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RESEARCH ARTICLE

Process Optimization for Manufacturing of Poly(D, L-lactide-co-glycolide) Microspheres of Naltrexone

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ABSTRACT

In this study, the use of biodegradable polymers for microencapsulation of naltrexone using solvent evaporation technique is investigated. The use of different process options and variables to prepare naltrexone microspheres is also studied. Naltrexone microspheres using biodegradable poly(D, L-lactide-co-glycolide) prepared using solvent evaporation technique had a significant amount of residual amount of solvents above allowable safety threshold. To reduce the level of solvents below safety limit in microspheres, process variables were explore, the effect of different process parameters, such as drug/polymer ratio and temperature at which evaporation is carried out, effect of lyophilization process, effect of pore forming agent, effect of final ethanol washing, etc. on the residual level of solvents in microsphere, morphology, total entrapped drug and in vitro drug release of microspheres was studied. The temperature at which the solvent evaporation was carried out did not have any significant impact on reducing the level of residual solvent in the microsphere. Similarly increasing the ratio of drug:polymer ratio did not have a major impact of residual solvents. However, a higher ratio of drug:polymer yielded higher % drug entrapped. Hence, a higher ratio of drug:polymer was selected so that higher entrapment can help in maintaining required % entrapment even with the use of pore forming agent and use of final ethanol washing procedure where % drug entrapment gets down with reduction of the level of residual solvents. With the use of pore forming agents during microencapsulation process helps in producing microspheres with more porous nature which in turn reduces the residual solvents. But as the microspheres are more porous, it also reduces the drug migration yielding lower % entrapped drug level. With the use of final ethanol washing process during microencapsulation process helps in reduces the residual solvents by extracting residual solvents from microsphere. But it also reduces the drug migration yielding lower % entrapped drug level. Wherever microspheres are more porous, % in vitro release also becomes faster. Desired level of residual solvents in microspheres along with required release profiles of naltrexone & % drug entrapped can be achieved using right process variables.

KEYWORDS

Naltrexone, Residual solvent, % drug entrapped, Pore-forming, Ethanol washing process, Temperature, Solvent evaporation

INTRODUCTION

Naltrexone is an opiate antagonist used mainly as an adjunct to prevent relapse in detoxified opioid-dependent patients. It is currently given orally as tablets or capsules in a daily dose of 50 mg. Naltrexone is orally active with a relatively short half-life and subject to extensive hepatic first-pass metabolism.1 Naltrexone provides no euphoric effects, and there are no observable

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pharmacological consequences when a patient discontinues the drug.2 For naltrexone treatment to be effective, a sufficient level of the drug concentration must be maintained. The minimum effective concentration of naltrexone for the treatment of opiate addiction is estimated to be in the range of 0.5 to 1.0 ng/mL.3,4 The patients who are committed to leaving alcohol addiction are advised to continue the naltrexone therapy for 4 to 8 months.5 This treatment typically requires the patient to self-administer dosages of the drug several times a week. The main drawback in naltrexone treatment protocol is patient compliance as the patients upon leaving alcohol addiction shows severe withdrawal signs, and it impacts the mental health of patients. A possible means of improving patient compliance and concomitant rehabilitation is the use of controlled drug delivery systems of opioid antagonists.6,7 Many efforts have been made to develop novel systems to maximize patient compliance.8-12 There have been different studies using biodegradable beads prepared by the National Institute on Drug Abuse on the use of naltrexone as an opiate antagonist in animals.13-16 Martin et al17 used naltrexone-zinc tannate complex, a sparingly soluble form, to increase the duration of the antagonistic effect. Negishi et al18 obtained 28 days of in vitro release of the antagonist by covalently coupling naltrexone to a biodegradable poly(α-amino acid) backbone. However, most attention has been focused on the preparation of polymeric injectable microparticles or implants of naltrexone. Sharon and Wise7 prepared 1.5-mm diameter beads composed of naltrexone and poly(lactide-co-glycolide). Microcapsules prepared from glutamic acid/ethyl glutamate copolymer released naltrexone at a rate of 20 to 25 μg/h for 30 days.19 Some effort has also focused on the preparation of morphine-triggered naltrexone delivery systems.8,20,21 These studies have provided valuable data on the usefulness of implantation for naltrexone delivery. Bhargave et al22 studied the effects of naltrexone pellet implantation on narcotic tolerance and physical dependence in rats. However, research on the application of naltrexone implants for human use has not been as convincing.23 More studies are needed to prepare a suitable naltrexone delivery system. The primary objective of the present study was to make naltrexone microspheres PLGA, a biodegradable polymer approved by the Food and Drug Administration for human use where microspheres should be complying with all required safety norms.

