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Aripiprazole loaded PLGA nanoparticles for controlled release studies: Effect of Co-polymer ratio

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Abstract

Poly (lactic-co-glycolic acid) nanoparticles loaded with Aripiprazole has been developed as a new therapeutic strategy to achieve its controlled release profile suitable for parenteral administration. Nanospheres composed of different lactic/glycolic acid ratios and drug compositions were synthesized and loaded with Aripiprazole by emulsion/solvent evaporation method and subsequently characterized by particle-size distribution, scanning electron microscopy, encapsulation efficiency and in–vitro drug release studies. Specific drug-polymer interactions are engineered by optimizing the lactide to glycolide ratio (L:G ratio) and including specific polymer hydrophobicity.

Keywords: PLGA Nanoparticles, Co-polymer Ratio, Controlled drug delivery

Introduction

Drug delivery has been improved with the translation of several nanoscale drug delivery systems into the clinic, although the full potential of these systems is only now starting to be explored. Nanoscale drug delivery systems have shown the ability to encapsulate a variety of therapeutic agents, such as small molecules (hydrophilic and/or hydrophobic), peptide protein-based drugs, and nucleic acids. By encapsulating these molecules inside a nanocarrier, the solubility and stability of drugs can be improved, providing an opportunity to re-evaluate the therapeutic potential of drugs previously discounted because of poor pharmacokinetics [1]. Biodegradable micro/nanoparticles are promising candidates for extended release of hydrophobic drugs. PLGA based microparticles have been studied extensively and several products are available on market [2, 3]. In recent years, biodegradable nanoparticles for controlled drug delivery became a valuable approach to overcome the potential serious side effects arising from lifelong, systematic administration of therapeutic agents [4, 5]. However for site specific controlled drug delivery, nanoparticles offer additional advantages due to their submicron size [6]. Due to its unique properties PLGA copolymers have been used in controlled delivery of many proteins, drugs and other factors, such as cytokines, harmones, enzymes and vaccines [7-11]. The controlled release of a model hydrophobic drug from PLGA nanoparticles depends on the characteristics of the particles, including particle size, size distribution, drug content, incorporation, and surface morphology [12]. The mechanism of drug release plays a vital role in determining how the above properties influence the in-vitro release behaviour.

Aripiprazole is an antipsychotic drug and is used mainly in the management of mental illness and is also used in the treatment of various forms of epilepsy [13-15]. An uninterrupted supply of antipsychotic medication therapy is vital for patient health. A long term controlled drug delivery parenteral formulations could be an ideal candidate to solve all these problems. Such medication is very useful in overcoming the problem of patient noncompliance and minimizes the incidence and severity of adverse side effect as with oral tablet dosage form. Nanospheres are one nanoparticulate delivery system and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability or stability, and to target drug to specific sites. Nanoparticles can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing dosing frequency and improving patient compliance.
The aim of this study was to prepare Aripiprazole loaded PLGA nanoparticles to achieve a controlled drug release profile suitable for parenteral administration. First some important formulation variables (polymer and drug ratio) were optimized to obtain spherical particles. Optimized formulations were further evaluated by surface properties, encapsulation efficiency, particle size distribution and Aripiprazole release rate. The influence of formulation variables on the nanosphere properties was examined and the nanosphere formulations suitable to achieve our goal were selected.

Materials and Experimental Methods

Materials

Lactic acid, glycolic acid and poly (ethylene glycol) were supplied by Sigma Aldrich chemicals (St. Louis, USA). Aripiprazole drug was received as a gift sample from BAL pharma, Bangalore, India.

All chemicals were AnalaR grade and were used as received. Throughout the experiment double-distilled water was used.

