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FORMULATION DEVELOPMENT AND EVALUATION OF TRIAMCINOLONE ACETONIDE INTRAMUSCULAR *IN-SITU* NANO SUSPENSION

M. Sunitha Reddy* and K. Sindoori[1]
Centre For Pharmaceutical Sciences, JNTU Kukatpally, Hyderabad-500085, Telangana, India

***Corresponding Author Email: baddam _sunitha@rediffmail.com**

ABSTRACT

Triamcinolone acetonide is a steroidal anti-inflammatory drug. The patients who are suffering from auto immune diseases like Rheumatoid arthritis treated with Triamcinolone Acetonide, which binds to the Glucocorticoid receptor leading to the activation of anti – inflammatory transcription factors there by it suppresses the activity of immune system. The main objective of the present work is to prepare and evaluate Triamcinolone Acetonide intra muscular in situ nano suspension. The nano suspension was prepared by Micro fluidization technology, with different polymers like poloxamer 407 and poloxamer 188 which forms in-situ gel at body temperature. The prepared in situ nano suspension was evaluated for official parameters. The release profile of optimized formulation has shown 101.06 ±0.68 release in 12 hours respectively with particle size 429.7±4.10 nm, pH 6.5, osmolarity 289osm/L. Three months accelerated stability studies was studied at (25±2C^0 / RH 60± 5%) and (40±2C^0/ RH 75± 5%) and no significant changes were observed.

KEY WORDS

Triamcinolone Acetonide, In-situ, High pressure homogenization, Micro fluidization, Poloxamer 407, poloxamer 188 etc.

INTRODUCTION

The parenteral drug delivery system is most predominant and preferable drug delivery system to by passing the drug related side effect via oral route (Acid degradation, enzymatic action at intestine and hepatic effect) and Pharmacokinetic (Absorption and solubility) problems. These routes can also facilitate the drug delivery directly to the specific area/region in the body by injecting the solution.

Different strategies, such as micronization, solubilization using co-solvents, oily solutions, the use of permeation enhancers,[1] surfactant dispersion which evolved earlier to tackle the formulation problems, have narrow use. Though there are reasonable success has been achieved in different formulations like microemulsions,[2] water-insoluble drugs using liposomes, emulsions, solid dispersion technology and inclusion complexes employing cyclodextrins. But there are still some deficiencies for the above formulations, such as low drug loading, high toxicity, poor stability, potential drug expulsion during storage and complex manufacturing method.[3]

Injectable *In situ* Gel forming Drug Delivery System

In situ gel system was introduced by Dunn and co-workers at Southern Research Institute in Birmingham, Alabama in 1987. It can be used to delivery for both parenteral and site-specific drug delivery. The term *in situ* obtained from the "Latin" phrase which translated as "in position". Prior to the administration of nanosuspension into the body looks like solution but after injecting into the body it forms in-situ gel due to body temperature.[4]

Classifications of *In-situ* Polymeric Systems

Natural polymers-

chitosan, Alginic acid, pectin, dextran, carboxymethyl chitin, gum, chitosan.

Synthetic polymers-
Aliphatic polyesters such as, poly (glycolic acid), poly- caprolactone and poloxamers.

Definition of Nanosuspension[5]

The colloidal dispersions of nanosized drug particles stabilized by surfactants are
known as nano suspensions. Or it can be defined as nano suspensions are biphasic system in which pure drug particles dispersed in an aqueous vehicle. In nano suspensions diameter of the particle is less than "1µm" in size.

To all drug compounds belonging to **biopharmaceutical classification system (BCS) classes II and IV** can be implemented by formulating nanosuspensions to increase the availability and solubility of drug at specific site.

Advantages:

- Poorly water-soluble drugs can be successfully formulated using nano suspensions and improved dissolution So, formulation of nanosuspensions require active moiety which is water insoluble.
- High drug loading can be achieved as a drug exists in the form of pure solids and can significantly reduce the administration volume of high dose.
- Nanosuspensions have the capacity to increase the chemical and physical stability of drugs.
- Intra muscular administration of nanosuspensions provides increased retention time of nanoparticles at muscular region and reduces the oral side effects.

