J. Pharm. Res. 2019;18(2):9–16

Print ISSN: 0973-7200
Online ISSN: 2454-8405

Journal of Pharmaceutical Research

ORIGINAL ARTICLE

Design and Evaluation of Injectable Suspension Containing Anti-inflammatory Glucocorticoids

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ARTICLE INFO

Article history:
Received 03.02.2019
Accepted 28.06.2019
Published 12.07.2019

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https://doi.org/10.18579/jopcr/v18.2.anil

ABSTRACT

Objective: The aim of the present work was to formulate an intramuscular injection of Betamethasone acetate and Betamethasone sodium phosphate. Betamethasone acetate, an insoluble substance, was formulated as a depot suspension for parenteral use, whereas betamethasone sodium phosphate was used for immediate action, with excipients such as buffering agents, surfactants, complexing agents, and preservatives.

Methods: The formulation involved batch optimisation of preservatives, surfactants, and homogenisation with consistent API quantities. The final formulation contained 3 mg each of Betamethasone acetate, betamethasone sodium phosphate, 3.4 mg monobasic sodium phosphate, 7.1 mg dibasic sodium phosphate, 0.01 ml polysorbate 80, 0.1 mg disodium edetate, and 0.2 mg benzalkonium chloride per 1 ml suspension. The in vitro assessments included compatibility, pH, assay, related substances, syringeability, particle size, zeta potential, sedimentation, resuspendability, dissolution, and SEM. All the in vitro evaluation parameters were within the limits specified by the USP.

Findings: Stability studies conducted according to ICH guidelines concluded that the optimised formulation was stable. The drug was found to be compatible and stable with the excipients.

Novelty: This study successfully developed a stable and effective intramuscular injection of betamethasone acetate and betamethasone sodium phosphate for depot action. This dual-action formulation, which combines immediate and sustained release properties, offers significant advancement in injectable corticosteroid therapies.

Keywords: Betamethasone acetate; Betamethasone sodium phosphate; DSC; SEM; Zeta potential; Depot suspensions

INTRODUCTION

Injectable suspensions are heterogeneous systems comprising a solid phase dispersed in a liquid phase, typically aqueous or nonaqueous. For pharmaceutical acceptability, they should be sterile, stable, resuspendable, syringeable, injectable, isotonic, and non-irritating[1]. Recent scientific advances in parenteral drug delivery have spurred the development of systems that allow sustained or controlled drug release[2]. Parenteral depot formulations have gained prominence owing to technological advancements[3], especially for drugs with limited bioavailability or narrow therapeutic indices in the gastrointestinal tract[4,5].

Glucocorticoids (GCs) are pivotal in medicine and exhibit anti-inflammatory, immunosuppressive, anti-allergic, and antitumour properties[6–9]. They mitigate conditions such as rheumatoid arthritis, inflammatory bowel disease, and COVID-19 by inhibiting inflammatory mediators[10]. GCs effectively suppress the immune response in autoimmune diseases and in transplant settings. They also alleviate allergic reactions such as rhinitis and urticaria, and enhance chemotherapy outcomes by reducing side effects and boosting efficacy[11–13].

A significant application of GCs is the management of asthma, a prevalent global inflammatory disease[14]. Inhaled glucocorticoids have revolutionised asthma treatment and become a cornerstone therapy for chronic disease management. Advances in the understanding of how GCs suppress inflammation offer the potential for developing improved therapies[15,16]. This study aimed to optimise the ingredients and process of drug formulation for long-acting depot

injections. It involved formulating the injectable dosage form, evaluating the final formulation, conducting accelerated stability studies, and comprehensively documenting all resulting data.

METHODOLOGY

Material and their sources

Betamethasone acetate, betamethasone sodium phosphate, dibasic sodium phosphate, monobasic sodium phosphate, disodium EDTA, benzalkonium chloride, polysorbate 80, peg 3350, water for injection, hydrochloric acid, potassium bromide, methanol, ether, and sodium hydroxide. These materials were obtained from the Strides Technology and Research Centre (STAR), Bangalore (Table 1).