Naltrexone microspheres were prepared using a solvent evaporation method. The effect of different formulation parameters on drug release from microspheres was studied.

Naltrexone microsphere preparation tried with the use of solvent extraction technique had a lot of challenges and required specific manufacturing infrastructures. However, yield with this method is always compromised which ultimately may lead to higher cost translated to end user.

Naltrexone microspheres were tried with solvent evaporation technique which is a very simple process which does not require very complicated manufacturing process with the benefit of significantly higher yield translating cost benefit to end user.

Developing the solvent evaporation process for naltrexone microspheres and the challenges associated with it is described here in this study. The main difficulties with solvent evaporation technique are to reduce residual solvent in final microspheres and still achieve the desired level of in vitro drug release.

MATERIALS AND METHODS

Materials

Naltrexone was procured from Sun Pharmaceuticals. PLGA polymers were supplied by EVONIC. Polyvinyl alcohol (PVA) was supplied by Nippon Gohsei. Dichloromethane (DCM) was purchased from Merck. Ethanol was purchased from Merck. Other materials were of analytical grade and were used as received.

Microsphere Preparation [Solvent Extraction Process]

The development described here is for 5g naltrexone microsphere. Emulsification/solvent evaporation method was utilized for the

preparation of naltrexone microspheres. The brief details of the process followed in this study are given below:

Table: 1 Formula of Naltrexone Microsphere [Solvent Extraction Process]

Sr. No.	Ingredients	Qty./vial	Overages	Qty/batch
1	Naltrexone	380 mg	Nil	1685mg*
2	Poly(DL-lactide-co-glycolide) 75:25 [7525DLG7A]	747.63 mg	--	3.315g*
3	Ethyl acetate	--	--	16.525g
4	Benzyl alcohol	--	--	3.93g
5	Water for Injection	--	--	q.s.

* Label Claim – Each gram of microsphere contains 337mg of Naltrexone and 663mg of Polymer

Manufacturing Process: The process followed for the batch manufacturing is described briefly as below:

Naltrexone was dissolved in Benzyl Alcohol [Phase A]

Polymer was dissolved in Ethyl Acetate [Phase B]

Mix polymer [Phase A] and drug solution [Phase B]. This phase is known as DP (Discontinuous Phase)

Note: The mixed viscous solution is not stable as Naltrexone acts as a nucleophile and reduces the molecular weight of the polymer. If there is some delay in the process, this phase may be stored at lower temperature to slow down the reaction of polymer molecular weight reduction

Prepare 100mL PVA solution by dissolving 1%w/v PVA by heating the solution at approx. 50-60oC. Add ethyl acetate – 7.5%v/v. This phase is also known as CP (Continuous Phase)

Note: Ethyl acetate is added at 7.5%v/v ratio to prevent migration of ethyl acetate from drug-polymer solution during homogenization which helps in keeping emulsion from immediate solidification

Add phase c) into phase d) and homogenize the same with IKA T25 homogenizer at 4000rpm for 2min. This emulsion phase is known as "Primary Emulsion."

Prepare 2.5L quench fluid by adding 75mL of ethyl acetate. Keep the quench phase at less than 10^{o}C.

Note: Ethyl acetate at approx. 3% v/v is added to quench fluid to slow down the migration of ethyl acetate during quench process. As ethyl acetate has higher solubility/miscibility in water as compared to benzyl alcohol, the faster ethyl acetate migration from droplet would solidify the droplet faster which then makes difficult to remove benzyl alcohol.

Mix the phase e) with the phase f) using flow through glass unit using a peristaltic pump and allow to extract the solvent for 4 hrs at a temperature less than 10^{o}C.

Note: Keeping temperature less than 10^{o}C is critical as the solidified microsphere at this stage has very low glass transition temperature (Tg) which if exposed to higher than Tg would lead to aggregation.

Separate the hardened microspheres using a centrifuge.

Rinse the collected microspheres using 1000mL (25%v/v ethanol) for three times for approx. 15min at approx. 10^{o}C.

The microspheres were collected and dried for 24 hrs at room temperature.