MANUFACTURING PROCESS OF NANO SUSPENSION[6]

Nanosuspension preparation can be broadly classified in to two categories 'top- down' and the 'bottom-up' processes. Top down processes include high pressure homogenization and media milling method. Bottom up technologies start with a molecular dispersion of a pharmaceutical ingredient.

Top down (Disintegration) Principle - High Pressure Homogenization

Microfluidizer[7]

Microfluidizers work on jet stream principle, the suspension is accelerated and is passed with high velocity through specially designed homogenization chamber. Two types of chamber were designed they are, In the A type chamber, the suspension changes a few times the direction of its flow leading to particle collision and shear forces. In the B type chamber, suspension flow is through the Y shape microchannels, the suspension stream is divided into two streams which then collide frontally. Microfluidizer technology is helpful for insoluble drug delivery particles, when passed through fluidizer results in submicronic nanosuspensions.

Mechanism of Microfluidizer[8]

Microfluidizer involves a technology called Patented mixing technology, which makes use of a device called microfluidizer. It involves a high-pressure positive displacement pump (5000 to 30000psi), which forces the product through the interaction chamber, which consists of small channels called microchannels. The formulation pass through these microchannels resulting in very fine particles of submicron range.

METHODOLOGY

Chemicals: Polysorbate 80 (tween 80), Sodium carboxy methyl cellulose, Sodium hydroxide, Sodium chloride, Poloxamer 407, Poloxamer 188, Benzyl alcohol, Hydrochloric acid, water for injection.

Instruments: Digital weighing balance, Homogenizer, Zeta sizer, UV- spectrophotometer, Bath sonicator, Cyclomixer, Differential scanning calorimeter, Microfluidizer, pH Meter, Brookfield Viscometer, Laminar air flow chamber, Magnetic stirrer, Dissolution test apparatus (USP II).

Preformulation Studies:

- Physical characterization of API
- Solubility studies of API
- Drug and excipient compatibility

Method:

Dissolution:

USP type II (paddle) apparatus was used to study the drug release patten.900mL of pH 7.4 +0.5% SLS Phosphate buffer was used as dissolution medium and the basket was rotated at 50rpm at temperature (37 ± 0.5ºC). At predetermined intervals, aliquot samples (5 mL) were collected and replaced with same volume of fresh medium into the baskets. The collected samples (5 mL) were filtered through 0.45µm membrane filter and the filtrate was diluted appropriately and was estimated using UV-Visible spectrophotometer at λmax238 nm.

FORMULATION DEVELOPMENT

Physical characterization of suspension

- Zeta potential
- pH
- Viscosity (cps)
- Osmolarity

- Gelation time

Table no: 01 Formulation

S. No.	Name of the Excipients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Triamcinolone Acetonide	40	40	40	40	40	40	40	40	40
2	Sodium CMC	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
3	NaCl	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
4	Polaxomer 407	7.5	10	12	-	-	-	3.75	5	6
5	Polaxomer 188	-	-	-	10	15	20	5	7.5	10
6	Tween 80	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
7	Benzyl alcohol	9.9	9.9	9.9	9.9	9.9	9.9	9.9	9.9	9.9
8	NaOH (0.1N)	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs
9	HCl (0.1 N)	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs
10	Water for injection	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs

Manufacturing procedure

1. About 60% of WFI was collected in a clean and dried beaker.
2. Weighed quantity of Tween 80, Benzyl alcohol and Sodium chloride were added to above WFI and stirred continuously on magnetic stirrer at 300rpm until clear solution was obtained.
3. Weighed quantity of Poloxamer and sodium carboxy methyl cellulose were added slowly to the above solution and stirred continuously using magnetic stirrer.
4. Required quantity of Triamcinolone Acetonide (API) was added to above beaker and stirred for 10 min at 300rpm on magnetic stirrer.
5. The above mixture was passed through Homogenizer at 6000rpm for 10 min and allowed to 7 cycles of micro fluidization using MF401 Microfliudizer at 10000 psi.
6. The pH of the suspension was adjusted between 5-7.5 with NaOH, HCL and final volume was adjusted with WFI.

