipitation	*	*	*
	4th hr	Precipitation	*	*	*
T3	Initial	Clear and colourless	0.068	99.14	6.19
	4th hr	Hazy solution	*	*	*
T4	Initial	Precipitation	*	*	*
	4th hr	Precipitation	*	*	*
T5	Initial	Clear and colourless	0.079	99.27	6.17
	4th hr	Clear and colourless	0.108	98.92	6.24
T6	Initial	Precipitation	*	*	*
	4th hr	Precipitation	*	*	*

*Due to hazy solution/precipitation, no further study were carried out.

Table 5. Optimized composition.

Ingredients	Optimized formulation
ASP (125 mg/5 mL)	2.5 g
Mannitol	5% w/v
PEG 400 (20% V/V)	20 mL
HCl/NaOH	Quantity sufficient for pH 6.0-6.4 adjustment
SWFI	Up to 100 mL

processing of a batch to start of lyophilization cycle. Therefore, prepared formulations were visually observed for their physical appearance at initially and after 4 hrs of time interval at 25°C. Physical appearance (color, transparency, precipitation, etc.) of prepared formulations were studied. Batch T5 (contain PEG 400 as a co solvent) appeared colourless and clear initially as well as after 4 hrs at 25°C. Absorbance was determined by measuring absorbance through UV Spectrophotometer. It should be NMT 0.5. Absorbance of batch T5 (contain PEG 400 as a co solvent) was measured at 430 nm, initially it was 0.079 and after 4 hrs it was 0.108. Percentage of transmittance was determined by measuring transmittance through UV spectrophotometer. It should be NLT 97.0%. Transmission of batch T5 (contain PEG 400 as a co solvent) was measured at 650 nm, initially it was 99.27% and after 4 hrs it was 98.92%. The pH of the prepared formulations was measured using Lab India pH meter at 25±1ºC. The pH of all the formulations was set initially in the range of 6.0-6.4. It is reported that adding ASP to basic solution (pH>7.4) will hydrolyze ASP to salicylic acid (Table 4).

It was found that Water - PEG 400 (4:1) provides an excellent medium for preparation of intravenous dosage form of ASP.

Compatibility of rubber closures with Aspirin injection

Adsorption of drug to rubber closures is a common problem associated with injectable solutions. Further, incompatibility could also arise between the vehicle components and the rubber formulation that could result in deformation of the rubber closure with subsequent loss of container closure integrity. Adsorption is generally prevented by using a Teflon-coated stopper that forms a barrier between the solution and the rubber components. The vials were placed upright and inverted in the stability chambers maintained at 25°C with 60% RH and 2-8°C for 6 hours. Results indicated that the formulation was compatible with the rubber stoppers. The recovery of ASP was nearly 100% from solutions stoppered with either Teflon-coated or uncoated stoppers. Vials containing punctured rubber stopper was stored inverted to confirm sealability of the rubber closures. No leakage was observed.

Effect of silicon tubing on Aspirin injection

Silicon tubing is generally used to transfer the product from one container to another and for vial filling operations. Drug adsorption into these tubing materials may result in substantial loss of potency. The nature of solvents may affect the ability of the tubing. The effect of sil-