The common observation was the microspheres yield were very less, and there was lump formation observed during the process.

The reason for lump formation during extraction process could be the rate of solvent migration from droplet to continuous phase is not proper. Few more trials were planned with the intention of reducing or avoiding the aggregation/lump

formation during quenching. There could be multiple reasons for aggregation/lump formation during quenching. The solvent extraction process is known to be very delicate, and it may need complete automation equipment setup. However, it is decided by the researcher to try the process/formula variable to address this issue before exploring the other type of process. Below mentioned factors may impact the aggregation/lump formation in solvent extraction process:

The agitation kinetic/magnitude

Ratios of Continuous Phase (CP) & DP (Discontinuous Phase) in Primary Emulsion

Ratios of Primary Emulsion to Quench liquid

Qty of Ethyl acetate in Quench liquid

Qty of Benzyl Alcohol to dissolve the drug

Few trials were planned with different factored varied by using "Solvent Extraction" process. The details of these trials are described in below table.

Table: 2 Trial Details of Naltrexone Microsphere [Solvent Evaporation Process]

Parameter Details	Trial NTII	Trial NTIII	Trial NTIV	Trial NTV
Formula / Composition				
Naltrexone	1685mg*	1685mg*	1685mg*	1685mg*
Poly(DL-lactide-co-glycolide) 75:25 [7525DLG7A]	3.315g*	3.315g*	3.315g*	3.315g*
Ethyl acetate	16.525g	24.1g	16.525g	16.525g
Benzyl alcohol	3.93g	3.93g	3.93g	3.93g
Polyvinyl Alcohol (PVA)	q.s.	q.s.	q.s.	q.s.
Water for Injection	q.s.	q.s.	q.s.	q.s.
Quench Phase Agitation	Over head stirrer (High speed)	Over head stirrer (High speed)	Over head stirrer (High speed)	Over head stirrer (High speed)
Continuous Phase (CP) Volume	100mL	100mL	100mL	100mL
Discontinuous Phase (DP) Volume	25.45g	33.03g	25.45g	25.45g
Primary Emulsion Parameters	Homogenization at 4000rpm for 2min	Homogenization at 4000rpm for 2min	Homogenization at 4000rpm for 2min	Homogenization at 4000rpm for 2min
Quench liquid composition	3%w/v EA	3%w/v EA	3%w/v EA	6%w/v EA
Quench liquid volume	2.5L	2.5L	1.25L	2.5L
Rate of addition of Primary Emulsion to Quench Liquid	5mL/min	5mL/min	5mL/min	5mL/min

* Label Claim – Each gram of microsphere contains 337mg of Naltrexone and 663mg of Polymer

EA – Ethyl acetate;

Note: Batch Size – 5g in all above trials

Table: 3 Formula of Naltrexone Microsphere [Solvent Extraction Process]

Parameter Details	Trial VI	Trial NTVII	Trial VIII
Formulation / Compositions			
Naltrexone	1685mg*	1685mg*	1685mg*
Poly(DL-lactide-co-glycolide) 75:25 [7525DLG7A]	3.315g*	3.315g*	3.315g*
Ethyl acetate	16.525g	16.525g	14.0g
Benzyl alcohol	3.0g	3.93g	3.93g
Polyvinyl Alcohol (PVA)	q.s.	q.s.	q.s.
Water for Injection	q.s.	q.s.	q.s.
Quench Phase Agitation	Over head stirrer (High speed)	Over head stirrer (High speed)	Over head stirrer (High speed)
Continuous Phase (CP) Volume	100mL	400mL	100mL
Discontinuous Phase (DP) Volume	24.53g	25.45g	22.93g
Primary Emulsion Parameters	Homogenization at 4000rpm for 2min	Homogenization at 4000rpm for 2min	Homogenization at 4000rpm for 2min
Quench liquid composition	3%v/v EA	3%v/v EA	3%v/v EA
Quench liquid volume	2.5L	2.5L	2.5L
Rate of addition of Primary Emulsion to Quench Liquid	5mL/min	5mL/min	5mL/min

* Label Claim – Each gram of microsphere contains 337mg of Naltrexone and 663mg of Polymer

EA – Ethyl acetate;

Note: Batch Size – 5g in all above trials

Microsphere Preparation [Solvent Evaporation Process]

Table: 4 Formula of Naltrexone Microsphere [Solvent Evaporation Process]

Sr. No.	Ingredients	Qty./vial	Overages	Qty/batch
1	Naltrexone	380 mg	35%	2275mg
2	Poly(DL-lactide-co-glycolide) 75:25 [7525DLG7A]	747.63 mg	--	3.315g
3	Methylene Chloride	--	--	20.0g
4	PVA	--		2.5g
5	Water for Injection	--	--	500mL

Manufacturing Process: The process followed for the batch is described briefly as below:

The polymer was dissolved in 20mL Methylene Chloride and kept on stirring for 4 hrs.