Manufacturing of Triamcinolone Acetonide intra muscular *in-situ* Nano suspensions:

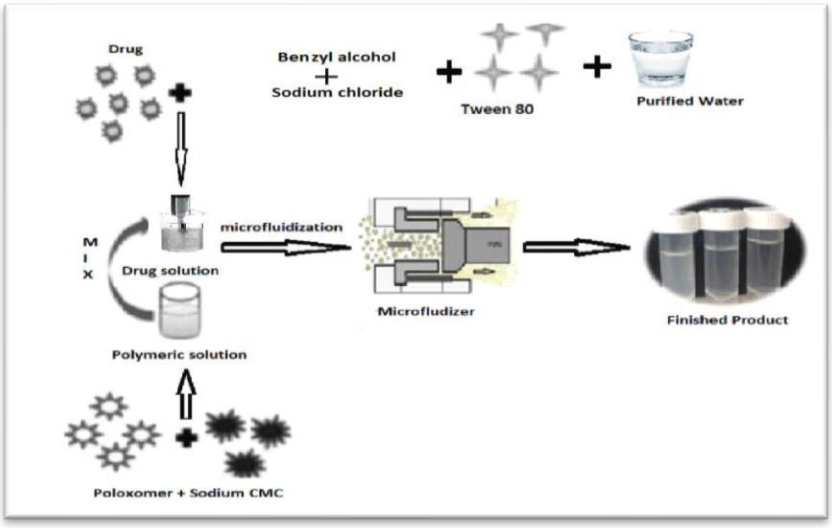

Figure 01: Preparation of Triamcinolone Acetonide intra muscular *in-situ* Nano suspensions by Microfludization

RESULTS AND DISCUSSION

PREFORMULATION STUDIES

Table no: 02 physical characterization of API

S.NO	Description	Result
1	Color	White crystalline powder
2	Odor	Odorless
3	Taste	Bitter
4	LOD	0.4% L

SOLUBILITY STUDIES OF API

Table no: 3 solubility studies of API

S.NO	Media	Solubility (mg/mL)
1	Purified Water	0.004
2	0.1N HCL	0.006
3	pH 4.5 Acetate Buffer	0.006
4	pH 6.8 Phosphate Buffer	0.004
5	pH 7.4 Phosphate Buffer	0.043
6	Water +0.5% SLS	0.344
7	0.1N HCL +0.5% SLS	0.171
8	pH 4.5 Acetate Buffer + 0.5%SLS	0.311
9	pH 6.8 Phosphate Buffer +0.5% SLS	0.292
10	pH 7.4 Phosphate Buffer +0.5%SLS	0.612

DRUG-EXCIPIENT COMPATABILITY STUDIES BY DIFFERNTIAL SACNNING CALORIMETRY (DSC)

The thermal properties of the drug and the mixture of drug and excipients are of important interest since this can help to assess the interaction among different components of the formulations. The DSC thermogram of pure Triamcinolone Acetonide showed a sharp endothermic peak at a temperature of 291°C which is in accordance with the reported value (292°C). This clearly indicated the purity of the drug (Figure 02).