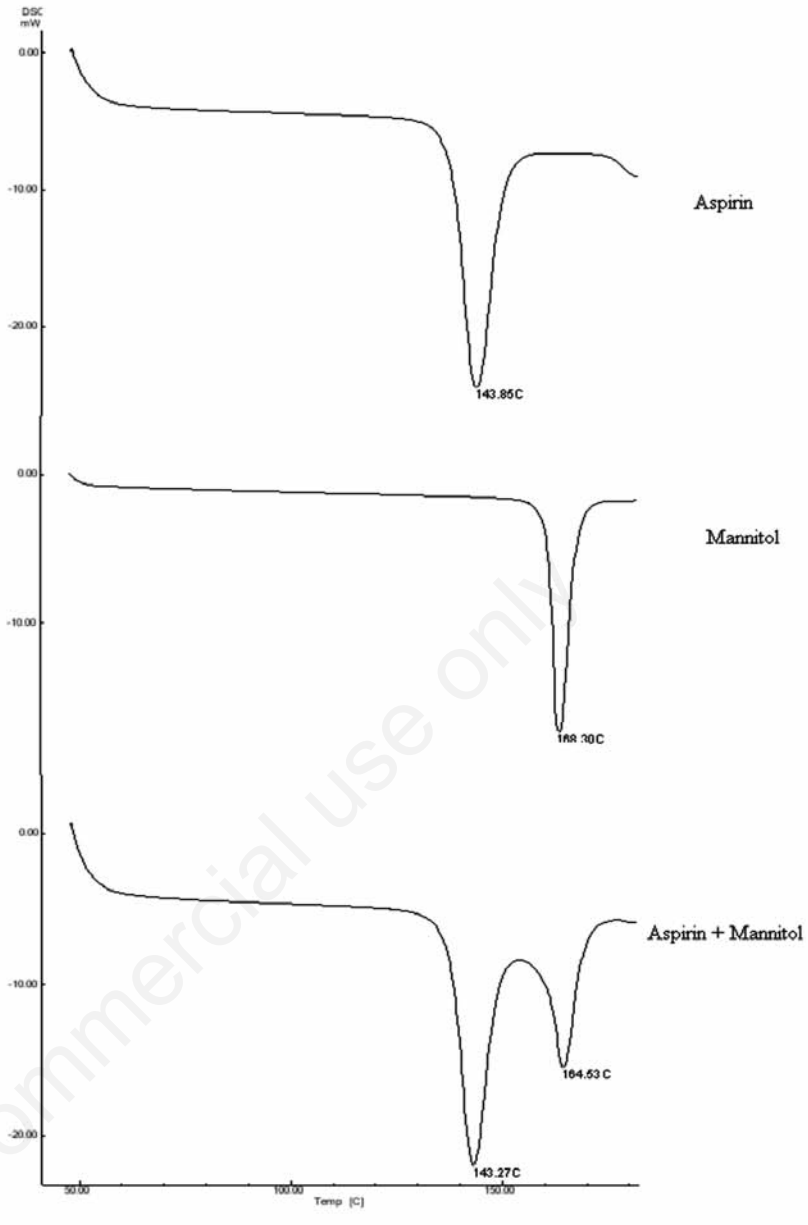

Figure 2. DSC Thermograms of ASP, Mannitol and ASP with Mannitol.

Table 6. Results for optimization of lyophilization cycle time.

Time of sample withdrawal from lyophilizer (hrs)	Moisture content (%)	Reconstitution time (sec)	Clarity of reconstituted solution
24	21.11±0.234	58	Clear
36	10.80±0.351	46	Clear
48	1.82±0.112	33	Clear
60	0.97±0.321	23	Clear
72	0.30±0.022	17	Clear

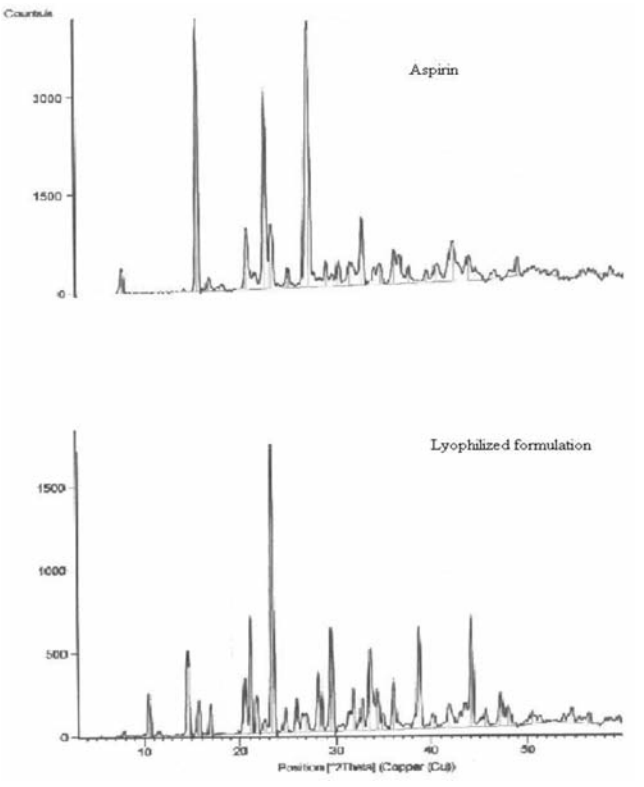

Figure 3. X-ray diffractograms of ASP and Lyophilized formulation.