Naltrexone was added in polymeric solution (Step a)

Note: The mixed viscous solution is not stable as Naltrexone acts as a nucleophile and reduces the molecular weight of the polymer. If there is some delay in the process, this phase may be stored at lower temperature to slow down the reaction of polymer molecular weight reduction

PVA solution was prepared by addition of PVA at approx. 80oC in approx.. 450mL WFI and cooled down to room temperature and volume was made to 500mL

Homogenization was done with slow addition of drug+polymeric solution (Step b) into PVA solution with homogenization with IKA T25 for 1min at around 4000rpm

The emulsion was then kept under slow stirring with overhead Remi stirrer with nitrogen sparging at room temperature to evaporate methylene chloride for approx. 4 hrs

The white hardened microspheres were separated using a centrifuge and washed 2-3 times with water (Approx. 200mL each) to remove residual PVA. Each wash is to be stirred for 5-10min before next wash

Collected microspheres were dried at room temperature by keeping at room temperature in a petri dish for 24-36 hrs.

The dried microspheres were sieved through 80# to break the flakes/soft slugs of microspheres.

The common issue observed in "Solvent Evaporation Process" was of low % entrapment and a higher level of residual solvents. Few trials were planned to very different process variables to address the issues of low % entrapment and higher residual solvent level

Table: 5 Trial Details of Microsphere Manufactured by "Solvent Evaporation Process."

Sr. No.	Ingredients	Batch No NTXI	Batch No NTXII	Batch No NTXIII
1	Naltrexone (% Overage)	2275mg (35%)	2275mg (35%)	2275mg (35%)
2	Poly(DL-lactide-co-glycolide) 75:25 [7525DLG7A]	3.315g	3.315g	3.315g
3	Methylene Chloride	20.0g	20.0g	27.0g
4	PVA	2.5g	2.5g	2.5g
5	Water for Injection	500mL	500mL	500mL
6	Evaporation Temperature	30ºC	40ºC	35ºC

Table: 6 Trial Details of Microsphere Manufactured by "Solvent Evaporation Process."

Sr. No.	Ingredients	Batch No NTXIV	Batch No NTXV	Batch No NTXVI
1	Naltrexone (% Overage)	2528mg (50%)	2528mg (50%)	2275mg (35%)
2	Poly(DL-lactide-co-glycolide) 75:25 [7525DLG7A]	3.315g	3.315g	3.315g
3	Methylene Chloride	20.0g	20.0g	20.0g
4	PVA	2.5g	2.5g	2.5g
5	Poloxamer 188	0.2g (4%)	0.1g (2%)	-
5	Water for Injection	500mL	500mL	500mL
6	Ethanolic wash	-	-	25% v/v ethanol wash (150mL) for 2hr

Table: 7 Trial Details of Microsphere Manufactured by Final & Optimized "Solvent Evaporation Process" [B. No. NTXVIII]

Sr. No.	Ingredients	Qty./vial	Overages	Qty/batch
1	Naltrexone	380 mg	50%	25.275g
2	Poly(DL-lactide-co-glycolide) 75:25 [7525DLG7A]	747.63 mg	--	33.15g
3	Methylene Chloride	--	--	200.0g
4	PVA	--		25g
5	Poloxamer 188	--	--	1.0
5	Water for Injection	--	--	5000mL

The results, observation and advantages and disadvantages of each factor are detailed in "RESULTS & DISCUSSION" section.

Microsphere Characterization[24-27]

The microspheres from the final optimized process were characterized for following parameters:

a. Morphology & Description of microspheres (Visual & Using Microscope)
b. % Entrapment (Using HPLC Method)
c. Water content (Using KF)
d. Residual solvent (Using GC Method)
e. Particle size distribution (Using Malvern)
f. In vitro release (Using Rotating Bottle Apparatus and HPLC Method)
g. Flow property
h. Sedimentation kinetic of reconstituted suspension
i. Re-suspensibility of reconstituted suspension
j. Syringeability of reconstituted suspension
k. Glass transition temperature (Tg)
l. Molecular weight

The details are given in "RESULTS AND DISCUSSION."