Preparation of Aripiprazole loaded PLGA nanoparticles

Aripiprazole loaded PLGA nanoparticles with different LA:GA (lactic acid:glycolic acid) ratios (G50; G25; G15) were prepared by oil in water emulsion/solvent evaporation method. Aripiprazole and 0.5 g of PLGA was dissolved in 3 ml of methylene chloride (DCM)/acetone as a solvent. This mixture was added to a 2% PEG aqueous solution with stirring (12,000 rpm) to achieve an O/W emulsion system. Then, the emulsified system was stirred with a magnetic stirrer under reduced pressure to evaporate the DCM and form polymer nanoparticles. The nanospheres were then isolated by filtration through vacuum and washed 4-5 times with distilled water and dried at room temperature for 24 hours.

Nanoparticle Characterization

Dynamic light scattering

The prepared nanoparticles were dispersed in a water solution, added to a cuvette and analyzed by dynamic light scattering (DLS, 90 plus particle size analyser, Brookhaven instruments, USA), in order to obtain size and its disparsity. When the PDI values varied between 0.01 and 0.7, the particles would have narrow distributions. The high values of PDI (PDI>0.7) indicated very broad distributions [16]. Zeta potential was determined by sonicating the samples in 1 mL of distilled water, and then subjecting to dynamic light scattering.

Scanning electron microscopy

Scanning electron microscopy (SEM, jeol, USA, 5kV) was used to determine the size, shape and surface texture of the nanoparticles as well as to verify the results obtained with dynamic light scattering.

Drug loading and encapsulation efficiency

The Aripiprazole content and entrapment efficiency were measured using HPLC (water, USA) with a reversed phase symmetry C18 5.0 micrometer column (4.6mm x 150 mm). The mobile phase used for the column was 38% acetonitrile and 62% 10 mM, pH 4.8 ammonium acetate solution. After being passed through the MCX cartridge, a 1ml of nanoparticulate suspension was dissolved in 40 ml of mobile phase and a 50 µl aliquot of this sample was injected in HPLC machine with an auto injector. The column effluent was detected at 254 nm by UV spectrophotometer. A calibration curve for Aripiprazole was obtained using series of Aripiprazole standards prepared in the mobile phase. The calibration curve was linear in the range of concentration measured. Actual drug loading and encapsulation efficiencies were calculated using the following equations.

$$\text{Theorical drug loading} = \frac{\text{drug (total)}}{\text{drug (total)} + \text{polymer}}$$

$$\text{Actual drug loading} = \frac{\text{drug (enc)}}{\text{drug (total)} + \text{polymer}}$$

$$\text{\% Encapsulation efficiency} = \frac{\text{Actual drug loading}}{\text{Theoritical drug loading}} X100$$

In-vitro release studies

In vitro release studies were performed at 37^0 C using the Tablet dissolution tester (Disso test, Lab India, Mumbai, India) equipped with six paddles at a paddle speed of 100 rpm. About 50 mL of phosphate buffer solution (pH=7.4) was used as the dissolution media. The sample was sealed in a dialysis bag, which was suspended in a sealed beaker containing release medium at constant stirring. At a fixed time intervals, 2 mL of the resultant release medium was sampled for the analysis of Aripiprazole content, then 2 mL of the fresh release medium was immediately added to maintain the original volume. The amount of Aripiprazole released was analyzed based on the standard curve using a UV Spectrophotometer at the λ_{max} of 246 nm. The cumulative amount of drug released into the media at each time point was evaluated as the percentage of total drug release to the initial amount of the drug.

Results and Discussion

Drug content and entrapment efficiency

As the initial Aripiprazol concentration is increased, the drug content first increases, then plateaus and finally decreases as shown in Figure 1a, while the drug incorporation efficiency remains constant and then decreases as shown in Figure 1b.

