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Formulation Development and Evaluation of Injectable Depot Suspension

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ABSTRACT

The aim of the present study was to develop sustained release parenteral depot drug delivery system of contraceptive drug (Medroxyprogesteron acetate). Parenteral depots formulations (suspension) are widely used to improve therapeutic value of water insoluble drug's & provide sustained drug release over a longer duration of time and solve the problem of daily intake of medicine. Long acting hormonal contraceptive depot formulation advanced the practice of contraception, where injectable & subdermal route of administration used. Objective of present study was to develop medroxyprogesteron acetate injectable depot suspension for contraceptive used. Medroxyprogesteron acetate injectable depot suspensions were prepared by sterile combining of API and excipient powders by rapid stirring method. Mixing speed and temperature dependent solubility of parabens were affect the particle size, specific surface area and dissolution rate of given suspension. Different excipients used in the formulation are Methylparaben & Propyllparaben used as preservative and re-suspending agent, sodium chloride were used Isotonisity modifier, PEG 3350 were used as suspending agent, Tween 80 used as surfactant. The resultant MPA suspension were evaluated for Physical properties, MPA content were 101.00 ± 2 %, viscosity were 8.58 ±1cps, pH 5.826 ±1 and osmolality were found to be 378 ± 5 Mosmol /kg.H2O respectively & further characterized for surface morphology, particle size, zeta potential sedimentation volume, syrengeability, hold time study, In vitro drug release. The suspensions were found to be spherical with smooth surface. Particle size, specific surface area & zeta potential were found to be (Particle size 13.4 μm with specific surface area 1530 cm^2/km, -22.66 mV). Interaction between the drug and polymer were investigated by Fourier Transform Infrared (FT-IR) Spectroscopy. The MPA injectable suspension could go through 24 gauge hypodermic needle smoothly with withdrawal volume 1.70, mL, MPA suspension re-dispersed within 1 minute without forming clogs. The FT-IR analysis confirmed the compatibility of MPA with the excipients without interaction. In- vitro drug release from these MPA injectable suspension showed sustained release over a period of 48 hours (Dissolution type-II apparatus), & 90 minute (Dissolution type- IV apparatus).

1. Background

Now a day, in our fast growing world many scientific advances have been made in the area of parenteral drug delivery for development of advanced system that allows sustained release or controlled release of drug [1]. Due to technological advances parenteral depot formulations and their drug delivery technologies have received much scrutiny in recent years [2]. Because parenteral route is most superior and common route of drug delivery for the drug which has limited bioavaibility and narrow therapeutic index in gastro intestinal track [3].

Long acting hormonal contraceptives depot formulations are the method of birth control that provides effective contraceptive action for longer period of time. Hormonal contraceptives advance the practice of contraception where injectable and subdermal routes of administration are used. When oral contraceptive shows the difficulty of maintaining daily intake of medicines, then there is need of long acting hormonal contraceptives method. Long acting hormonal contraceptive formulations taken every two to three month and solve the problem of daily intake of medicine. The development of long-acting steroidal contraceptive systems this is believed to be an important solution to the problems of effectiveness, safety, and acceptability of steroidal contraceptives, especially in developing countries [4].

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Parental suspension is useful dosage form for administering insoluble or poorly soluble drugs [5]. The larger surface area of disperse drug may help to ensure a high degree of avaibility for absorption. Parental suspension provides more prolonged release from the injection site than comparable to solution. Parenteral suspension are thermodynamically unstable heterogeneous disperse system, in which insoluble finally divided fine or coarse powders disperse in the dispersion medium (In liquid medium, i.e. aqueous or non- aqueous). Parenteral suspension should be sterile, pyrogen-free, non-irritating, bio-compatible, bio-degradable and non-toxic preparation of active constituent in suitable dispersion medium [6].

A parenteral depot injection is an injection, usually subcutaneous or intramuscular, of a pharmacological agent which releases its active compound in a consistent way over a long period of time [7]. Depot preparation can be define, as a formulation which is aqueous or oleaginous suspension (or) oleaginous solution administered by parental route i.e. by sub-cutaneous or intra muscular. Basically there are four type of depot formulations such as, (a) dissolution controlled depot formulation, (b) Adsorption type depot formulation, (c) Encapsulation type depot formulation, (d) Esterfication type depot formulation [8]. Advancement of depot also offers; reduce the dosing frequency, decrease dose related unwanted adverse-effect, increase the efficacy-dose relationship, enhancing patient compliance, alleviation of pain during administration, improve the drug utilization and reduce the costing of parenteral medicines [9].

Medroxyprogesterone Acetate (MPA) as parenteral depot suspensionmost commonly -used aslong acting steroidalhormonal contraceptive drugs usedfor prevention of pregnancy in women and also used in endocrine therapy for prevention ofadvanced or recurrent breast cancer. MPA act on the gonadotropin and it inhibit the secretion of gonadotropin there by prevent the follicular maturation and ovulation result in endometrial thinning, this action of MPA produce its contraceptive effect [10]. It is known that MPA can cause serious adverse effects such as thrombosis as well as other side effects such as weight gain, loss of bone mineral density, hypertension, nausea and cushingoid effects [11]. Although a higher frequency of toxicity has been seen at higher doses. MPA metabolites are excreted in the urine as glucuronide conjugates with only minor amounts excreted as sulphates [12].

The objective of this paper was to develop MPA parenteral depot suspension, by rapid stirring method and characterize. The paper discusses the development of parenteral depot drug delivery system for parenteral formulation of MPAto reduce number of daily repeated administrations. It would be cost effective & beneficial in reducing the frequency of dosing, overcoming dose related toxic side effect of conventional dosage form such as; (MPA pills, MPA tablet), thereby improving patient compliance to treat the contraceptive disease.

2. Materials & Methods

2.1 Materials

Medroxyprogesteron acetate was purchased from Farmabios, Italy. PEG3350, Methylparaben, Propylparaben, Hydrochloric acid and sodium hydroxide were obtained from Merck chemical Ltd; Mumbai, Tween 80 (LQ-MH) purchase from Sigma Aldrich. All other chemical and reagent used are of parenteral and analytical grade.

2.2 Methods

2.3 Preformulation study

Preformulation study is one of the important pre-requisite in development of any drug delivery system. It gives the information needed to define the nature of the drug substance and provide a framework for the drug combination with pharmaceutical excipients in the dosage form. This study includes following points as [13].

2.3.1 Confirmation of Drug

Confirmation of drug was carried out by melting point method, UV Spectroscopy, Infrared spectroscopy (IR) and Differential scanning calorimetry.

2.3.2 *Differential Scanning Calorimetry*

Thermal analysis of pure MPA were recorded on DSC colorimeter (DSC-60, Shimadzu Corporation, Kyoto, japan). The accurately weighed sample (5±2 mg) hermetically sealed in an aluminium pan(Sample sealer and crimper SSC- 30 Shimadzu Corporation, Kyoto, Japan). The DSC runs were performed over a temperature range 25ºC to 300ºC, at heating rate 10ºC/min under pure dry nitrogen flow of 50 ml/min using an empty aluminium pan as reference [14].

2.3.3 *UV Spectroscopy*

The UV spectrum of MPA in 96% ethanol was scanned at (800 nm to 400 nm using a 1 cm cell. The maximum absorbance was determined using UV- visible Spectrophotometer (UV 1700, Shimadzu, Japan) to confirm the λmax of the drug.[15]

2.3.4 *FT-IR Spectroscopy*

The chemical structure of drug MPA were analysed by using measured by using FTIR spectrophotometer (FTIR- 8400S; Shimadzu, Asia Specific Pvt. Ltd. Singapore) by KBr press pellet technique. For that Sample (2 mg) was mixed with (50 mg) KBr and formed pellet in manual press. The pallet (KBr press; 1.5 kg pressure) was placed in the sample holder and spectral scanning was taken in the wavelength region between 4000-700 cm/swith scan speed of 1 cm/s [19].