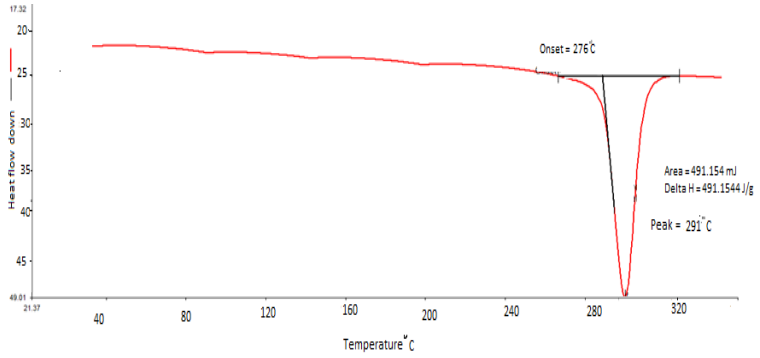

Figure 02: DSC thermogram of pure Triamcinolone Acetonide drug

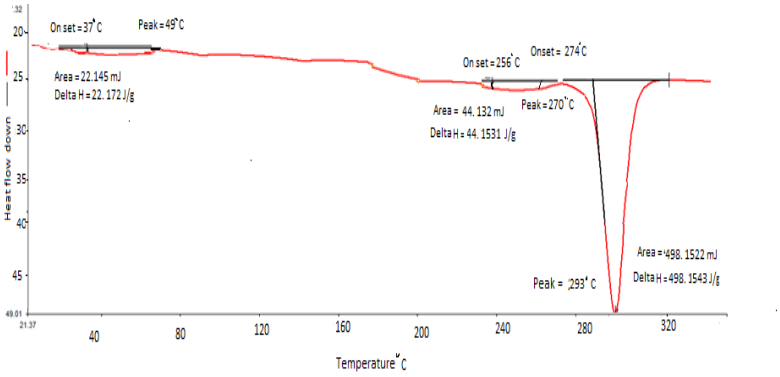

Figure 03: DSC thermogram of SLS and Drug Physical mixture

The DSC thermogram of physical mixture (Figure 03) was showing a sharp endothermic peak of drug at a temperature of 293 °C, and the presence of hump was noticed at temperature 49°C.

ASSAY

Assay percentage of the Innovator product was 100.44±1.02, Assay percentage of optimized formulation F8 showed assay percentage of 99.06±0.81 which is in limit and matches the marketed product.

Table no: 04 Drug Content

Test	Assay (96 – 103 % USP)
RLD	100.44±1.02
F1	100.7±0.52
F2	99.86±0.31
F3	100.4±1.05
F4	99.63±0.21
F5	100.4±0.02
F6	100.2±1.03
F7	100.5±0.42
F8	99.06±0.81
F9	100.4±1.05

(n=3)

DISSOLUTION

The theoretical drug release profiles are obtained for the innovator product and different formulations F1, F2, F3, F4, F5, F6, F7, F8, F9 and the optimized F8 formulation shows the cumulative % drug release 0, 41 ± 0.82, 64 ±0.63, 72 ± 1.54, 83 ± 0.98, 89 ± 1.03, 94 ± 1.11 and 101.1 ± 0.68 respectively. The innovator product shows drug release 0, 42 ± 1.32, 60 ± 1.82, 73 ± 2.1, 81 ± 3.1, 88 ± 0.8, 92 ± 1.99 and 98 ± 2.1 respectively. These are carried out at certain intervals of time duration 1, 2,3,4,6,8,12(hours). Of all the formulations performed F8 shows cumulative % drug release after 12th hour like that of innovator.