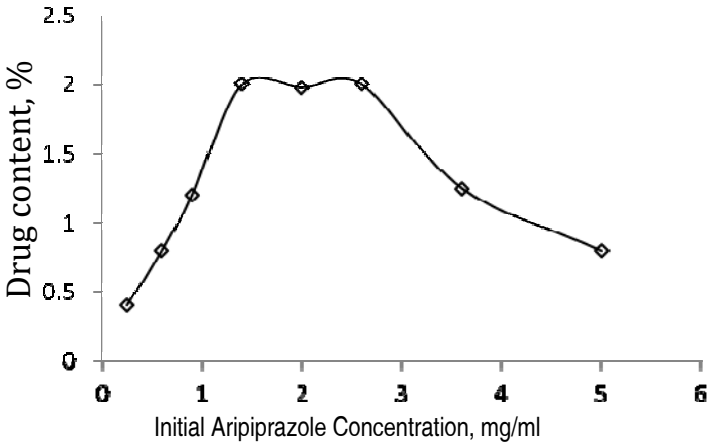

Figure 1a. Effect of initial Aripiprazole concentration on the drug content.

The drug content in the nanoparticles is affected by the drug-polymer interaction and the drug miscibility in the polymer. The importance of drug miscibility in the polymer has been discussed [16] for a hydrophobic drug-polymer system of dexamethasone loaded PLGA/PLA nanoparticles. They have reported that higher drug-polymer miscibility leads to higher drug incorporation. In our results as the L:G ratio is increased from 50:50 to 85:15, the drug content and incorporation increases. This happens due to increased hydrophobicity of polymer at higher L;G ratios, which increases the hydrophobic interaction of Aripiprazole with PLGA.

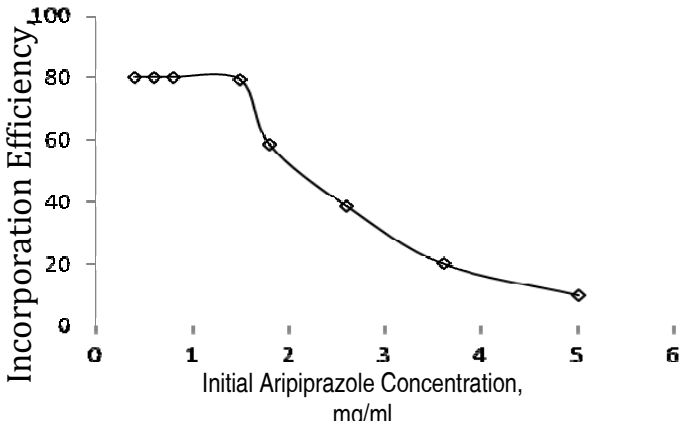

Figure 1b. Effect of initial Aripiprazole concentration on the drug incorporation efficiency.

Particle size and size distribution

To determine the effect of lactic:glycolic acid ratio on the PLGA polymer different weight ratios (50:50, 75:25 and 85:15) were used. Exactly the same preparation method was used for the three polymers and the results showed that the PLGA with a higher lactic amount gives a smaller size as well as a zeta potential value closer to zero. All nanosphere lots presented a consistent size distribution histogram and the encapsulation efficiency ranged from 20-40% (Table 1). It can also be observed that the presence of drug during the preparation of the nanoparticles, gives a smaller and less negative value of the zeta potential.

Table 1. The effect of the lactic:glycolic acid ratio on the size, PDI, zeta potential and encapsulation efficiency PLGA nanoparticles

Sample	Size	PDI	Zeta potential	EE (%)
PLGA 50:50	105.3±1.7 nm	0,11±0.03	-15.8 ± 7.2 mV	18.75
PLGA75:25	101.7±1.1 nm	0,13±0.02	-7.3 ± 3.7 mV	21.39
PLGA 85:15	97.9±0.5 nm	0,15±0.01	-3.7 ± 1.4 mV	36.0

Scanning electron microscopy

SEM images of the PLGA nanoparticles (G50, G25 and G15) loaded with Aripiprazole were shown in Figure 2. The particles had spherical in shape and smooth surface with diameters of 10 µm, and narrow size distributions which could be related to the data measured by size analyser.