2.3.5 Solubility Study

Solubility study of MPA was measured in different solvent like; water, 0.001 N HCL, 0.1 N HCL, 4.5 pH Acetate buffer, 6.8 pH buffer and 0.35% Sodium lauryl sulphate [16].

2.3.6 *Drug -Excipient Interaction Study*

The possible chemical interactions of drug with excipients were assessed using their mixture with Active pharmaceutical agent (MPA-1mg, Methylparaben, Tween 80 and PEG3350) and transfer in to 2 ml effendrop Tube), effendrop bottle was kept in stability chamber at 25ºC±2ºC. Drug excipient compatibility carried out for a period of 3 weeks [17]. At the end of three weeks physical mixture of excipient with drug were analysed by using FTIR spectrophotometer (FTIR- 8400S; Shimadzu, Asia Specific Pvt. Ltd. Singapore) Sample (2 mg) was mixed with KBr (50 mg) and formed pellet in manual press(KBr press- 1.5 kg pressure) was placed in the sample holder and spectral scanning was taken in the wavelength region between 4000-700 cm/s with scan speed of 1 cm/ [18].

3. Method

Detail manufacturing process of MPA suspension was captured in figure 1.

3.1 *Manufacturing of Part A*

Approximately 90 % v/vWFI was collected in 5 litter manufacturing vessel (SS, 316L). Then temperature of WFI was bring down up to the room temperature, out of 90% v/v of WFI 67%, v/vof WFI was kept in manufacturing vessel (SS, 316L) and remaining WFI was removed and kept aside in a closedvessel (SS, 316), close with SS lid. Thus from cooled WFI was used for volume make up and for preparation of 0.1 N Hydrochloric acid solutions and 0.1N sodium hydroxide solution. Then batch quantity of polyethylene glycol 3350, Tween 80 HP was added and dissolved (Table 1 composition) in initial 67%, v/v of WFIby continuous stirringinto (SS, 316L) clear solution was obtained. Then solution was filtered by using PVDF (0.22 μm-47 mm, catalogue no: GDWPO4700) and labelled it as "filter part A". Record the in process parameter, given in (Table 2). Bulk solution in manufacturing vessel was blanket with continuous low pressure nitrogen purging.

3.2 *Preparation of API Slurry*

About 30%, v/v of filtered part A' solution was collected into sterilized manufacturing vessel and stirs the solution for NLN 5 min with magnetic stirrer (Heidolph, RPM: 500, Time: 5 min). Then batch quantity of sterile medroxyprogesteron acetate was added gradually into the phase A" solution with continuous stirring using overhead stirrer for minimum 60 min. after completion of sterile MPA addition, labelled this solution as slurry.

3.3 *Preparation of Manufacturing Part B*

37% v/v, bulk suspension of required quantity of cool WFI was

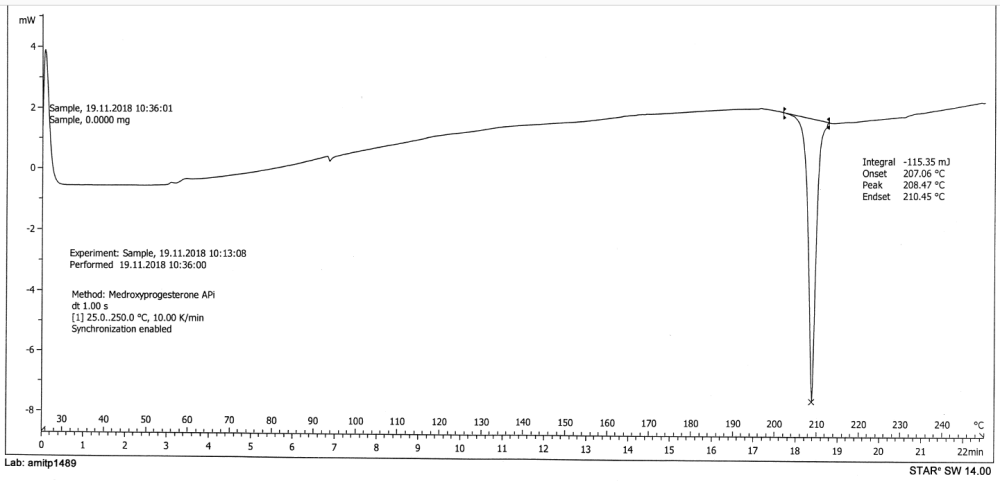


Figure 1: Manufacturing method of MPA suspension

Table 1: Composition of medroxyprogesteron acetate injectable suspension 150 mg/ml (Batch Quantity: - 2900 ml)

S.No	Ingredients	Quantity gm
1	MedroxyprogesteroneAcetate, USP	442.322
2	Polyethylene glycol 3350	83.810
3	Tween 80 HP	6.989
4	Sodium chloride	25.172
5	Methylparaben	3.973
6	Propylparaben	0.435
7	Sodium Hydroxide	q.s. to pH 3-7
8	Water for injection	q.s. to 2900 mL

Table 2: In-process parameter for medroxyprogesterone suspension phase A

S.No	Parameter	Observation
1	Description	Clear solution
2	pH,	5.2
3	Stirring speed	1200 RPM
4	Stirring time	1.30 hrs

RPM= Resolution Per Minute

collected in suitable vessel. Then batch quantity of sodium chloride in the WFI was added with continuous stirring until clear solution obtained. The temperature of solution vessel was maintained up to 24°C to 25°C, batch quantity of Methylparaben, Propylparaben was added into above solution by continuous stirring until clear solution was obtained. Then above solution was filtered by using PVDF (0.22 μm- 47 mm, catalogue no: GDWPO4700) filter, rinse the container with WFI, and transfer filtered solution stainless steel vessel and labelled as part B". Then measured quantity of API slurry was added into filtered part B solution in manufacturing vessel with continuous stirring NLT 15 min with overhead stirrer (high shear stirrer-1200 rpm speed). pH of solution was adjusted up to (6.50 to 6.80) with 0.2 μm filtered 0.1 N NaOH solution and filter through PVDF (0.22 μm- 47 mm, catalogue no: GDWPO4700) filter 0.1 N HCL solution as per requirement.

3.4 *Heat Treatment to Bulk Suspension*

Final batchvolume of above bulk suspension (Part B phase) wasmake up using WFI. Then bulk suspension was transfer in to stainless steel jacket with continuous stirring and heating, and maintaining the temperature up to 80°C for 90 min, under nitrogen purging. Bulk suspension was cooled up to room temperature and filled in USP type –I clear glass vial (2 mL, Scott khaisa) with filled volume 1.7 mL/Vial.

4. valuation Parameter

4.1 *Assay of Medroxyprogesteron Acetate*

MPA (1.0 gm) injectable suspension was transferred into 100 mL of volumetric flask, 50 mL diluents (Water/Acetonitrile, 30/70) dissolve and sonicate until MPA suspension was dissolved and make volume up to the mark with diluents. Further withdraw 4.0 mL above solution in to 50 mL (Borosil, USP type I glass bottle) volumetric flask and make up the volume up to mark with diluents and, mix well, and MPA injectable suspension concentration was determined by using high performance liquid chromatography (HPLC, Agilent 1200). The HPLC system was equipped with an ultraviolet detector (240 nm wavelength for medroxyprogesteron acetate analysis). A Syncronis C18 (5.0 μm, 4.6 mm ×150 mm Stainless steel column) analytical column was used with flow rate 1.4 mL/min., (Water/Acetonitrile, 30/70)mobile phase for isocratic flow, Run time: 15 min. Retention time: 35 min. for MPA) [19].