Stability Study

The microspheres from the final optimized process were kept on stability to assess that the critical attributes remain un-impacted on stability. The details of the stability parameters are given in "RESULTS AND DISCUSSION."

Animal Pharmacokinetic

The microspheres from the final optimized process were also subjected to "Animal Pharmacokinetic Studies" on a rat model. This study was performed by CPCSEA guideline, and the protocol was approved from IAEC of Shree S. K. Patel College of Pharmaceutical Education & Research (protocol number SKPCPER/IAEC/2014-01/06). The microsphere equivalent to 380 mg naltrexone is reconstituted with 3.4mL diluent is administered to rat through "Intra-muscular" route at a dose of 50mg/Kg. The details of the study and results are given in "RESULTS AND DISCUSSION".

RESULTS AND DISCUSSION

As discussed in earlier sections, preparation of naltrexone long-acting microspheres was having a lot of challenges. There are mainly two possible processes which can be tried, i) Solvent Extraction and ii) Solvent Evaporation Process. Few of the challenges were, i) Acceptable level of yield in the solvent extraction process, ii) Lower % Entrapment of Drug, iii) Acceptable morphology of the microspheres, iv) Acceptable level of Residual Solvents (i.e. Methylene Chloride) in the solvent evaporation process. As higher molecular weight polymer is used, removal of methylene chloride which is used in the process to an ICH acceptable level is a challenge. Due to high molecular weight, methylene chloride takes time for complete removal/migration. The results are presented in the following table for few trials taken to resolve the issue of higher methylene chloride level.

Table: 8 Results / Observations of Microspheres manufactured by Solvent Extraction Process

Parameter Details	Trial NTII	Trial NTIII	Trial NTIV	Trial NTV
Process observation	-	-	-	-
Aggregation/lump formation	Approx. 50-60%	Approx. 50-60%	Approx. 30-40%	Approx. 30-40%
Wt of Microsphere observed	2.5g	2.1g	3.0g	2.8g
Physical observation of microsphere	Normal	Fluffy microsphere	Fluffy microsphere	Fluffy microsphere
% Entrapment	Not done	Not done	Not done	Not done
Total drug content	Not done	Not done	Not done	Not done
% Free drug	Not done	Not done	Not done	Not done
Remarks If any	Yield is too low. Need further improvement	Yield is too low. Microspheres were fluffy. Need further improvement	Yield is too low. Microspheres were fluffy. Need further improvement	Yield is too low. Microspheres were fluffy. Need further improvement

Note: As the morphology/yield is not up to the expectation, other relevant parameters (i.e. % Entrapment/% Free drug/% Total drug) were not analyzed.

Table: 9 Results / Observations of Microspheres manufactured by Solvent Extraction Process

Parameter Details	Trial NTVI	Trial NTVII	Trial VIII
Process observation	-	-	-
Aggregation/lump formation	Approx. 50-60%	Approx. 50-60%	Approx. 50-60%
Wt of Microsphere observed	2.6g	2.7g	2.5g
Physical observation of microsphere	Normal	Normal	Normal
% Entrapment	Not done	Not done	Not done
Total drug content	Not done	Not done	Not done
% Free drug	Not done	Not done	Not done
Remarks If any	Yield is too low. Need further improvement	Yield is too low. Need further improvement	Yield is too low. Need further improvement

Note: As the morphology/yield is not up to the expectation, other relevant parameters (i.e. % Entrapment/% Free drug/% Total drug) were not analyzed

From the above trials taken using solvent extraction technique, it is concluded that most of the process variable could not lead to acceptable yield/morphology. Solvent extraction technique is always known as very delicate. As there are no favorable results obtained with solvent extraction technique, it does not mean that the technique is not working. As the technique is very delicate in nature, it may need more automatic equipment setup which may not be feasible at small scale.

It concluded that "Solvent Evaporation Technique" using Methylene Chloride as a solvent would be explored to manufacture Naltrexone Microsphere.