Compatibility studies with excipients

Conducting an excipient compatibility screen at the early pre-formulation stage of development provides valuable information about potential incompatibilities between the drug and excipients. The compatibility between drugs and excipients can be affected by many factors, such as moisture content, physical form, particle size, surface area, morphology, and trace impurities of either component. Stressed storage conditions are used to accelerate reactions between the drug and excipients, so that measurable changes occur within a short time frame. Compatibility studies of API and selected excipients were conducted for a duration of one month. The drug and excipients were placed in 1:2 ratios in glass vials, kept at an accelerated temperature of 40°C±2°C/75%±5% RH, and analysed by IR.

Preparation and optimization of the injectable suspension

Method of preparation

The manufacturing process was carried out under aseptic conditions, including washing and sterilization of vials, rubber plugs, and disinfection of aluminum seals. The vials were filled into class 100 laminar air cabinets. Ethylenediaminetetraacetic acid (EDTA), monobasic sodium phosphate, and dibasic sodium phosphate were added to 80% water and injected with continuous stirring using a magnetic stirrer to obtain a clear solution. The pH of the solution was determined and polysorbate 80 was added with stirring to obtain a clear solution. Subsequently, a benzalkonium chloride (BKC) solution was added and stirred until a clear solution was obtained. The required quantity of betamethasone sodium phosphate was slowly added to the solution under continuous stirring until a clear solution was obtained. The pH of the solution was determined again, and the solution was made up to 98% with water for injection. The solution was then filtered through a sterilized 0.22 micron membrane filter. The required quantity of

Table 1: Formula for trial batch and optimized lab scale batch

a. Formula for trial batch

Sr. No.	Ingredients	Batch no.		
		I	II	III
1	Betamethasone acetate	3 mg	3 mg	3 mg
2	Betamethasone sodium phosphate	3 mg	3 mg	3 mg
3	Dibasic sodium phosphate	7.1 mg	7.1 mg	7.1 mg
4	Monobasic sodium phosphate	3.4 mg	3.4 mg	3.4 mg
5	Polysorbate 80	-	0.01 ml	-
6	Peg 3350	-	-	10 mg
7	Disodium edetate	0.1 mg	0.1 mg	0.1 mg
8	Benzalkonium chloride	0.2 mg	0.2 mg	0.2 mg
9	Water for injection	Q.S. 1 ml	Q.S. 1 ml	Q.S. 1 ml

b. Formula for optimized lab scale batch

Sr. No.	Ingredients	Quantity per ml	Quantity per liter
1	Betamethasone acetate	3 mg	3 gm
2	Betamethasone sodium phosphate	3 mg	3 gm
3	Dibasic sodium phosphate	7.1 mg	7.1 gm
4	Monobasic sodium phosphate	3.4 mg	3.4 gm
5	Polysorbate 80	0.01ml	10 ml
6	Disodium edeate	0.1 mg	100 mg
7	Benzalkonium chloride	0.2 mg	200 mg
8	Water for injection	Q.S. 1 ml	Q.S. 1L

betamethasone acetate was added to the filtered solution under continuous stirring in an aseptic area. Finally, the volume was made up with water for injection and the suspension was filled into USP Type I amber tubular glass vials.

Potency calculation for active pharmaceutical ingredient (API)

Potency calculation for API was calculated using the formula:-

$$Equivalent\ factor = \frac{Molecular\ weight\ of\ betamethasone\ acetate}{Molecular\ weight\ of\ betamethasone\ acetate}$$

= 1.112

$$Quantity\ required\ for\ batch = actual\ quantity \times 1.112$$

= 3gm X 1.112

Quantity req for batch = 3.336 gm

$$Quantity\ required\ for\ batch = \frac{Qty/batch \times 100}{Assay}$$

$$Quantity\ required\ for\ batch = \frac{3.336 \times 100}{99.4}$$

= 3.356 gm

$$Equivalent\ factor = \frac{Molecular\ weight\ of\ betamethasone\ sodium\ phosphate}{Molecular\ weight\ of\ betamethasone}$$

= 1.315

$$Quantity\ required\ for\ batch = \frac{Qty/batch \times 100}{Assay}$$

$$Quantity\ required\ for\ batch = \frac{3.94 \times 100}{99.8}$$

= 3.947 gm

$$Equivalent\ factor = \frac{Molecular\ weight\ of\ betamethasone\ sodium\ phosphate}{Molecular\ weight\ of\ betamethasone}$$

Evaluation of injectable suspension

Description

The appearance of the suspension was observed under visible light.