Standard curve was $(Y = 17394x + 10659, R2 = 0.9999)$.

Calculation: % Assay of MPA

$$Au / AS \times \text{Wstd}/200 \times 100 / \text{W}sam \times 50 / 4 \times 1 / LC \times P / 100 \times wt / ml$$

Where,

Au = Mean area of MPA peak from sample solution.

AS = Mean area of MPA peak from standard solution.

Wstd = Weight of working / reference standard of MPA in mg.

Wsam = weight of sample taken in gm.

LC = Label claim of MPA in mg/mL (150 mg /mL).

P = Potency of MPA.

wt / ml = Weight per mL.

4.2 *pH*

The pH of MPA injectable suspension was carried out by using thermo scientific micro electrode pH meter (Orion star, Thermo scientific, USA). Transfer 5 mL of medroxyprogesteron acetate injectable suspension into suitable dry clean glass beaker (10 mL) and dip the pH electrode in beaker (At room temperature, 25°C). Allow it to stabilize and observed the pH of MPA suspension [20].

4.3 Osmolality

Osmolality is a measurement of number of dissolve particles of solution. Osmolality depend upon the no of particles in the solution, not depend upon the charge and nature of solution. Osmolality of MPA suspension was performed by using single sample micro osmometer (Advance Osmometer- 3350). Transfer 250 μL suspension in curette and measure the osmolality of sample and record the result [21].

4.4 Viscosity

Viscosity of MPA suspension was performed by using Brookfield viscometer (Model: DV2T). Weigh accurately 20 mL suspension and

transfer in to sample holder, (Temperature: 25°C, Adapter: Parallel plate SC4-18, for low viscous suspension, RPM: 100) and record the viscosity of MPA suspension[21].

4.5 Sedimentation Volume

Original height (H_0) of 10 mL medroxyprogesteron acetate suspension in 50 mL graduated measuring cylinder (Borosil, grade A, USP type I glass) after shaking was recorded. After standing for 5 min, 2.5 h, 15 days the height (H)of the sediment was measured. The sedimentation rate of medroxyprogesteron acetate suspension was calculated according to following equation [22].

$$F = \frac{H}{H_0}$$

Where,

F = Sedimentation volume.

H = Ultimate volume of sediment (final volume).

H_0= Initial height of suspension

4.6 *Bulk Hold Time Study (SS 316 L vessel)*

Bulk holding study was performed by keeping the MPA injectable suspension in one liter, (Stainless steel -316 L) vessel at room temperature up to 48 hours. Withdraw samples from stainless steel -316 L, vessel at pre-determine time interval and analyse for pH, Osmolality & Assay.

4.7 *Tube Holding Study*

Tube holding study of MPA injectable suspension was performed by pouring the MPA injectable suspension in platinum silicon tubing,(Sani-Tech Sil-250, Saint Gobain) and Pharmapure tubing (Pharma 50, Saint Gobain). 20 ml suspension was pouredin each tubing andclose both the ends of tubing kept for 24 hours at room temperature at static condition from closed tubing required quantity of samples was withdrawnat pre-determine time interval and analyse for pH osmolality & Assay.

4.8 *Syringe Ability Test*

The Syringeability of MPA injectable suspension was performed by using 24 gauge needle of (BD hypodermic needle, 1 inch length) with needle level down and tipped the vial at (45°) 1 mL suspension withdrawing in to syringe, depressed the plunger with steady pressure untill the syringe is empty. The syrengeability was evaluated by smooth degree of extraction and the withdrawal volume per minute with shaking, finally observed any clogging, drainage [23].

4.9 *Re-suspendibility Study*

MPA injectable suspension 10 mL was kept in a 50 ml (Borosil, Grade A, USP type- I glass) graduated measuring cylinder up to one week for settling of suspended particles, and then layered suspension was shocked under rocker shaker (MR-12, Biosan) with rotation at 20 rpm/min., after 1 min. And 1.5 min measured its re-dispersed time [24].

4.10 *Morphological study (Scanning electron microscopy, Microphotograph by optical microscope)*

The morphology of the MPA acetate injectable suspension was examined by optical microscope (BX41, Olympus Japan) and scanning electron microscope (SEM, JEOL-JSM-IT100 and SEM JOEL-6360LV, Tokyo, Japan). A small drop of suspension was tiled at glass slide to observe the morphology of micro-suspension under the high times optics microscope. Samples place on the glass surface were air dried and then follow by oven drying quickly [18]. After oven-drying, the samples were fixed on an SEM stab using double-sided adhesive tape and coated with gold at 20 mA for 2 minute using an auto fine coater (Ion sputter JFC 1600). After coating, the digital images of the samples at 1800*x*, 2500*x*, 2700*x*, 3000*x*, 4000*x*, 9000*x*, resolution were obtained by the SEM with secondary electron detector with accelerating voltage 100 kV [25].

5. Particle Size Analysis by Laser Diffraction Particle Size Analyser

Particle size of MPA injectable suspension was determined by Laser diffraction particle size analyser (Malvern; Master sizer hydro-3000, instrument, Malvern, UK). Suspension was diluted with double distilled water to produce a suitable scattering intensity prior to the measurements, the z-average cell was used having 10 mm diameter, which were equilibrating for 120 seconds [25].

5.1 *In vitro Drug Release Studies*

Flow through cell (USP type IV Dissolution apparatus)

Drug release from MPA injectable suspension was performed using dissolution medium (0.35% sodium lauryl sulphate) for 90 minute in USP type IV dissolution apparatus(Flow through cell-Sotax 7-Pro, 2.7 μm size glass microfiber (Maker: Milli pore) filter follow by 100 mg glass wool, 13 gm of glass beads in each cell). Dissolution medium was prepared according to US Pharmacopeia and office of generic drug (OGD media). Transfer 1000 μl of MPA injectable suspension with the help of micropipette on glass bead in flow through cell (volume: 14 mL). Fix the assembly and set the pump flow [26] (16 ml/min) and temperature 37°C [26]. Then run the apparatus as per dissolution parameters reported in Table 5 and Collect the sample at given time intervals. Mix each solution properly and directly inject in to HPLC (Agilent, 1200) system. (Retention time: About 8.5 min for MPA, Run time:15 minute column: Syncronis C18150 x 4.6 mm, 5.0 μm, flow rate: 1.4 ml/min, Mobile phase for isocratic flow (Water/Acetonitrile 40/60), Diluent: 0.35% SLS).[26]

USPII Dissolution Apparatus

Drug release from MPA injectable suspension was performed using dissolution medium (0.35% sodium lauryl sulphate) for 48 hr. in tablet dissolution tester (Electro lab-India, EDT-08Lx, Syringe pump (ESP: 124) and sample collector ESC: [27]. Dissolution medium (0.35% SLS, 900 ml in each bowl) was prepared according to US Pharmacopeia and office of generic drug (OGD media). Load the dissolution program as per parameter reported in Table 6, and allow to achieve the bath temperature 37°C. Transfer 1000 μL of suspension in to bowl and run the dissolution. Aliquots of dissolution withdraw at predetermine time points and filter the aliquots through 0.22 μm nylon filter paper (Milli pore), filter solution Mix each solution properly and directly inject in to HPLC (Agilent, 1200) system. (Retention time: About 8.5 min for MPA, Run time:15 minute column: Syncronis C18150 x 4.6 mm, 5.0 μm, flow rate: 1.4 ml/min, Mobile phase for isocratic flow (Water/Acetonitrile 40/60), Diluent: 0.35% SLS) [28].