Table no: 05 Percentage (%) drug release profiles

Trials	Time(hr)							
	0	**1**	**2**	**3**	**4**	**6**	**8**	**12**
F1	0	55 ± 1	77 ± 3	88 ± 2	92 ± 1	97 ± 1.3	97 ± 2.3	96 ± 1.2
F2	0	37 ± 2	56 ± 1	70 ± 3	81 ± 2	87 ± 1.9	92 ± 2.7	97.5 ± 1.86
F3	0	35 ± 1.9	53 ± 3.1	64 ± 2.1	69 ± 1.4	73 ± 2	79 ± 1.23	83 ± 1.53
F4	0	48 ± 1.12	59 ± 2.1	84 ± 1.65	94 ± 2.31	96 ± 2.1	97 ± 2.6	98.2 ± 0.23
F5	0	39 ± 1.51	58 ± 1.42	74 ± 1.49	83 ± 2	90 ± 1.21	95 ± 1.98	98.6 ± 0.86
F6	0	28 ± 1.87	43 ± 1.22	55 ± 2	60 ± 1.02	70 ± 1.67	87 ± 0.68	92 ± 0.98
F7	0	51 ± 1.52	67 ± 1.65	78 ± 2.13	88 ± 1.09	96 ± 1.43	96 ± 1.21	98 ±0.87
F8	0	41 ± 0.82	64 ±0.63	72 ± 1.54	83 ± 0.98	89 ± 1.03	94 ± 1.11	101.1 ± 0.68
F9	0	27 ± 1.14	36 ± 2.01	47 ±1.13	58 ±0.98	64 ± 1.21	71 ± 0.98	82 ± 1.25
RLD	0	42 ± 1.32	60 ± 1.82	73 ± 2.1	81 ± 3.1	88 ± 0.8	92 ± 1.99	98 ± 2.1

(n=3)

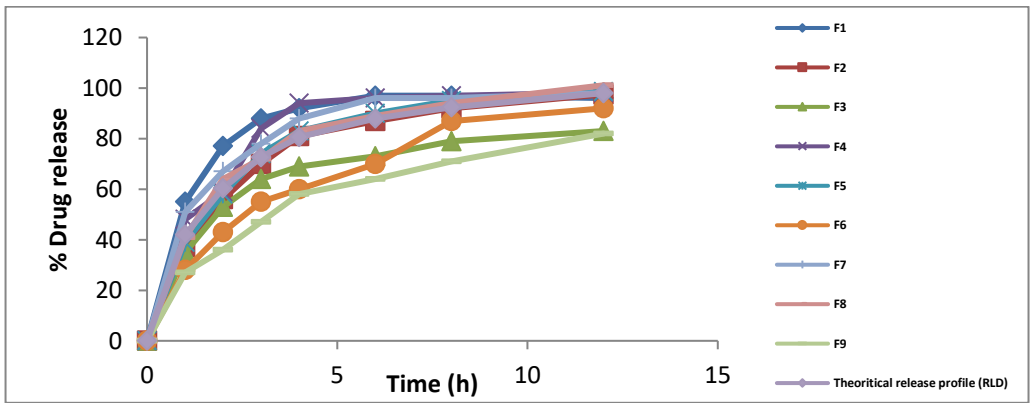

Figure 04: In-Vitro dissolution profile of all formulations

Table No: 06 Percentage (%) drug release of RLD and F8

Time	RLD	F8
0	0	0
1	42 ± 1.32	41 ± 0.82
2	60 ± 1.82	64 ±0.63
3	73 ± 2.1	72 ± 1.54
4	81 ± 3.1	83 ± 0.98
6	88 ± 0.8	89 ± 1.03
8	92 ± 1.99	94 ± 1.11
12	98 ± 2.1	101.1 ± 0.68

(n=3)