Syringeability

Syringeability was determined by measuring the time for a volume (2 ml) of a composition to be transferred through 24 gauges X 1.5-inch (0.55 X 40 mm) needle into a syringe from an inverted vial with the formulation.

Resuspendability

Ability of the suspension to uniformly disperse with minimal shaking after keeping it for some time.

PH of the injectable suspension

The pH of the injectable suspension was determined by using digital pH meter.

Sedimentation volume

The sedimentation volume was determined as the ratio of the final sedimentation volume to the initial sedimentation volume. A 100 ml of suspension was placed in a 100 ml graduated measuring cylinder, the initial volume of sedimentation was noted as Vo, it was kept overnight, and the final volume of sedimentation was denoted as Vu.

Scanning electron microscopy

The sample was then placed in an evacuated chamber and scanned in a controlled pattern using an electron beam. The interaction of the electron beam with the specimen produces a variety of physical phenomena that are used to form images and provide elemental information regarding the specimens.

Particle size analysis

The particle size should be less than 5-micron meter in an injectable suspension. It was analyzed using a Malvern particle size analyzer. Particles in the size range of colloids display constant random thermal motion known as Brownian motion. This motion causes the intensity of the light scattered by the particles to vary with time. The larger the particle, the slower the motion, and hence the smaller the variation in the intensity of scattered light. Photon-correlation spectroscopy uses the rate of change in intensity to determine the size distribution of the particles.

The Zetasizer has a correlator with 64 channels, each of which measures the changes in light fluctuation over a defined time span. The time span is known as the sample time or delay time, and the correlator measures the light intensity by counting the photons. For a very short time period, the changes in light intensities will be very small, as the particles have very little time to move. The positions of the particles are statistically correlated. In contrast, with a long sample time, the particles move randomly from their initial positions. Therefore, the particles can be statistically described as not being correlated.

Zeta potential

The zeta potential of the suspensions was measured using a Malveren Zetasizer. For the zeta potential measurements, the laser light was split to provide an incident and reference beam. The incident laser beam passed through the centre of the sample cell and scattered light at an angle of approximately 130 was detected. When an electric field is applied to the cell, any particles moving through the measurement volume cause the intensity of light to fluctuate with a frequency proportional to the particle speed, which is passed to the digital signal possessor and then to a computer. Zetasizer software produces a frequency spectrum from which the electrophoretic mobility can be calculated; hence, the zeta potential was calculated.

Assay

Assay was carried out by HPLC method to determine the exact amount of betamethasone acetate and betamethasone phosphate present in the specified amount of injectable

suspension.

Dissolution

The dissolution of betamethasone acetate injectable suspension was carried out in 7.4 phosphate buffer with 0.1% SLS using a type-4 dissolution apparatus. The assembly consists of a reservoir containing the release medium and a pump that forces the release medium upward through the vertically positioned flow through the cell and water bath. The pump usually has a delivery capacity flow rate between 2 and 16 ml per minute. Usually, the bottom cone of the cell is filled with small glass beads of approximately 1 mm in diameter and with a bead of approximately 5 mm in diameter positioned at the apex to protect the fluid entry tube, whereas a filter is positioned at the inner top of the cell. At the regular interval of time sampling has been done and drug release is studied by using HPLC analysis.

Stability Studies

Accelerated testing studies were designed to increase the rate of chemical or physical degradation of the drug substance/product by using exaggerated storage conditions as per USFDA, and rapid detection of deterioration of the drug in different formulations can be known in short time. Stability studies were performed according to the ICH guidelines. The accelerated study was carried out at temperatures of 40°C±2°C/75%±5% RH, and the sample was withdrawn at one-month intervals and analyzed for evaluation parameters, such as assay, pH, and impurities.