Table: 10 Results / Observations of Microspheres manufactured by Solvent Evaporation Process

Sr. No.	Ingredients	Batch No NTXI	Batch No NTXII	Batch No NTXIII
1	Physical appearance	Free flowing round microsphere (Acceptable appearance)	Free flowing round microsphere (Acceptable appearance)	Free flowing round microsphere (Acceptable appearance)
2	Yield	4.2	4.1g	4.0
3	% Entrapment	95.9%	60.2%	85.7%
4	Residual solvent (ppm)	31363ppm	25913ppm	23395ppm

It looks very clear from the above trials and its results that varying the evaporation temperature or methylene chloride qty does not lead to resolution of the issue. Increasing evaporation could slightly reduce the methylene chloride qty but did not resolve the issue. In fact, it also reduces the % entrapment. Hence it can be concluded that increasing the temperature during evaporation with the intention of reducing the residual solvent, is not helping

It is evident from the literature that pore forming agents can contribute to lessening the level of methylene chloride in microsphere by creating the channels. It is also clear that using ethanol as the last wash to extract out the methylene chloride from microsphere. Few trials were planned with this concept using pore-forming

agents and/or ethanolic wash. The results/observations are given below:

Table: 11 Results / Observations of Microspheres manufactured by Solvent Evaporation Process

Sr. No.	Ingredients	Batch No NTXIV	Batch No NTXV	Batch No NTXVI
1	Physical appearance	Free flowing round microsphere (Acceptable appearance)	Free flowing round microsphere (Acceptable appearance)	Free flowing round microsphere (Acceptable appearance)
2	Yield	4.2	4.1g	4.0
3	% Entrapment	87.7%	102%	85.7%
4	Residual solvent (ppm) – Before lyophilization	15462ppm	15163ppm	25155ppm
5	Residual solvent (ppm) – After lyophilization	2141ppm	4772ppm	3127ppm
6	In vitro release (%)			
	1day	26.3	24.0	29.9
	3day	53.3	69.3	37.1
	7day	75.8	72.9	40.1
	14day	92.4	83.4	65.5
	21day	95.5	91.10	79.2
	28day	95.9	94.10	83.7
	35day	--	96.00	86.5

It looks very clear from the above trials and its results that varying the addition of pore-forming agents or ethanolic wash helps in reducing the residual methylene chloride level. In fact, it also reduces the % entrapment. Using 2% poloxamer looks comparatively better.

Table : 12 Results / Observations of Microspheres manufactured by Final & Optimized "Solvent Evaporation Process" [B. No. NTX VIII]

Parameters	Results
Description	White free flowing microspheres
% Entrapement	103.2%
Water content (By KF)	2.2%
Related substances * Single unknown * Total Impurities	 0.19% 0.58%
Residual solvents * Methylene chloride	 3110ppm
Particle size distribution (By Malvern) * D (0.1) * D (0.5) * D (0.9)	 31micron 72micron 97micron
% In vitro release (Using Rotating Bottle Apparatus) * Initial * 1 Day * 3 Day * 7 Day * 14 Day * 21 Day * 28 Day * 35 Day	 0% 17.4% 58.3% 70.2.9% 82.4% 91.7% 93.9% 97.1%
Angle of Repose (Flow properties)	36.5 degree
Sedimentation Rate & Sedimentation Volume	Sedimentation rate – 0.5mL/Hr Sedimentation volume – 0.55
Re-suspensibility	4-5 inversions required
Syringeability	The syringeability of reconstituted microsphere when withdrawn and delivered through 20G ½ Inch needle, was smooth
Glass Transition Temperature (Tg)	55.3°C
Molecular weight	98513 dalton
SEM (Scanning Electron Microscope) Analysis	Images taken. Shape of the microsphere is round in shape and surface is smooth

From the above results, it can be inferred that the microspheres manufactured by final and optimized solvent evaporation process when subjected to exhaustive characterization as outlined above, it met all expected performance criteria.