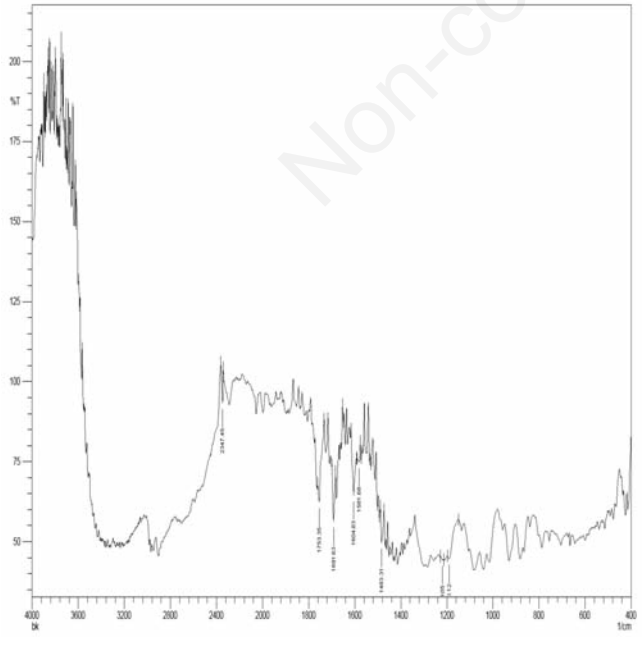

Figure 4. FTIR spectrum of final lyophilized formulation.

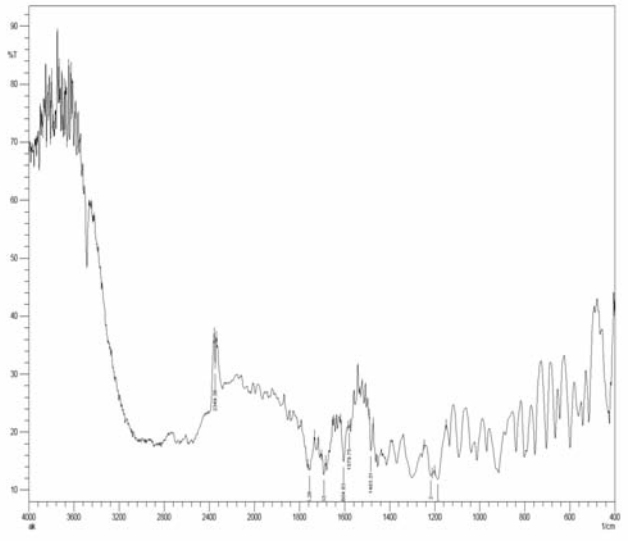

Figure 5. FTIR Spectrum of ASP.

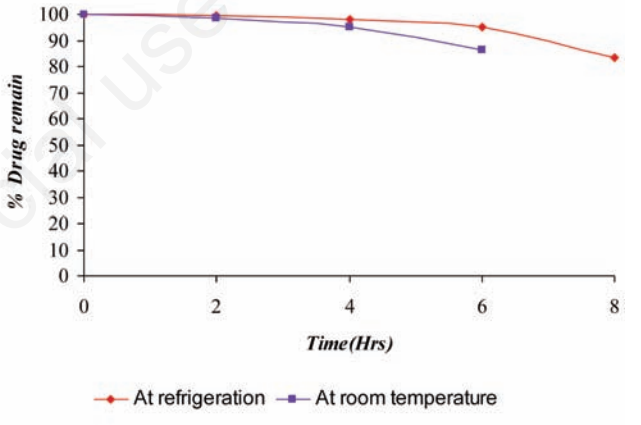

Figure 6. Stability of ASP after reconstitution of product at refrigerated condition and at room temperature.

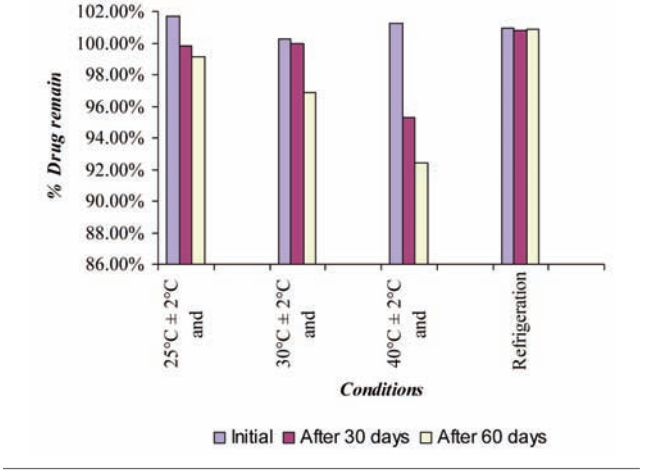

Figure 7. Schematic representation of % drug remaining after 60 days.

Table 7. Physical stability study.