5.2 *Related Substances*

Composite mixture of five vial of MPA injectable suspension was transferred into 50 mL scott glass bottle, shake to form uniform suspension, transferred 700 μm MPA suspensionwith the help of micropipette into the 50 mL volumetric flask, Add 20 ml, diluent (Water/Acetonitrile, 30/70) dissolve and sonicate until MPA suspension dissolve and make volume up to the mark with diluent. Related compound in MPA injectable suspension concentration was determined by using high performance liquid chromatography (HPLC, Water). The HPLC system was equipped with an ultraviolet detector (254 nm wavelength for medroxyprogesteron acetate analysis). A Nova-Pak(4.0 μm, LiChrosorb, 3.9 mm ×300 mm stainless steel column WAT011695) analytical column was used with flow rate 1 mL/min (Isocratic flow) Run time: 20 minute, Retention time: 35 minute, Water/Acetonitrile, 30/70, mobile phase for isocratic flow was used [29].

5.3 *Zeta Potential*

The zeta potential of MPA injectable suspension was measured by using the Malvern Zetasizer (Nano ZS 90, Malvern, UK). The measurements were performed with diluting MPA suspension in double-distilled water. It was measured using Dip cell with applying field strength 20 *V/cm* and the average of the zeta potential was given from 30 runs [21].

5.4 *Stability Study*

Accelerated Stability

Stability MPA injectable suspension was checkedat (long term, 25 ± 2°C/60 ± 5% RH) and (accelerated condition, 25 ± 2°C/60 ± 5% RH) as per ICH guideline (Q1A). Product has been expose upward condition to compare the adsorption of drug product as well as leaching impurity from rubber closure and evaluated on the basis of assay dissolution and physical parameter [30].

Photo Stability

Photo stability of MPA injectable suspension was performed on three different group of product as per ICH guideline Q1B. In that samples were exposed with light providing overall illumination of NLT 1.2 million lux hours an integrated near ultra violet energy of not less than 200 watt hours/square meter in photo stability chamber. Sample vial place horizontally with respect to the light source, which provides for the most uniform exposure of the light. Photo-stability parameter given in (Table 3) [31].

6. Result and Discussion

6.1 *Melting Point (DSC)*

Melting point of MPA was determined by DSC and endothermic peak of MPA was observed at 210.45°C, (Figure 2) shows DSC thermogram peak of MPA, observed and reported melting point reported in (Table 4), respectively.

6.2 *UV Spectroscopy*

The UV spectrum of MPA, in 96% ethanol was scanned at 800 nm to 400 nm. The maximum absorbance of MPA was found to be241 nm and UV spectrum of MPA in 96% ethanol reported in (Figure 3).

6.3 *FTIR Spectroscopy*

FT-IR Spectrum ofMPA (Sterile API) was determined by using KBr press pellet technique and spectrum is shown in (Figure 4) Medroxy progesterone acetate shows characteristics absorption bands at 3335.03 cm^{-1} for O-H, 2947.33 cm^{-1} for C-H stretching, 1731.17 cm^{-1} for C=O stretching And 1363.72 cm^{-1} C=C bending.

Table 3: Photo Stability Parameter

Photo stability Condition	Sample Packed in	Time
1.2 Million lux hours and 200 UV watt hours	Primary pack (vials)	4 days
	Secondary pack (Carton)	4 days
	Aluminium wrapped vial	4 days

6.4 *Solubility Study*

The solubility of MPA was assessed in different solvent system viz. Water, 0.001 N HCl, 0.1 N HCl, 4.5 pH Acetate buffer, 6.8 pH buffer, 0.35% SLS. It was found that MPA shows 0.1438±0.001 mg/ml maximum solubility in 0.35% SLS whereas very less solubility was found in phosphate buffers pH 6.8 and acetate buffer. Solubility of MPA reported in (Table 5).

6.5 *Drug Excipient Compatibility Study*

FTIR method was adopted to study the compatibility with drug and excipients. MPA shows characteristics absorption bands at 3335.03 cm^{-1} for O-H, 2947.33 cm^{-1} for C-H stretching, 1731.17 cm^{-1} for C=O stretching and 1363.72 cm^{-1} for C=C bending. There were no extra peaks observed in the spectrum when API in combination with excipient. FTIR Spectra Figure 5 (a) MPA, (b) Physical mixture of MPA and PEG 3350, (c) Physical mixture of MPA and Propylparaben, (d) Physical mixture of MPA and Methylparaben), represents the no compatibility between drug and the excipients mixture.

6.6 *Physical Properties Of Suspension*

The MPA injectable suspension was white homogenous suspension. MPA content were 101.00±2 %, viscosity were 8.58±1 cps, pH 5.826±1 and osmolality was found to be 378±5 Mosmol/kg.H_2O respectively. Physical properties of suspension were reported in (Table 6).

6.7 *Sedimentation Volume Study*

Sedimentation volume and sedimentation rate of MPA injectable suspension after 15 days was 5.8 ml, 1 min. respectively with clear solution. Properties of suspension are reported in (Table 7).