This batch was also kept on stability to evaluate the critical parameters on stability. The results are given below

Table: 13 Accelerated Stability of Final Batch of Naltrexone Microsphere

Parameters	Specification	1 Month	2 Month	3 Month
Description	White free flowing microspheres filled in clear glass vials	Complies	Complies	Complies
Water content	To be reported	1.6 %	2.1%	2.2%
Particle size distribution (By Malvern)	To be reported	Not performed	Not performed	19micron (D0.1) 56micron (D0.5) 93micron (D0.9)
Residual solvents (By GC) Methylene Chloride	To be reported	Not performed	Not performed	2772ppm
Assay (By HPLC) - % Entrapment	Not less than 90%	100.2%	101.2%	101.3%
Related Substances (By HPLC) * Single unknown * Total impurities	To be reported	0.26% 0.89%	0.30% 0.91%	0.25% 1.03%

Table: 14 Real Time Stability of Final Batch of Naltrexone Microsphere

Parameters	Specification	1 Month	2 Month	3 Month
Description	White free flowing microspheres filled in clear glass vials	Complies	Complies	Complies
Water content	To be reported	2.3 %	2.0%	2.8%
Particle size distribution (By Malvern)	To be reported	Not performed	Not performed	29micron (D0.1) 65micron (D0.5) 98micron (D0.9)
Residual solvents (By GC) Methylene Chloride	To be reported	Not performed	Not performed	3612ppm
Assay (By HPLC) - % Entrapment	Not less than 90%	99.3%	99.8%	99.4%
Related Substances (By HPLC) * Single unknown * Total impurities	To be reported	0.23% 0.74%	0.20% 0.81%	0.25% 83%

From the results mentioned above, it can be concluded that most of the desired attributes are met for the microspheres produced with the final formula and process and stability carried out for 3M (Accelerated and Real Time) looks beautiful.

This final product was also subjected to "Animal Pharmacokinetic Study" to understand the In vivo behavior of the product to assure that it releases the naltrexone in a sustained manner to maintain a blood level of naltrexone over a period of 1 month time.

The study was carried out on final product and innovator product (Brand Name – VIVITROL) sourced from US market, in 04-04 rats at a dose of 50mg/Kg through intramuscular dose

Both the products were dosed at 50mg/Kg dose levels. The details are given in below Table

Table: 15 Details of Animal Pharmacokinetic Study of Naltrexone Microsphere

Group No	Treatment	B.No.	Dose (mg/Kg)	Strength of suspension (mg/mL)	Dose Volumes (mL/Kg)	No of Animals
G-IIA	Test Product	NTXVIII	50	100	0.5mL	04
G-IIB	Reference Product	402-3668AA	50	100	0.5mL	04

Table: 16 Blood Profiles of Reference Product

Reference Product Concentration (ng/mL)

Reference Product		Concentration (ng/mL)				
Day	Time (day)	R1	R2	R3	R4	Mean
Day 1	1	7.991	6.832	8.210	10.230	**8.4**
Day-2	2	11.934	12.332	13.221	14.500	**13.4**
Day-3	3	15.221	14.992	16.339	17.221	**16.18**
Day-7	7	13.081	12.562	11.902	15.309	**13.26**
Day-14	14	11.922	10.234	13.214	15.921	**13.12**
Day-21	21	13.220	12.231	11.231	14.231	**12.6**
Day-28	28	6.023	5.901	7.020	4.990	**5.97**
Day-35	35	0.223	0.341	0.334	0.305	**0.33**

Table: 17 Blood Profiles of Reference Product

Test Product Concentration (ng/mL)

Test Product		Concentration (ng/mL)				
Day	Time (day)	R1	R2	R3	R4	Mean
Day 1	1	8.232	9.212	9.013	11.234	**9.8**
Day-2	2	14.230	15.230	16.030	14.987	**15.4**
Day-3	3	18.050	17.990	19.043	20.020	**19.02**
Day-7	7	12.220	13.541	10.032	13.222	**12.27**
Day-14	14	11.113	9.782	10.230	13.230	**11.08**
Day-21	21	8.920	7.230	10.040	8.450	**8.6**
Day-28	28	4.230	6.980	5.345	3.456	**5.26**
Day-35	35	0.320	0.234	0.331	0.235	**0.27**

Figure: 1 PK Profile of Reference Product (Vivitrol)

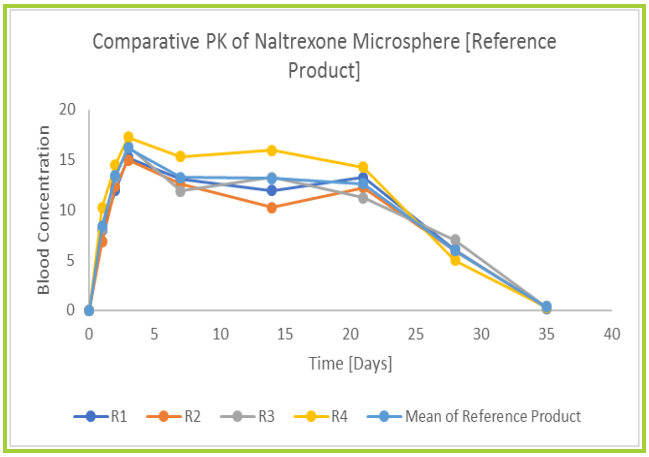

Figure: 2 PK Profile of Test Formulation [B. No. NTX VIII]

