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Aripiprazole loaded poly(caprolactone) nanoparticles: Optimization and in vivo pharmacokinetics

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ABSTRACT

In the present investigation, a Quality by Design strategy was applied for formulation and optimization of aripiprazole (APZ) loaded PCL nanoparticles (APNPs) using nanoprecipitation method keeping entrapment efficiency (%EE) and particle size (PS) as critical quality attributes. Establishment of design space was done followed by analysis of its robustness and sensitivity. Characterization of optimized APNPs was done using DSC, FT-IR, PXRD and TEM studies and was evaluated for drug release, hemocompatibility and nasal toxicity. PS, zeta potential and %EE of optimized APNPs were found to be 199.2 ± 5.65 nm, −21.4 ± 4.6 mV and 69.2 ± 2.34% respectively. In vitro release study showed 90 ± 2.69% drug release after 8 h. Nasal toxicity study indicated safety of developed formulation for intranasal administration. APNPs administered via intranasal route facilitated the brain distribution of APZ incorporated with the $AUC_{0\rightarrow 8}$ in rat brain approximately 2 times higher than that of APNPs administered via intravenous route. Increase in C_{max} was observed which might help in dose reduction along with reduction in dose related side effects. The results of the study indicate that intranasally administered APZ loaded PCL NPs can potentially transport APZ via nose to brain and can serve as a non-invasive alternative for the delivery of APZ to brain.

1. Introduction

Schizophrenia is a brain disease that interferes with normal brain functioning. It is a mental disorder often characterized by abnormal social behaviour and failure to recognize what is real and is now representing one of the largest and fastest growing areas of unmet medical need. Schizophrenia has been described as one of the most incapacitating across all mental illness diagnoses, leading to significant annual financial costs on health care system budgets globally [1]. The challenge to their clinical application is limited oral bioavailability and limited CNS penetration posed by the blood-brain barrier (BBB) following parenteral administration [2]. In the last decade, intranasal (IN) administration has attracted considerable interest, since it provides a non-invasive method for bypassing the BBB and delivering therapeutic drugs directly to the CNS. The advantages include its rich vasculature, large surface area, highly permeable membrane for rapid absorption and avoidance of first pass metabolism; in addition, this delivery route is needleless, maximizing patient comfort and compliance [3]. Many substances have been shown to reach the cerebrospinal fluid (CSF), the olfactory bulb and other parts of the brain after nasal administration in experimental animals [4,5]. The administered molecule absorbed nasally could be delivered directly to the central nervous system (CNS) within minutes along both the olfactory and trigeminal nerves [6]. Intranasal administered peptide hormones (melanocortin and insulin) have been reported to be delivered directly to the CSF in human [7].

In order to deliver drugs via intranasal route, there arises a need to develop a delivery system which can efficiently bypass the mucosal barriers of nasal pathway as well as protect drug from degradation. Nanomedicine or nanoparticle based delivery systems have gained lot of attention in the present time owing to their unique characteristics which includes stability in blood, non-toxicity, non-immunogenicity, non-inflammatory, non-activation of neutrophils, biodegradability, avoidance of reticulo-endothelial system and its usefulness as a carrier for various molecules such as drugs, proteins, peptides, or nucleic acids [8]. An attractive polymer for developing such delivery system is poly(ε-caprolactone) (PCL), approved by the Food and Drug Administration (FDA). Nanoparticles composed of PCL hold promising potential as drug delivery system due to their high colloidal stability in a biological fluid, facile cellular uptake by endocytosis, low toxicity in vitro and in vivo, and controlled release of their cargo [9,10].

Aripiprazole (APZ) is a second generation or atypical antipsychotic which selectively binds to central dopamine D2 and serotonin (5-HT2c) receptors, appears more effective on the associated negative symptoms of schizophrenia and has a lower propensity to cause extra pyramidal symptoms [11]. It undergoes extensive hepatic metabolism and P-glycoprotein efflux which leads to increased dose related side effects including neuroleptic malignant syndrome, hypotension, QTc

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Table 1
Effect of polymer:drug ratio on particle size and %EE.

Polymer:drug ratio	Aqueous:organic ratio	Surfactant concentration (% w/v)	Particle size* (nm)	Entrapment efficiency* (%)
1:1	1:4	0.5	Not formed	Not formed
2:1			Not formed	Not formed
4:1			188.6 ± 6.98	42.4 ± 3.27
6:1			206.3 ± 5.64	48.4 ± 2.35
8:1			222.8 ± 4.05	56.6 ± 3.24
10:1			236.2 ± 5.66	69.2 ± 3.21
12:1			267.6 ± 4.76	72.4 ± 3.16

* Mean ± SD; $n = 3$.

prolongation, high blood sugar (in those having diabetes), hypotension, dry mouth, tremor, akathisia and somnolence [12–15].