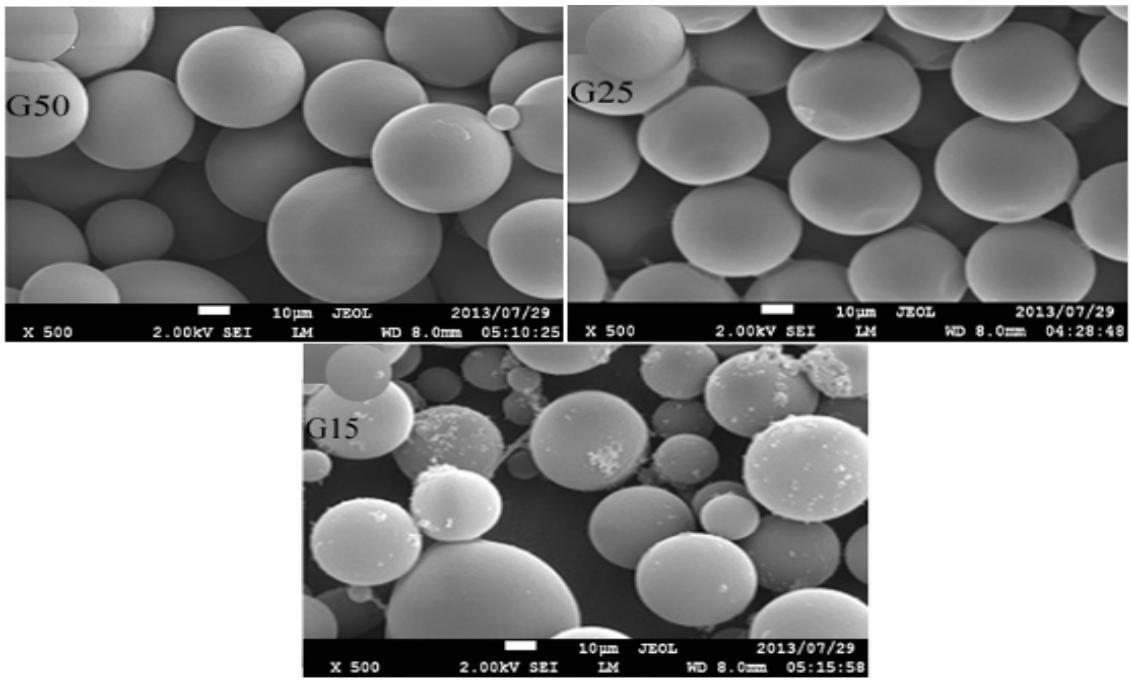

Figure 2. Scanning electron micrographs of Aripiprazole loaded PLGA nanoparticles G50, G25 and G15

In-vitro release studies

In vitro release kinetics of Aripiprazole from G50, G25 and G15 PLGA nanoparticles are shown in Figure 3. The drug release process over a long period of time is expected to be influenced by the polymer L:G ratio since the process is controlled by degradation rate of polymer, which is affected by polymer hydrophobicity. Strong L:G dependence of release profile has been reported [17] for a hydrophobic drug (paclitaxel) incorporated in PLGA nanoparticles. In this case the drug release mechanism was a combination of drug diffusion and polymer degradation.

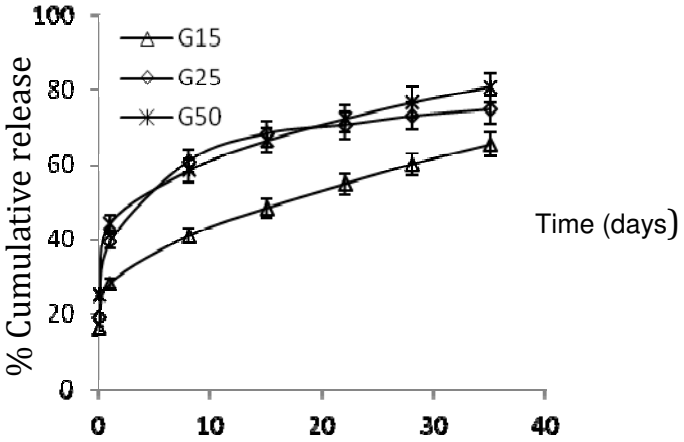

Figure 3. In vitro release kinetics of Aripiprazole from G50, G25 and G15 PLGA nanoparticles