RESULTS AND DISCUSSION

The goal of the present study was to develop an injectable suspension of the potent anti-inflammatory glucocorticoid drug betamethasone acetate, evaluate its physicochemical properties to investigate its release pattern and stability, and optimise its final formulation which is effective in the treatment of arthritis. Any formulation work must be preceded by pre-formulation studies, including analytical investigations and choice of the analytical method, and there is a need for the selection of preservatives and surfactants which are compatible with drugs and among themselves, as well as physiologically safe and biocompatible. Preliminary ideas about the behaviour of the dosage form were formulated using the prepared and selected ingredients, and their singular and collective effects on the physicochemical and pharmaceutical properties of the dosage form also need to be generated during this phase. Similarly, betamethasone acetate and betamethasone sodium phosphate were first subjected to a purity study using their melting points and characteristic IR spectra. The drug conformed to pharmacopoeia standards with respect to melting point and characteristic IR peaks. Furthermore, there was no discernible shift/disappearance/appearance of peaks in the drug-excipient combined spectra, indicating good drug-excipient compatibility.

Another objective of this study was to increase the duration of action and bioavailability of betamethasone acetate, which is widely used for the management of arthritis. As a primary step, the drug was subjected to pre-formulation studies according to the USP specification. The drug was found to be a white crystalline powder, which complies with the standard. Solubility was determined, and it was found that the drug was practically insoluble in water, soluble in alcohol and dichloromethane, and freely soluble in acetone. The drug was identified by the IR spectrum, which complied with the standard drug spectrum. The assay was carried out by HPLC, which was found to be 99.4%, and each single impurity and total impurity were 0.10% and 0.18%, respectively. The betamethasone acetate drug exhibits a 2.1% loss on drying and a melting point of 233^0 C. The specific rotation was +1230. These results are within the USP limits. Drug excipient compatibility studies were carried out with different excipients selected in a 1:2 ratio for one month under accelerated stability conditions. The IR spectra confirmed that all selected excipients were compatible with the drug. Injectable suspension was adopted to formulate parenteral dosage form of betamethasone acetate based on the physicochemical properties of the drug and other excipients, the excipient selected were polysorbate 80 as a surfactant, disodium edetate as complexing agent, dibasic sodium phosphate and monobasic sodium phosphate as buffering agent, benzalkonium chloride as a preservative and water for injection as a vehicle (Table 2).

The formulation was carried out in three different batches by varying the surfactant and maintaining constant quantities of all the active pharmaceutical ingredients. In batch I, the formulation contained no surfactant and showed poor syringeability, poor sustained release, and brisk shaking to resuspend. To improve the sedimentation properties, syringeability, and resuspendability of polysorbate 80, PEG3350 was used in batches II and III. The polysorbate 80 formulation exhibited good syringeability, resuspendability, high zeta potential, and sustained release for 24 h. Therefore, batch II was used in further studies.

Evaluation of the injectable suspension involved several key parameters across the three batches. The injectable suspension in all three batches was observed to be a white-coloured suspension, as shown in Table 4. The syringeability of batches I, II, and III was 26, 16, and 21 seconds, respectively, indicating that batch II exhibited the best syringeability. Resuspendability for all three batches was found to be within 4–8 seconds, with batches II and III showing rapid resuspendability within just 4 seconds. The pH of the suspension in all batches fell within the USP limits of 6.8 to 7.8, indicating the absence of stability issues, such as hydrolysis and esterification. The sedimentation volumes of batches I, II, and III after one day were 0.35, 0.48, and 0.55, respectively, with batches II and III displaying

Table 2: Preformulation data of the drugs and compatibility data for the drug and excipients

a. Betamethasone acetate

Mfd. By: NEW CHEM LAB. Batch no: L1939T

Mfg. Date: 05/2007 Exp. date :05/2012

Tests	Specifications	Results
Description	White or almost white crystalline powder	Complies
Solubility	practically insoluble in water; soluble in alcohol and in dichloromethane; freely soluble in acetone	Complies
Identification (ir)	The infrared absorption spectrum of the potassium bromide dispersion of sample must correspond that of similar preparation of API	Complies
Loss on drying	NMT 4.0%	2.1%
Specific rotation	+120 to $+128^0$	$+123^0$
Assay	97.0% - 103%	99.4%
Related substance (HPLC) Each single impurity Total imputity	NMT 0.5 % NMT 1.25%	0.10% 0.18 %
Residual Solvents (GLC) Methanol Isopropyl ether Dichloro methane DMF Pyridine	NMT 3000 ppm NMT 5000 ppm NMT 600 ppm NMT 880 ppm NMT 200 ppm	n.d n.d n.d 505 ppm n.d
Particle size	NLT 99.0 % < 10 micro meters	100 %