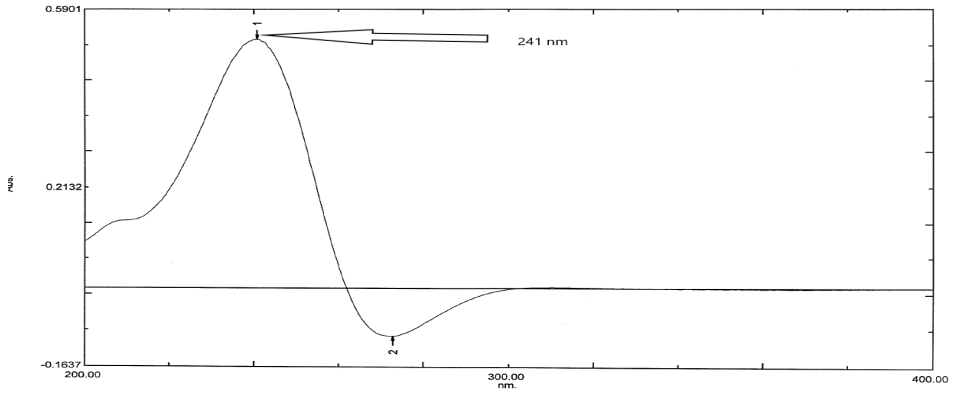


Figure 2: DSC thermogram of MPA

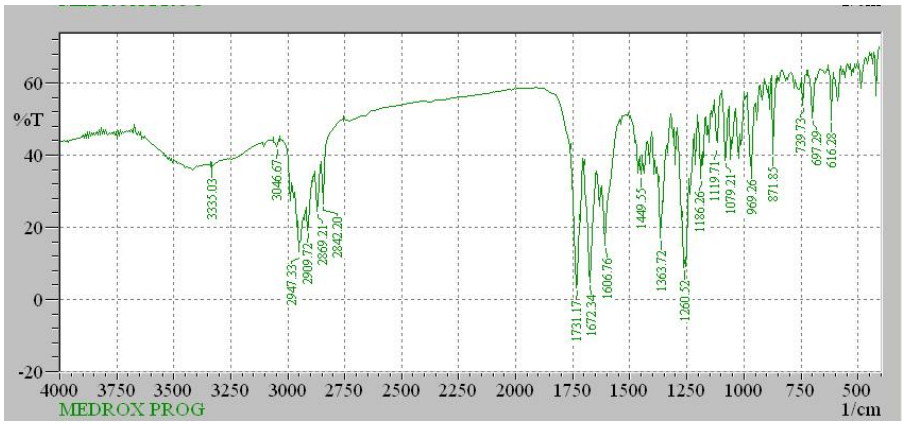


Figure 3: UV Spectrum of MPA in 96% ethanol

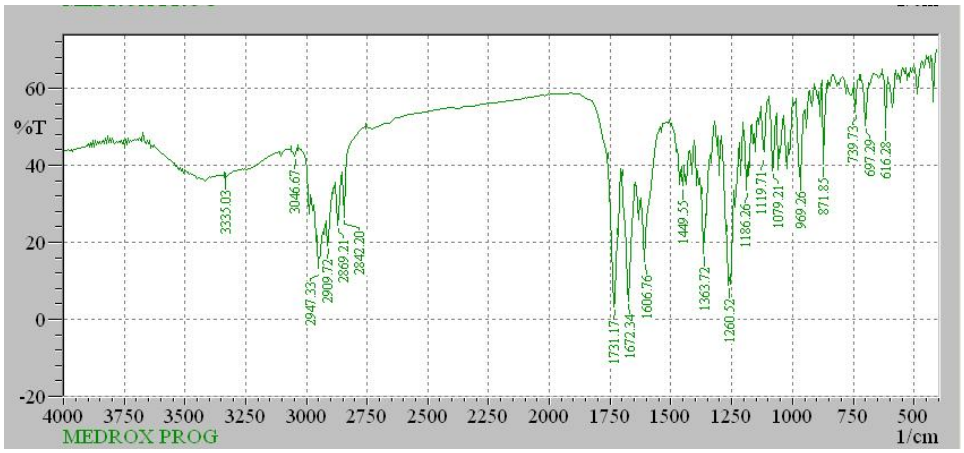


Figure 4: FT- IR Spectrum of MPA (Sterile API)

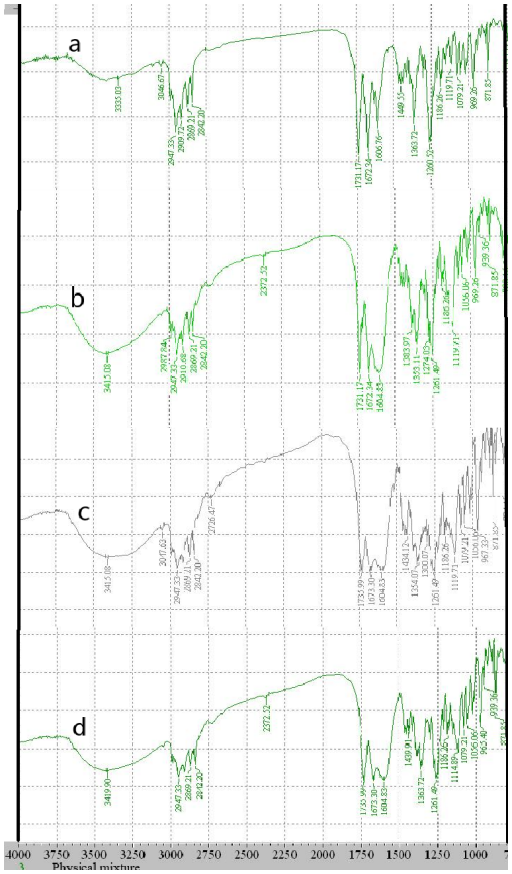


Figure 5: Drug excipient intraction study FT- IR Spectra of physical mixture (a) MPA, (b) Physical mixture of MPA and PEG 3350, (c) Physical mixture of MPA and Propylparaben, (d) Physical mixture of MPA and Methylparaben

6.8 *Bulk Hold Time Study (SS 316 L vessel)*

Analytical data for bulk holding study showed that all physiochemical parameters are well within limit of specification after 48 hours so that MPA suspension was found to best able with stainless steel 316L vessel for a period of 48 hours andresults were reported in (Table 8).

6.9 *Tube holding Study*

The finished MPA injectable suspension in platinum curved silicontubing, Pharmapure tubing at room temperature (25°C) up to 24, hours, analytical data show that all physiochemical parameters are well within limit of specification after 24 hours so MPA suspension was found to be stable for a period of 24 hours, compatible with platinum curved silicon tubing, Pharmapure tubing at room temperature (25°C) and 24 hours results were reported in (Table 9).

Table 4: Melting point of MPA

S.No	Method	Observed melting point	Reported melting Melting Point
1	DSC	210.45	214°C

Table 5: Solubility of MPA in Different Solvent System

S.No	Solvents	Observed solubility (mg/ml)
1.	Water	0.0015±0.001
2.	0.001N HCL	0.0015±0.001
3.	0.1N HCL	0.0014±0.001
4.	Acetate buffer pH 4.5	0.0013±0.001
5.	Phosphate buffer pH 6.8	0.0011±0.001
6.	0.35% SLS*	0.1438±0.01

Table 6: Physical Evaluation Parameter

MPA Suspension	Evaluation parameter			
	Assay (%)	pH	Viscosity (cps)	Osmolality(Mosmol)
	101.00 ±2	5.826±1	8.58±5	378± 5

6.10 *Syrengeability Test/Injectability Test*

The medroxyprogesteron acetate injectable suspension could gothrough 24 gauge hypodermic needle smoothly with withdrawal volume 1.70 mL after shaking. No clogging, drainage was observed in any of the sample during the study.

7. Re-suspendibility Study

The re-dispersed time after 15 days storage of layered MPA suspension was only 1 minute under a magnetic shaker rotating at 20 mi/min. Result were reported in (Table 10).

7.1 *Morphological Study*

Medroxyprogesteron acetate injectable suspension was visualized by using the optical microscope (BX41, Olympus Japan) at 10x & 40x, zoom optical microscope images (Figure 6a-10xzoom; 6b-40x zoom) showed that no agglomerations in MPA suspension. SEM images (Figure 7a-SEM image at 1800x, resolution; 7b-SEM image at 2500x, resolution; 7c-SEM image at 2700x, resolution; 7d-SEM image at 3000x, resolution; 7e-SEM image at 4000x, resolution; 7f-SEM image at 9000x, resolution;) showed that the particles of MPA suspension were irregular with smooth surface and well distributed with uniformed particle size (5 μm, 10 μm) and no agglomerations..

Table 7: Sedimentation Volume Study

S.No	Time	Volume of sediment (mL)	Sedimentation rate	Description of supernanent
1	Initial	10	1	White suspension
2	15 min	9.4	0.94t	Hazy suspension
3	30 min	8.2	0.82	Hazy suspension
4	45 min	7.6	0.76	Slightly hazy suspension
5	1 hr.	6.8	0.68	Slightly hazy suspension
6	90 min	6.8	0.68	Slightly hazy suspension
7	2 hr.	6.2	0.62	Slightly hazy suspension
8	2.5 hr.	5.85	0.855	Clear solution
9	3 Days	5.8	0.85	Clear solution
10	4 Days	5.8	0.85	Clear solution
11	5 Days	5.8	0.85	Clear solution
12	6 Days	5.8	0.85	Clear solution
13	7 Days	5.8	0.85	Clear solution
14	8 Days	5.8	0.85	Clear solution
15	9 Days	5.8	0.85	Clear solution
16	10 Days	5.8	0.85	Clear solution
17	11 Days	5.8	0.85	Clear solution
18	12Days	5.8	0.85	Clear solution
19	13 Days	5.8	0.85	Clear solution
20	14 Days	5.8	0.85	Clear solution
21	15 Days	5.8	0.85	Clear solution