The objective of the present investigation was to develop PCL based nanoparticles of APZ and determine their potential for delivering APZ to brain via intranasal route. Optimization of the process and formulation variables for development of APZ loaded PCL nanoparticles was done using Design of Experiments (DoE) [16], with the aim of to enhancing the desired quality attributes of nanoparticles such as particle size and entrapment efficiency. The robustness of the optimized formulation was evaluated to create precise design space and overlay plots. The formulated APZ loaded PCL nanoparticles were characterized for their size, shape and in vitro drug release profile. Furthermore, they were evaluated for hemocompatibility, nasal toxicity and in vivo pharmacokinetic profile.

2. Materials and methods

2.1. Materials

Aripiprazole was generously gifted by Alembic Pharmaceuticals Pvt. Ltd. Vadodara, India. Poly(caprolactone), Poloxamer 188 and Poloxamer 407 were purchased from Sigma Aldrich (India). Acetone, acetonitrile, methanol, dimethyl sulphoxide (DMSO) and hydrochloric acid were purchased from S. D Fine chemicals (India). All other chemicals and reagents used were of analytical grade.

2.2. Formulation of APZ loaded PCL nanoparticles

APZ loaded PCL nanoparticles (APNP) were formulated using nanoprecipitation method [17]. Briefly, APZ and PCL were dissolved in suitable organic solvent viz. acetone, acetonitrile and tetrahydrofuran (suitable solvent was selected after preliminary investigation). Firstly, PCL was dissolved in organic phase and surfactant (Poloxamer 188/ Poloxamer 407) was dissolved in aqueous phase (distilled water). Thereafter, the organic phase was injected into the aqueous phase using syringe (24 gauge) under magnetic stirring at 1000 rpm. The organic phase diffused slowly into the aqueous phase leading to formation of nanoparticles. The formulation was kept for 5 to 6 h stirring at 1000 rpm for removal of the organic solvent. The nanoparticle suspension was centrifuged at 15,000 rpm for 30 min at 8 °C (REMI centrifuge, India). The obtained nanoparticle pellets were washed thrice with double distilled water and kept for lyophilization.

2.3. Particle size analysis

Particle size analysis of APNP was carried out using a Zetasizer (Nano ZS 90, Malvern Instruments Ltd., Malvern, UK) equipped with a 4.0 mW internal laser, which works on the principle of dynamic light scattering. The samples were diluted with double-distilled water in a disposable polystyrene cell prior to the measurements to obtain a nanoparticle suspension with a concentration below 0.5 mg/ml (to avoid multiple scattering). All the measurements were performed at 25 °C, at a scattering angle of 90°. The intensity-weighed mean diameter of the bulk population of particles was given by the z-average diameter value of the particles. Each sample was analyzed in triplicate.

2.4. Zeta potential analysis

The zeta potential (ZP) was measured using a Zetasizer (Nano ZS 90, Malvern Instruments Ltd., Malvern, UK) and a folded capillary cell. The sample was diluted with double-distilled water in a disposable polystyrene cell prior to the measurements to obtain a nanoparticle suspension with a concentration below 0.5 mg/ml. The ZP measurement was carried out subsequently. All tests were conducted at 25 °C in triplicate.

2.5. Encapsulation efficiency (%EE) and loading efficiency (%LE)

Entrapment efficiency was calculated by determining the unentrapped drug in supernatant. Briefly, the nanoparticle suspension was centrifuged at 15,000 rpm for 30 min. The supernatant was withdrawn, filtered and analyzed by UV–Visible spectrophotometer (UV 1700, Shimadzu, Japan) at 259 nm to measure the amount of free drug. For measurement of entrapped drug, the nanoparticle pellet was dissolved in acetonitrile and then analyzed at 254 nm in UV–Visible spectrophotometer (UV 1700, Shimadzu, Japan). The %EE and %DL were calculated using the formula:

$$\%EE = \left(\frac{D-t-Df}{Dt}\right) * 100$$

$$\%DL = \left(\frac{Dt-Df}{Wn}\right) * 100$$

Table 2
Effect of aqueous:organic phase ratio on particle size and %EE of APNPs.

Aqueous:organic ratio	Polymer:drug ratio	Surfactant concentration (% w/v)	Particle size* (nm)	Entrapment efficiency* (%)
1:1	10:1	0.5	243.2 ± 2.71	43.2 ± 1.87
1:2			267.3 ± 3.16	48.4 ± 1.45
1:3			202.6 ± 3.12	64.4 ± 2.81
1:4			196.5 ± 4.47	68.8 ± 1.92
1:5			207.9 ± 2.28	69.2 ± 1.71
1:6			287.1 ± 3.24	52.2 ± 2.13
1:8			323.4 ± 4.14	50.4 ± 1.64
1:10			350.4 ± 3.87	44.3 ± 2.51

* Mean ± SD; $n = 3$.