b. Betamethasone sodium phosphate

Mfd. By: NEW CHEM LAB. Batchno:M0691

Mfg. Date: 04/2008 Exp.date :04/2011

Tests	Specifications	Results
Description	White or almost white very hygroscopic powder	Complies
Solubility	Freely soluble in water	Complies
Identification (ir)	The infrared absorption spectrum of the potassium bromide dispersion of sample must correspond that of similar preparation of API	Complies
Loss on drying	NMT 8.0%	6.8 %
Specific rotation	+98 to $+104^0$	$+102^0$
Assay	97.0% - 103%	99.8%
Related Substance (HPLC) Each single impurity Total impurity	NMT 2.0 % NMT 3.0 %	n.d 0.2 %
Residual Solvents (GLC) Acetone Methanol THF Ethyl acetate Pyridine	NMT 5000 ppm NMT 3000 ppm NMT 720 ppm NMT 5000 ppm NMT 200 ppm	295 ppm 350 ppm n.d n.d n.d

c. Compatibility data for the drug and excipients

Sr. no.	Combination	Results
1	Beta-beta	Complies
2	Beta-beta + dibasic sodium phosphate	Complies
3	Beta-beta + monobasic sodium phosphate	Complies
4	Beta-beta + EDTA	Complies
5	Beta-beta + BKC	Complies
6	Beta-beta + polysorbate 80	Complies
7	Beta-beta +dibasic +monobasic+edta+bkc+poly-80	Complies

better stability owing to higher sedimentation volumes. The zeta potential measurements for batches I, II, and III were 2.92, 13.6, and 12.1, respectively, with batch II showing the highest stability owing to the highest zeta potential. The assay of the injectable suspension, conducted using the HPLC method, showed results within the range of 98–103%, which is within the USP specification limits of 90–120% (Fig. 1, Fig 2 & Fig. 3). Analysis of related substances, also performed using the HPLC method, indicated that all batches were within the USP limits. Dissolution studies were conducted in phosphate buffer (pH 7.4) using a Sotax type 4 dissolution apparatus, revealing that batch I achieved complete release within 14 hours, whereas batches II and III exhibited sustained release up to 24 hours (Table 3). Scanning electron microscopy (SEM) of batch II demonstrated that the particles in the suspension were uniform in size, forming a flocculated suspension that led to a lower resuspending time. Accelerated stability studies were carried out according to ICH guidelines at 40°C ± 2°C / 75% RH ± 5% RH over a three-month period. During this period, the optimized injectable suspension formulations were analyzed for all evaluation parameters, including syringeability, resuspendability, pH, assay, and related substances.