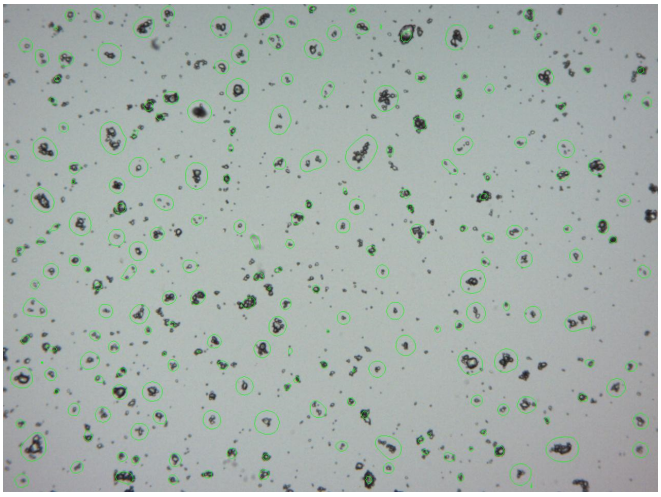


Figure 6A: Optical images of MPA suspension (a) 10 x zoom

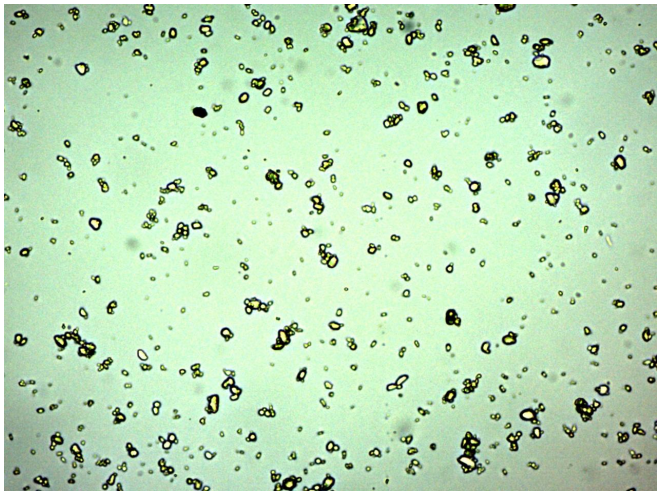


Figure 6B: Optical images of MPA suspension (b) 40 x zoom

Table 8: Bulk hold time study (SS 316 L vessel)

S.No	Parameter	Specification	Storage Condition (25°C to 30°C)		
Bulk hold time study in (SS 316 L vessel)			0 Hour	12 Hour	48 Hour
1	Description	White homogeneous suspension	White homogeneous suspension	White homogeneous suspension	White homogeneous suspension
2	pH	Between 5 to 7	5.81	5.82	5.82
2	Assay	96% to 103 %	101	101.1	101.9

Table 9: Tube Holding Study (Tubing)

S.No	Parameter	Specification	Storage Condition (25°C to 30°C)	
Bulk hold time study in (Tubing)			4 Hour	24 Hour
1	Description	White homogeneous suspension	White homogeneous suspension	White homogeneous suspension
2	Ph	Between 5 to 7	5.81	5.82
2	Assay	96% to 103 %	101	101.1

Table10: Resuspendability Result

S.No	Specification (Must meet with assay limit)	Assay (%)	Resuspendability hold time after shaking (% Assay)	
1	Between 90% and 110 % of label claim	101	30 second	60 second
2	Formulation		100.00	100.41