In contrast, the nanoparticles studied here release the Aripiprazole without a detectable change in effective diameter. Thus, the mechanism governing release from these nanoparticles is predominantly drug diffusion. During the drug release process, the drug diffuses through the hydrated polymer matrix into the aqueous phase. The process of hydration relaxes the polymer chains and enhances the diffusion of drug molecules. The rate of water uptake (hydration) of polymer particles increases with the hydrophilicity of polymer. Hence the initial burst is higher for more hydrophilic PLGA 50:50 particles than less hydrophilic PLGA 85:15 particles. The induction period is also effected by polymer hydrophobicity. Decreasing the hydrophobicity increases the rate at which the diffusion of the release medium moves from the surface to the core, which makes more drug available for diffusion in a less time and thus reduces the induction period.

Mathematical modeling of drug release

The drug release from polymeric micro/nano-particulate systems is usually considered as a combination of Fickian (diffusion) and non-Fickian movement of drug molecules through polymer chains [18]. Ritger and Peppas [19, 20] gave the semi-empirical equation to describe the release of solute when the prevailing mechanism is a combination of Fickian and non-Fickian mechanisms.

$M_t/M = Kt^n +$ (1)

Where M_t is the drug released at time t, M is the quantity of the drug released at infinite time, K is constant, n is an exponent, and represents the drug released at zero time and accounts for the initial burst [21]. The value of n is related to both the geometrical shape of the formulation and the release mechanism. For drug release from spherical particles, the value of n is equal to 0.43 for pure Fickian and 0.85 for pure non-Fickian mechanisms.

We fit our experimental release data to theoretical release profiles given by equation 1 and determine the value of the exponent n. The values of different parameters corresponding to the best-fit lines are given below.

$M_t/M = 0.053\ t^{0.433} + 46$ (2)

$M_t/M = 0.078\ t^{0.438} + 23$ (3)

$M_t/M = 0.112\ t^{0.435} + 4$ (4)

The value of n is ~0.43 for various particle sizes indicating that the drug release is diffusion controlled. The experimental and theoretical profiles for PLGA 50:50 particles start deviating at ~10 days, after which the release becomes slower than predicted by the diffusion equation. This deviation of release profiles starts at a much later time for PLGA 85:15 particles. This deviation suggests that the release mechanism is diffusion controlled for the initial few days, after which the role of polymer degradation becomes important in PLGA 50:50 particles. The polymer degradation is faster for PLGA particles. The polymer degradation is faster for PLGA 50:50 particles than for PLGA 85:15 particles and hence the deviation from experimental profiles is observed much earlier for PLGA 50:50 particles.

Conclusions

Aripiprazole loaded PLGA nanoparticles were produced by emulsion/solvent evaporation method and tested for their in-vitro release behavior. The three most important properties affecting the release behavior were identified as: polymer hydrophobicity (L:G ratio), drug content and particle size. The mechanism of drug release was confirmed to be diffusion controlled by the application of mathematical models and the corresponding drug diffusivities were established to be a function of both polymer hydrophobicity and particle size. Hence the release profile from Aripiprazole loaded PLGA nanoparticles can be tailored to achieve desired objectives by selective manipulation of particle properties.

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Author's contribution

The work is a product of the intellectual environment of the whole team; and that all authors have contributed in various degrees to the experiment design, to the research concept, and to the analytical methods used.

Conflict of interest

We have no interest of any conflicts regarding to the whole manuscript.

Corresponding author declaration

I [Dr. K. Chowdoji Rao] , the corresponding author of this manuscript, certify that the contributors and conflicts of interest statements included in this paper are correct and have been approved by all co-authors.

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