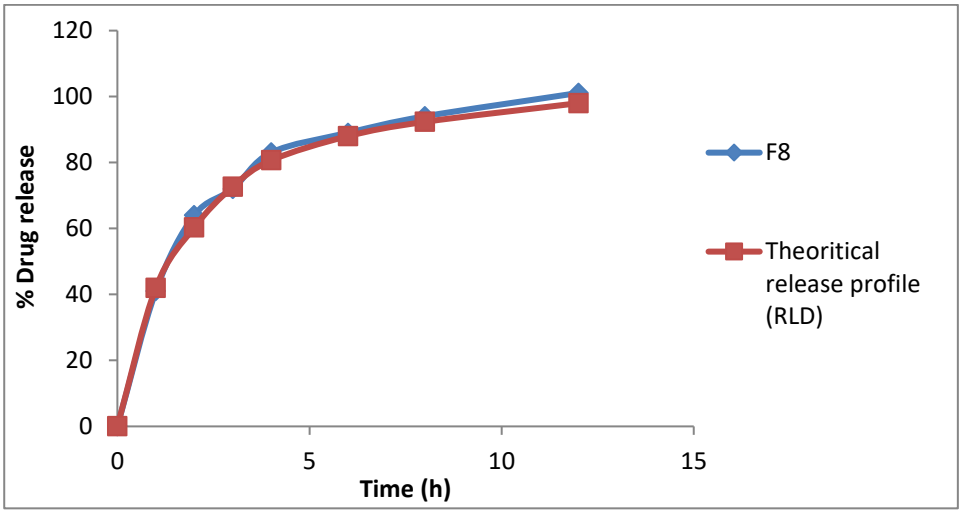

Figure No 05: In-Vitro dissolution profile of RLD and optimized formulation (F8)

PHYSICAL CHARACTERS OF PREPARED *IN-SITU* NANO SUSPENSION

Table :07 Physical characterization

Formulation	Size (nm)	PDI	Zeta Potential (mV)	pH	Osmolarity (osm/L)	Viscosity (cps)	Gelation time (min)
F1	716±4.41	0.181±0.02	-34.7±0.56	5.42	299	12	7
F2	489.7±4.10	0.171±0.04	-14.6±1.26	6.51	307	14	6
F3	469.6±32.7	0.332±0.11	-26.9±2.57	6.43	300	15	6
F4	642.5±24.4	0.230±0.06	-25.4±1.38	6.50	296	18	4
F5	429.3±33.2	0.318±0.03	-33.7±1.86	6.64	297	19	7
F6	424±6.53	0.425±0.05	-21.9±0.22	6.65	295	10	4
F7	636±4.41	0.181±0.02	-33.7±0.56	6.51	301	20	6
F8	429.7±4.10	0.171±0.04	-14.6±1.26	6.5	289	21	5
F9	448.6±32.7	0.312±0.11	-24.9±2.57	6.54	296	21	4

(n=3)

ACCELRATED STABILITY STUDY DATA (3 MONTHS)

Table :08 Stability data of 3 months

Formulation	F8	F8	RLD	RLD
Stability conditions	**25 C / RH 60±5%**	**40 C/ RH75±5%**	**25 C / RH 60±5%**	**40C/ RH75±5%**
Size (nm)	419.7±4.10	422.7±4.10	1379.6±32.72	1379.6±32.72
PDI	0.191±0.04	0.161±0.04	0.422±0.13	0.412±0.11
ZP (mV)	-17.6±1.21	-15.2±1.31	-24.9±2.57	-25.9±2.57
Drug content	100.06±0.81	98.06±1.81	99.44±1.12	101.44±1.22
pH	6.4	6.43	6.48	6.48
Osmolarity	285	282	281	285
Dissolution	100.1 ± 0.68	101.3 ± 0.68	98 ± 2.1	98 ± 2.1

(n=3)

Report:

After 3 months stability at **25 C / RH 60±5%** and **40 C/ RH75±5%** no significance changes was observed in optimized F8 formulation and RLD. It indicates optimized formulation has good stability in different environment conditions.

CONCLUSION

The purpose of this study was to develop an intra muscular administrable *in situ* nanosuspension of poorly water-soluble drug, Triamcinolone acetonide using Microfluidizer and optimized formulation in comparison to innovator product**.** Drug-excipient compatibility studies were conducted and the thermograms showed no incompatibility between the drug and excipients selected. After Preliminary trials with microfluidizer, 9 batches were selected to carried out for the characterization of pH, Osmolarity, Viscosity (cP) Size, PDI, ZP, and Dissolution. All the formulations were evaluated and the formulation F8 was found to be the optimized formulation. Three months accelerated stability studies was studied at (25±2C^0 / RH 60± 5%) and (40±2C^0/ RH 75± 5%) and it was found to be 100.1 ± 0.68 101.3 ± 0.68 at the end of 3 months respectively.

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***Corresponding Author:**
M. Sunitha Reddy*
Email: **baddam _sunitha@rediffmail.com**