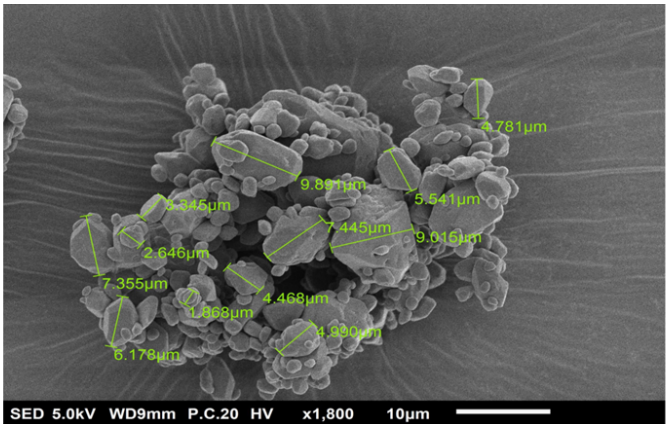


Figure 7(a): SEM images of MPA injectable suspension at resolution 1800x

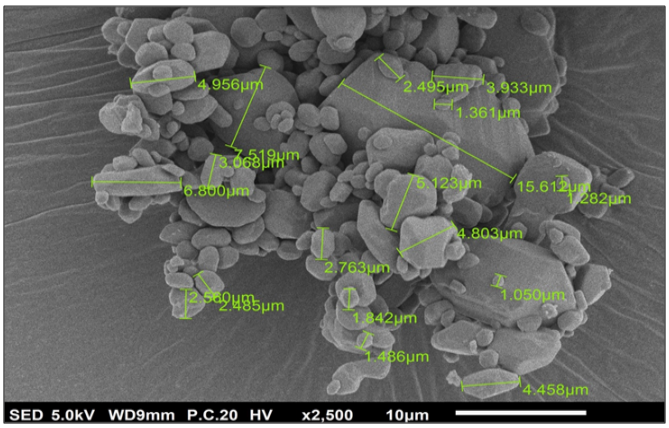


Figure 7(b): SEM images of MPA injectable suspension at resolution 2500x

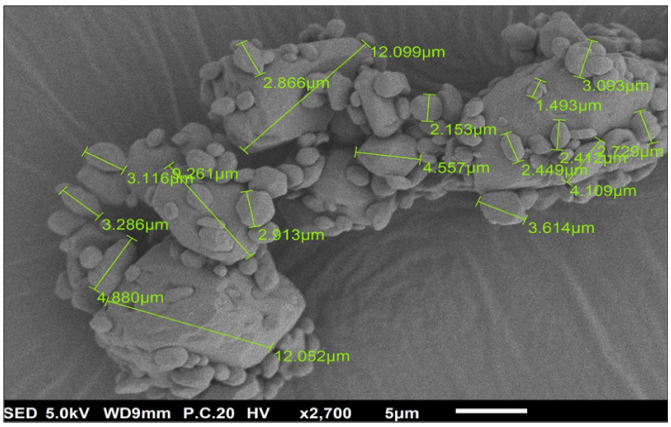


Figure 7(c): SEM images of MPA injectable suspension at resolution 2700x

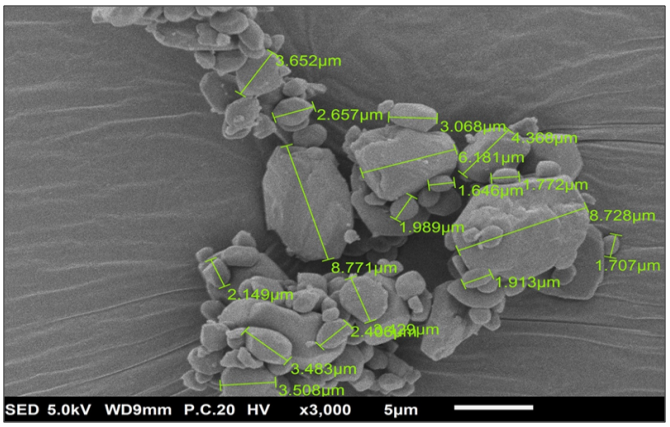


Figure 7(d): SEM images of MPA injectable suspension at resolution 3000x

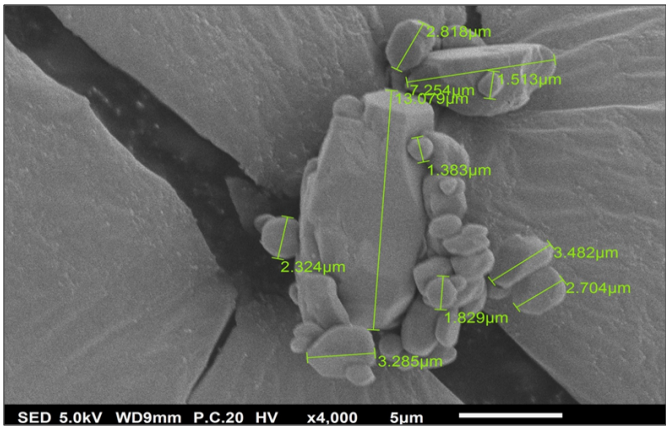


Figure 7(e): SEM images of MPA injectable suspension at resolution 4000x

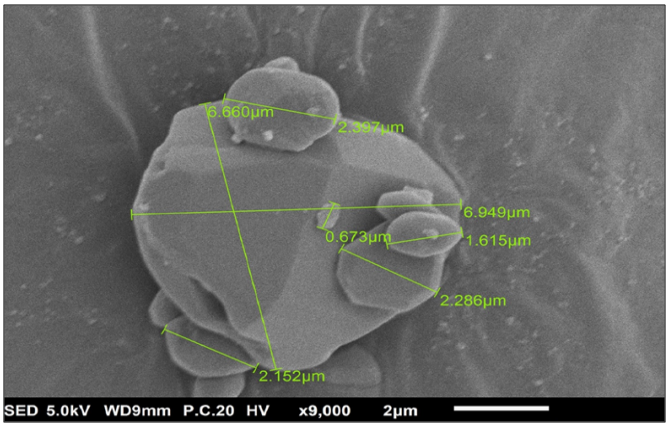


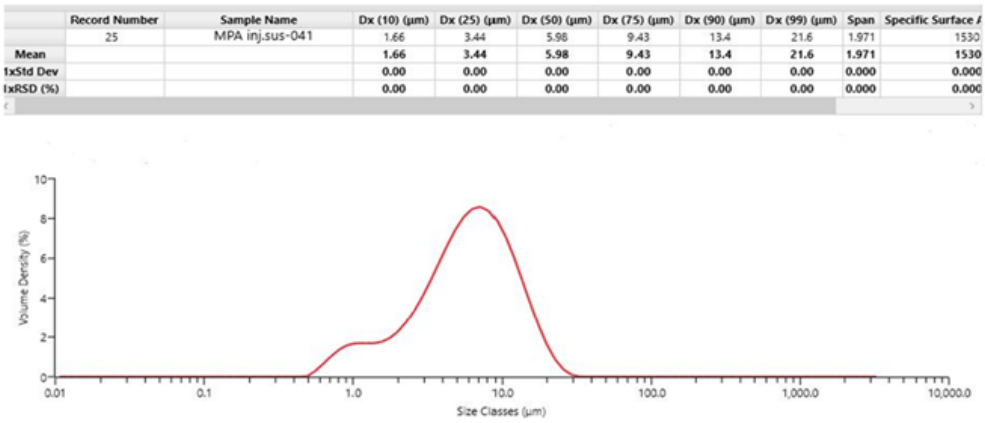
Figure 7(f): SEM images of MPA injectable suspension at resolution 9000x

7.2 *Particle Size Analysis*

Particle size analysis (Histogram figure 8) of MPA injectable suspension showed that mean particle size of micronized suspension was 13.4 μm with specific surface area 1530 cm^2/km. Particle size and surface area reported in (Table 11).

7.3 *In-vitro Drug Release (USP type - II, IV- Dissolution Apparatus)*

In-vitro drug release of MPA injectable suspensionthrough USP type IV- dissolution apparatus was illustrated in (Figure 9), the suspension showed a synchronous release of MPA suspension after 5 min. with sustained release after 80 min. Cumulative drug release of MPA suspension after 90 min. was illustrated in (Table 12) respectively, drug release completely in 90 minute. *In vitro* drug release of MPA injectable suspensionthrough USP type II- dissolution apparatus) was illustrated in (Figure 10), the suspension showed a synchronous release of MPA suspension within burst release within 5 min. (76% CDR), with sustained release after 24 h. Cumulative drug release of MPA suspension after 48 hours was illustrated in (Table 13) respectively.



	Record Number	Sample Name	Dx (10) (µm)	Dx (25) (µm)	Dx (50) (µm)	Dx (75) (µm)	Dx (90) (µm)	Dx (99) (µm)	Span	Specific Surface A
	25	MPA inj.sus-041	1.66	3.44	5.98	9.43	13.4	21.6	1.971	1530
Mean			1.66	3.44	5.98	9.43	13.4	21.6	1.971	1530
1xStd Dev			0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000
1xRSD (%)			0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000

Figure 8: Particle size histogram of MPA injectable suspension

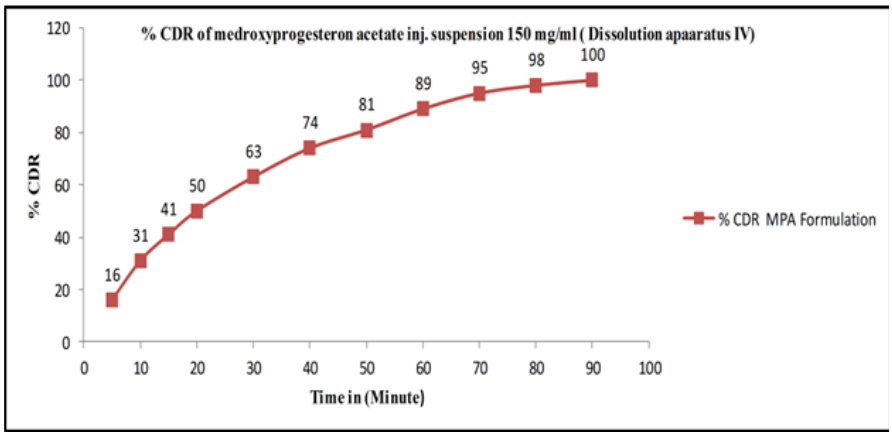


Figure 9: % CDR of MPA injectable suspension (Dissolution apparatus IV)

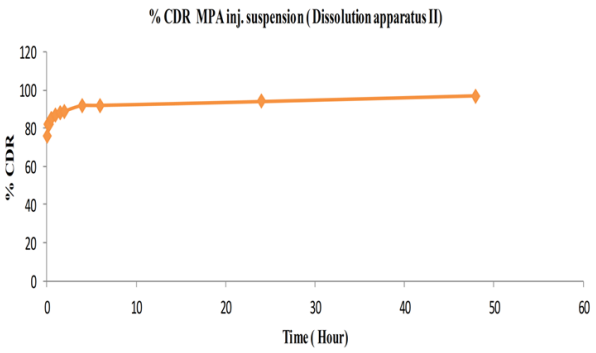


Figure 10: % CDR of MPA injectable suspension (Dissolution apparatus II)

Table11: Particle Size of MPA Suspension in Micron (μm)

S.No	Batch no	Particle size in micron (μm)			
		D10	D50	D90	Specific surface area (m^2/km)
1	MPA suspension	1.66	5.96	13.4	1530

Table 12: % CDR of MPA Suspension (USP Type IV apparatus)

S.No	Time (Min)	% cummulative Drug release
1	5	16
2	10	31
3	15	41
4	20	50
5	30	63
6	40	74
7	50	81
8	60	89
9	70	95
10	80	98
11	90	100

Table 13: % CDR of MPA Suspension (USP type II apparatus)

S.No	Time (Min)	% cummulative Drug release
1	0.08	76
2	0.16	82
3	0.25	82
4	0.5	85
5	1	87
6	1.5	88
7	2	89
8	4	92
9	6	92
10	24	94
11	48	97

7.4 ***Related Compound***

Analysis of related compound in finished product of MPA injectable suspension was carried out by chromatographic method by using the HPLC and observed impurity in MPA injectable suspension were reported in (Table 14).