Conditions	Colour of lyophilized cake		pH		After reconstitution with 5 mL of WFI Clarity	
	Initial	After 60 days	Initial	After 60 days	Initial	After 60 days
25±2°C and 60±5% RH	White	White	6.18	6.23	Clear	Clear
30±2°C and 65±5% RH	White	White	6.21	6.19	Clear	Clear
40±2°C and 75±5% RH	White	White	6.25	6.57	Clear	Clear
Refrigeration (2-8°C)	White	White	6.14	6.15	Clear	Clear

icon tubing on ASP formulation was tested by immersing the tubing in the drug solution for 4 hours at controlled room temperature. The solution was analyzed for loss of drug content. Percentage of drug remaining was 101.37.

Effect of SS-316 vessel on Aspirin injection

The effect of SS-316 vessel on ASP formulation was tested by keeping the drug solution in SS-316 vessel for 4 hours at controlled room temperature. The solution was analyzed to determine any loss of drug. Percentage of drug remaining was 99.66%.

Optimization of lyophilization cycle time

The bulk solution prepared as per Table 5 was divided into 5 mL of solution per vial. The vials were then subjected to lyophilization process in a Virtis bench top lyophilizer.

Vials were kept on the condenser surface. Condenser temperature was allowed to get reduced to –70°C. Vials were freeze in the condenser for 1 hr to get equilibrated at –70°C. After that, vials were immediately transferred to the shelf with the half closed slotted rubber stopper. The vacuum was allowed to reach at 100 mTorr. Lyophilization cycle was run for 72 hr. Samples (3 vials) were withdrawn at the time interval of 24, 36, 48, 60 and 72 hr. Moisture content, reconstitution time and clarity of reconstituted solution were determined. Average value of the three samples was considered. Results are shown as in Table 6. Forty-eight hrs lyophilization cycle time were optimized for injectable formulation of ASP as percentage moisture content is less than 2%.

Characterization of the developed lyophilized formulation

The X-ray diffractograms of the drug and lyophilized sample are shown in Figure 3.

The drug sample showed intense peaks due to crystallinity. The peaks in the lyophilized sample showed less intense peaks at same or nearer 2θ values which shows that the crystallinity of drug is reduced.

FTIR spectra of formulation are as per Figure 4. Active drug in the final lyophilized formulation play a vital role for the final potency of the formulation.[18] From Figure 5 it has been observed that there is no chemical interaction between ASP and the excipients used as formulation has given identical peaks as the standard drug sample for different functional groups. Reference drug sample has Carboxyl OH peak at 2350 cm^{-1}, while lyophilized formulation has same peak at 2348 cm^{-1}. Also reference drug sample has Vinyl ester C=O and Aromatic acid C=O peaks at 1756 cm^{-1} and 1693 cm^{-1} respectively while lyophilized formulation has same peak at 1754 cm^{-1} and 1692 cm^{-1}.

Stability of Aspirin in solution after reconstitution of lyophilized product

After reconstitution of lyophilized cake with 5 mL of WFI, stability of ASP was studied for 8 hrs under room temperature and refrigerated conditions (Figure 6).

The above results indicated that the ASP is stable in the bulk solution for 4 hr at room temperature and 6 hr under refrigerated conditions. Thus, the same solution can be used within 4 hr and 6 hr respectively in unavoidable conditions.

Stability of Aspirin injection

The physical stability study results showed that there is no change in physical parameters (pH, colour and clarity) after 60 days in different temperature and humidity conditions [25±2°C and 60±5 RH; 30±2°C and 65±5% RH; Refrigrator (2-8°C)].

The chemical stability study of the developed formulation shows that there is no significant change after 60 days at 25±2°C and 60±5% RH and Refrigerator (2-8°C) (Figure 7 and Table 7).

Conclusions

Objective of research done in the field of pharmaceuticals is to serve the society's needs by developing such a formulation which is highly effective and safe. The solubility and stability of ASP was evaluated to design a suitable parenteral formulation. Because of its poor aqueous solubility and hydrolyses problem, the formulation was prepared in a system consisting of 20% v/v PEG-400 and 80% v/v water. Manufacturing issues such as compatibility with silicon tubing, rubber stoppers, SS-316 vessel were also addressed. Lyophilized injectable formulation of ASP was prepared by using mannitol (5% w/v) as a bulking agent and PEG 400 as a co solvent. XRD results shows reduced crystallinity of the lyophilized product than the drug. From the FTIR spectra of lyophilized formulation it has been observed that there is no chemical interaction between ASP and the excipients used. Results indicated that the ASP is stable in the bulk solution for 4 hr at room temperature and 6 hr under refrigerated conditions. Thus, the same solution can be used within 4 hr and 6 hr respectively in unavoidable conditions. Stability studies results indicates that the lyophilized injection was physically and chemically stable. The study opens the chances of preparing lyophilized formulation of poorly-water soluble drugs.

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