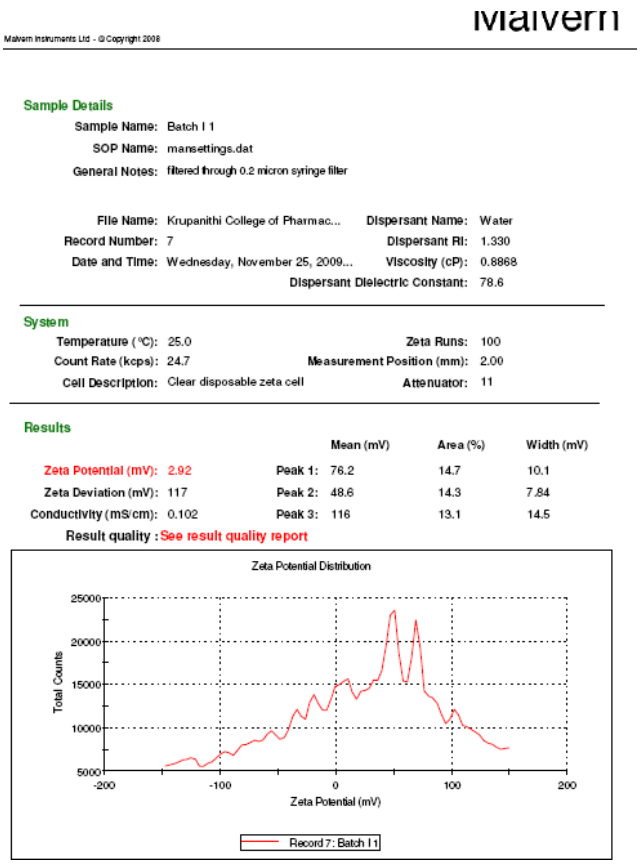

Fig. 1: Zeta potential of batch I

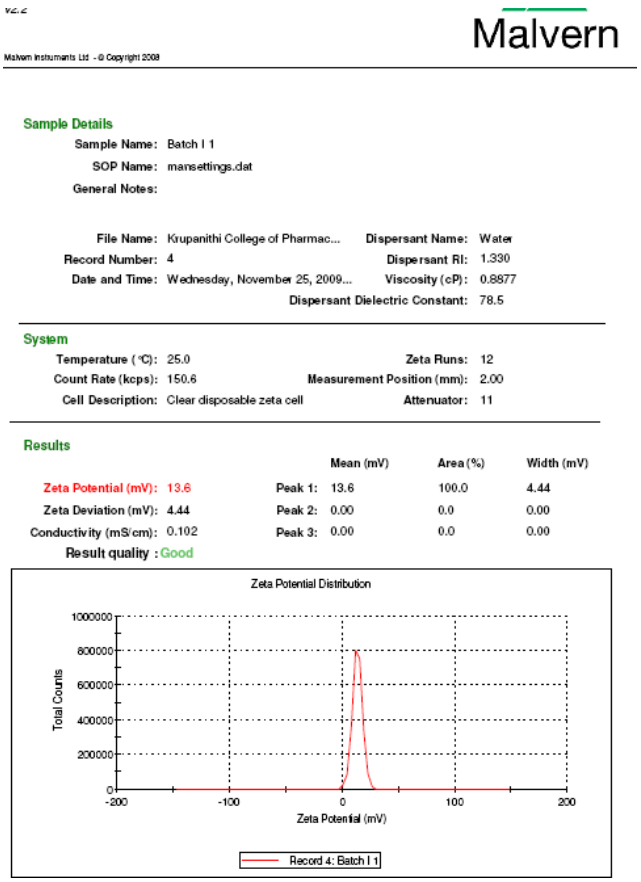

Fig. 2: Zeta potential of batch II

In the 3-month stability analysis, the colour of the product was the same as that of the initial product, and no colour change was observed. The syringeability of the product was determined to be 17 seconds. The entire suspension can be resuspended within 6 seconds. The pH of all the solutions was found to be 7.24 and not much difference was observed compared to the initial pH. The assay was analyzed by HPLC and the difference was found to be within the USP limits.

From the above results, it was found that all the evaluated parameters were within the USP limits, even after three months of stability studies. Finally, it was concluded that formulation number two was the best formulation.

CONCLUSION

This study aimed to create a novel injectable dosage form of betamethasone acetate that is insoluble in water. The project aimed to overcome these drawbacks and create a stable formulation as a depot injectable suspension. Surfactants such as polysorbate 80 and PEG 3350 were selected to improve syringeability, resuspendability, and sedimentation characteristics. Monobasic and dibasic sodium phosphates were used as buffers, disodium edetate was used as a complexing agent, and benzalkonium chloride was used as a