7.5 ***Zeta Potential***

Zeta potential of MPA injectable suspension was found to be -22.66 (mV) Zeta potential of formulation were reported in (Figure 11).

7.6 ***Accelerated stability study***

Accelerated stability study of MPA injectable suspension was carried out according to International Conference on Harmonisation (ICH) Q1A (R2) guidelines. Stability study was performed at 25±2°C/60±5% RH, 3 month 40±2°C/75±5% RH, 3 Product has been expose upward and inverted condition to compare the adsorption of drug product as well as leaching impurity from rubber closure. Result was reported in (Table: 15), result show that there is no significant change in assay, In-vitro dissolution, particle size (Figure 12), and other physical parameter.

7.7 ***Photo Stability***

Based on the photo stability result for all three group along dark control samples, it can be conclude that MPA injectable depot suspension was stable when exposed to light 1.2 million lux hours an integrated near ultra violet energy of not less than 200 watt hours/ square meter of cool fluorescent light in its primary packs.

8. Conclusion

Medroxyprogesteron acetate injectable depot suspension was successfully developed by using rapid stirring method. Mixing speed and temperature dependent solubility of parabens were affect the particle size, specific surface area and dissolution rate of MPA injectable suspension. The resultant MPA suspension were evaluated for Physical properties, MPA content were 101.00±2%, viscosity were 8.58 ±1 cps, pH 5.826±1 and osmolality were found to be 378 ± 5 Mosmol/ kg.H_2O respectively & further characterized for surface morphology of suspensions were found to be spherical with smooth surface, Particle size, specific surface area & zeta potential were found to be (Particle size 13.4 μm with specific surface area 1530 cm^2/km, -22.66 mV), sedimentation volume. The MPA injectable suspension could go through 24 gauge hypodermic needle smoothly with withdrawal volume 1.70, mL, MPA suspension re-dispersed within 1 minute without forming clogs. Interaction between the drug and polymer were investigated by Fourier Transform Infrared (FT-IR) Spectroscopy. The FT-IR analysis confirmed the compatibility of MPA with the excipients without interaction. In- vitro drug release from the MPA injectable suspension through dissolution apparatus IV showed a synchronous release of MPA suspension after 5 minute (16%) with sustained release after 80 minute (98%) and Cumulative drug release of MPA suspension after 90 minute was found to be (100%), and dissolution apparatus II showed sustained release over a period of 24 hours (94%) and Cumulative drug release of MPA suspension after 48 hours was found to be (97%). 3 month stability data at (25±2°C/60 ± 5% RH, 40±2°C/75±5% RH) and

Table 14: Reported RC of Medroxyprogesteron Acetate Injectable Suspension

S.No	Related compound	USP Limit	Reported RC of (F)
1	6β-hydroxymedroxyprogesteron (A)	NMT 1.0%	0.03
2	17a∞-methyl-keto-D-Homomedroxyprogesteron (B)	NMT 1.0%	0.19
3	Hydroxymedroxyprogesteron(C)	NMT 1.0%	0.01
4	Medroxyprogesteron	NMT 1.0%	0.05
5	D-homoanalogous(D)	NMT 1.0%	0.02

Table15: Accelerated Stability Result

S.No	Parameter	Initial MPA suspension	3M, 25 ± 2°C /60 ± 5% RH	3M, 40 ±2°C/75 ±5% RH
1	Description	White homogeeous suspension	White homogeeous suspension	White homogeeous suspension
2	pH	6.1	5.80	5.74
3	Assay of mpa	100.	99.00	98 .00
4	Dissolution % CDR (At 90 min run time by dissolution apparatus type-IV)	100	96	97
5	Particle size	13.4 μm	13.8 μm	14.2 μm

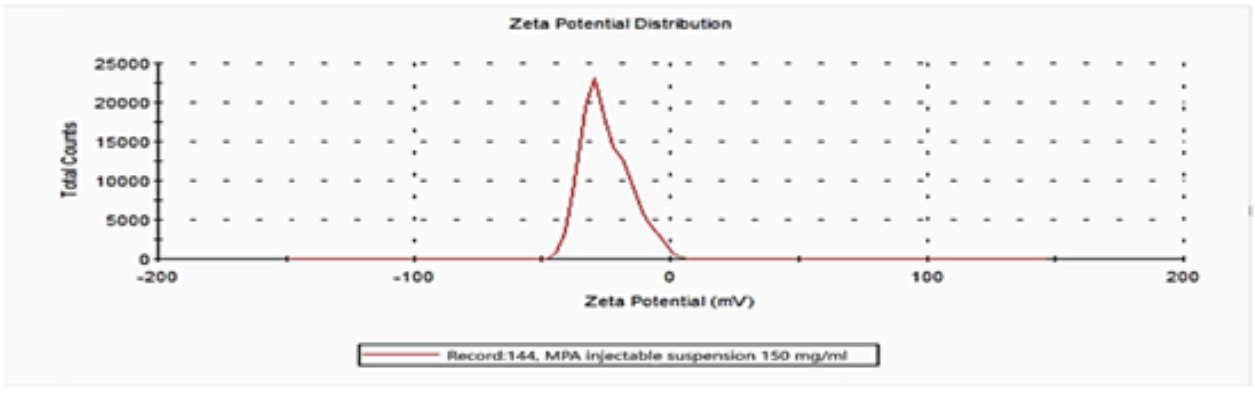


Figure 11: Zeta potential of MPA injectable suspension

Sample Name	Dx (10) (µm)	Dx (25) (µm)	Dx (50) (µm)	Dx (75) (µm)	Dx (90) (µm)	Dx (99) (µm)	Span	Specific Surface Area (m^2/kg)	D [4,3] (µm)
mpa 3m stability	1.63	3.36	6.15	9.96	142	22.3	2.048	1507	7.20
	1.63	3.36	6.15	9.96	142	22.3	2.048	1507	7.20
	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.00

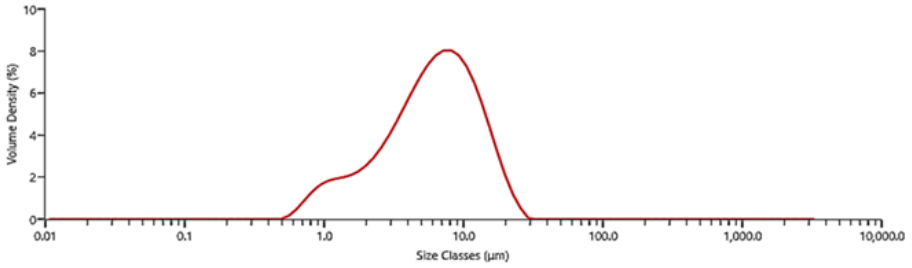


Figure 12: Particle size histogram MPA injectable suspension (Stability Sample)

photo stability data showed good stability. Finally, it is conducted that the processes adopted for the manufacturing of medroxyprogesteron injectable depot suspension provide all quality characteristics. The prepared medroxyprogesteron acetate suspension had uniform particle distribution, excellent sedimentation rate, syrengeability, redispersibility, good stability and excellent sustained-release effect.

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Ethics Approval And Consent To Participate

Not Applicable.

Human and animal right

No animals/Humans were used for studies that are base of this research.

Consent for Publication

Not Applicable.

Conflict Of Interest

The authors confirmed that this article content has no conflict of interest.

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