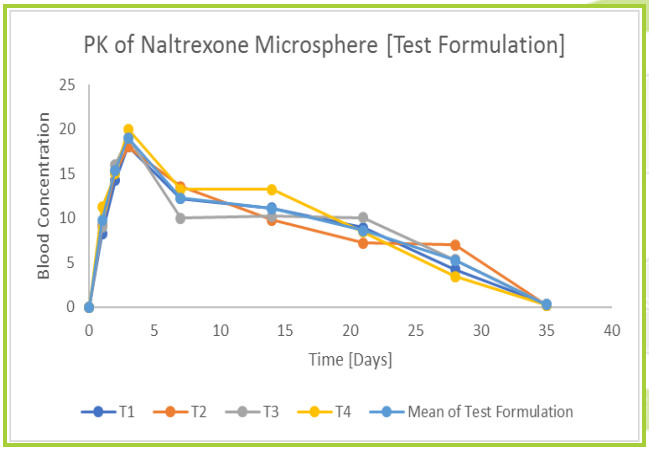

Figure: 3 Comparative PK Profile of Test Formulation [B. No. NTX VIII] Vs Reference Product [Vivitrol]

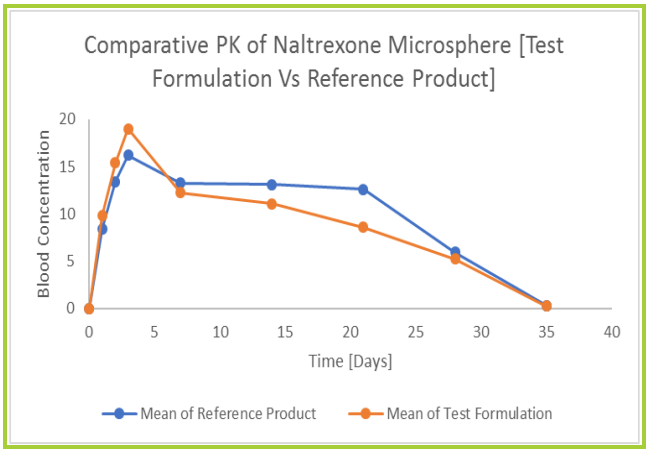

Table: 18 Comparative PK Parameters of Test Formulation [B. No. NTX VIII] Vs Reference Products

Mean PK parameter (0-35 days)		Test Formulation	Reference Formulation	RATIO(T/R)
		B. No NTXVIII	B. No 402-3668AA	
Parameter	Unit			
Cmax	ng/mL	19.02	16.18	1.17
AUC (0-35days)	day*ng/mL	16402.97	17356.12	0.945

From the data mentioned above, it can be concluded that the in vivo profile of developed Test Product of Naltrexone LAR is similar to Reference Product (VIVITROL)

CONCLUSION

Microspheres can be manufactured by different manufacturing processes based on physicochemical nature active moiety, desired profile target and based on available manufacturing set up.

Two widely used microsphere manufacturing processes are tried in this research work, i) Solvent Extraction Process and ii) Solvent Evaporation Process.

Based on the fact that solvent extraction process is bit delicate and not scale up the fast process, solvent evaporation method was chosen in this research work. Moreover, solvent extraction process did not yield the right quality of microsphere with good yield. There was a lot of lump formation issue in the process whereas solvent evaporation process yielded good return with distinctive morphology. However, there were some issues of little entrapment of drug in the microsphere and higher residual solvent level associated with solvent evaporation process which was addressed with process optimization.

The final batch manufactured with solvent evaporation process was subjected to stability study as per ICH conditions, and all critical parameters remained un-impacted on stability.

The final batch also was subjected to "Animal Pharmacokinetic Study" as well to understand the in vivo behavior. The animal PK study was carried out on final test batch Vs Reference product (VIVITROL). The outcome of the study proved that final product had same PK profile as of reference product.

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