Table 3: Evaluation

a. Description

Batch-I	Batch-I	Batch-III
White color suspension	White color suspension	White color suspension

b. Syringibility

Batch-I	Batch-II	Batch-III
26 sec	16 sec	21 sec

c. Resuspendibilty

Batch-I	Batch-II	Batch-III
Resuspendible in 8 sec	Resuspendible in 4 sec	Resuspendible in 4 sec

d. pH

Batch-I	Batch-II	Batch-III
7.14	7.2	7.26

e. Sedimentation volume

Duration	Batch-I	Batch-II	Batch-III
Initial	1.0	0.0	1.0
After 2 hrs	0.75	0.84	0.82
1 day	0.35	0.48	0.55

f. Zeta potential

Batch-I	Batch-II	Batch-III
2.92	13.6	12.1

g. Assay and related substances

Batch no.	Assay	Related substance			
	Beta acetate	Beta sod phosphate	Impurity A	Betamethasone 17-acetate	Unknown impurity	% total impurity
1	98.5	99.6	0.28	0.09	0.05	0.49
2	99.6	101.8	0.26	0.09	0.05	0.32
3	102.4	101.2	0.26	0.09	0.05	0.36

h. Dissolution

Time (hrs)	Batch-I	Batch-II	Batch-III
0.5	19.58	13.52	16.67
1	43.59	24.39	30.19
3	88.99	45.75	51.85
6	98.5	59.63	72.16
9	100.18	74.4	85.56
12	100.83	86.68	95.67
15	101.2	92,14	98.23
18	101.2	97.92	100.73
21	101.2	101.3	102.1
24	101.2	104.2	103.96

i. Stability studies of batch -2

Storage condition	Sampling interval	Assay	Related substance				
		Beta acetate	Beta sod phosphate	Imp A (NMT 8%)	Beta 17 acetate (NMT 1%)	Unknown impurity (0.2%)	% total impurity (NMT 4%)
$40^0 \pm 2^0$ C / 75% ± 5% RH	Initial	99.6	99.8	0.74	0.14	0.04	0.55
	1st week	99-4	99.8	0.68	0.16	0.07	0.58
	1st month	99.6	101.2	0.73	0.11	0.04	0.69
	2nd month	101.4	102.0	0.58	0.19	0.05	0.73
	3rd month	99.6	97.9	0.68	0.18	0.07	0.64

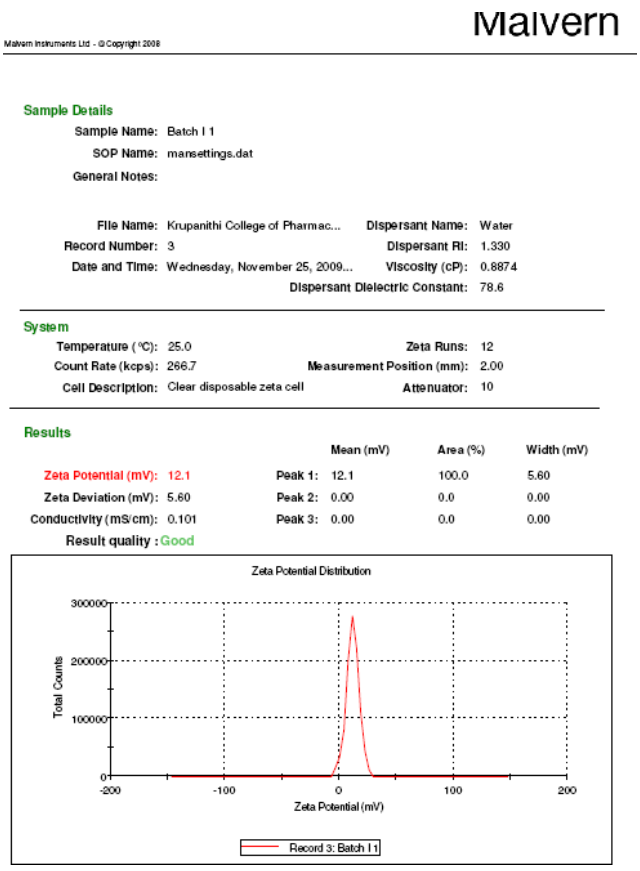

Fig. 3: Zeta potential of batch III

preservative. The batch-II formulation was selected because of its good appearance, syringeability, resuspendability, drainage, zeta potential, assay, pH, and related impurities. The drug release from the suspension was prolonged for 24 hours. Stability studies were conducted over three months, revealing the stability of the formulation.

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