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Original Article

FORMULATION AND EVALUATION OF ASPIRIN-LOADED PLGA NANOPARTICLES FOR OPHTHALMIC USE

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ABSTRACT

Objective: The objective of this work was to increase the bioavailability of Aspirin to the retina by increasing its bioavailability to blood. This was achieved by forming aspirin-loaded PLGA nanoparticles

Methods: Aspirin-loaded PLGA nanoparticles were prepared by a solvent evaporation process. The PLGA was dissolved in the proper solvent and added dropwise to the Aspirin-albumin solution revolving at 3000 rpm. Glutaraldehyde was used as a cross-linker at 20% concentration. The nanoparticles were obtained after passing the solution through HPH and subsequent centrifugation.

Results: The prepared nanoparticles were found to be spherical with the smooth surface as seen in SEM. and with a size of 160.9 nm. Aspirin-loaded PLGA nanoparticles showed *in vitro* drug release of 71.4 % and ex-vivo permeation of 66.2 %. The formulation was found to be stable for six months.

Conclusion: The developed aspirin-loaded polymeric nanoparticles could be effective for the controlled delivery of aspirin in the early prevention of diabetic retinopathy**.**

Keywords: Nanoparticles, Ocular, Aspirin, PLGA, Eye diseases

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INTRODUCTION

Up to 60% of all causes of visual impairment globally are cataracts and age-related macular degeneration [1]. In general, vitreoretinal disorders, which include side effects such as diabetic retinopathy (DR), diabetic macular edema, cataracts, and glaucoma, have emerged as significant contributor to visual loss in the elderly [1]. Due to the complicated structure and physiology of the eye, conventional methods of drug delivery may fail to produce the necessary therapeutic benefit. Ocular formulations must be made with the highest care in terms of sterility, stability, non-harmful excipients, and the absence of particles without sacrificing efficacy because they are delicate and crucial organ. In order to facilitate treatment efficacy while minimizing side effects related to such disorders, a drug delivery system must be created [2].

Aspirin (ASP) works by inhibiting the enzyme cyclooxygenase, which lowers the number of prostaglandins produced (PGs) [3]. Regarding its function as a physiological regulator, the control of PG production in the eye appears to be of great significance. Albumin-bound ASP was created to lessen the negative effects connected with free ASP since systemic administration of high dosages of ASP generates substantial side effects, including hemolytic breakdown.

Albumin nanoparticles that are biodegradable and biocompatible have been demonstrated to be very effective at delivering a variety of ophthalmic medications [4]. Aspirin and other medicines have binding sites in albumin [5].

The most promising type of drug delivery technologies for ophthalmic applications is polymeric ophthalmic nanoparticles because they improve medication absorption [6, 7]. The advantages of suggested nanomedicine in the treatment of diabetic retinopathy are highlighted by the employment of innovative nanoparticle-based drug delivery systems in conjunction with established therapeutic modalities [8]. The Polylactic-Co-Glycolic Acid (PLGA) class of polymers has received FDA approval [9]. It is a copolymer made of polyglycolic acid (PGA) and polylactic acid (PLA), and it is widely used to deliver medicines, proteins, and macromolecules [10, 11]. By boosting the formulation's retention through bioadhesion, nanoparticle formulations lengthen a novel drug's exposure time while protecting pharmaceuticals that are susceptible to degradation or denaturation in the pH range of extremes [12, 13].

By attaching ASP to albumin and encasing the ASP-albumin complex in PLGA, the current study sought to create ASP-loaded PLGA nanoparticles. It has been proposed that ASP's binding to albumin will lessen the negative effects of free ASP [4, 14]. The generated nanoparticles were evaluated for their zeta potential, polydispersity index, and particle size. Additionally investigated were the transcorneal permeability utilizing goat skin and the *in vitro* release kinetics over the cellophane membrane.

MATERIALS AND METHODS

Materials

Acetylsalicylic acid (aspirin, ASP) was obtained from Research Labs Fine Chemical Industries Ltd., Mumbai, India. Bovine serum albumin (BSA) was purchased from Sisco Research Laboratories Pvt Ltd, Mumbai, India. Resomer RG 504 H (PLGA, having 50:50% ratio of lactide and glycolide components) was purchased from Evonik Degussa, Bloomberg, USA. Aqueous solution of glutaraldehyde (25%) and Acetonitrile HPLC were procured from Research Lab Fine Chem Industries Ltd., Mumbai, India. Milli-Q ultrapure water filtered through Millipore system was used for the HPLC system and preparation of reagents/solutions. All other chemicals used were of analytical grade.

Methods

Preparation of aspirin-loaded PLGA nanoparticles (ASP-PLGA-NPs)

According to a procedure outlined by Das *et al.*, the ASP-PLGA NPs were made by solvent evaporation followed by cross-linking with a 25% aqueous solution of glutaraldehyde [13] In a lab incubator, aspirin was incubated with the necessary quantity of BSA solution (2% w/v in H_2O) for one hour at 37 °C. With constant stirring at 3000 rpm [15]. In order to guarantee that there would be enough amino groups available for binding with aspirin even after all of the

glutaraldehyde had bonded to albumin, glutaraldehyde solution was added at a concentration of 1.56 g/ml albumin solution. The solution was homogenized 10 times at 10,000 psi using an Avestin-EmulsiFlex-C5 high-pressure homogenizer while still being stirred at room temperature. Once the supernatant was clear and the temperature was between 2-4 °C, it was centrifuged at 12,000 rpm [16, 17]. As indicated in table 1, different batches were created and optimized by varying the protein: polymer ratio and the solvent.

Characterization of prepared ASP-PLGA-NPs

Analytical method

Reverse-phase high-performance liquid chromatography was used to purify aspirin in its pure form and formulation (RP-HPLC). Utilizing a C18 column (Kromasil, 150 x 4.6 mm, 5) and acetonitrile and water eluents (60:40 v/v, pH adjusted to 3.0 with orthophosphoric acid) at flow rates starting at 1 ml/min, chromatographic separation was carried out. A UV detector set to 235 nm was used to keep an eye on the peaks [18].

Particle size, polydispersity index (PDI) and zeta potential

The lyophilized nanoparticles were resuspended in phosphate-buffered saline (pH 7.4) to obtain a 0.5% solution according to the method described by Gupta *et al.* [17]. Particle size, polydispersity and zeta potential measurements were performed using a particle size analyzer (Zetasizer ZS90, Malvern Instruments, UK).

Scanning electron microscopy (SEM)

Microscopic images were taken with a scanning electron microscope (Philips, CM 200, acceleration voltage 200 kV, resolution 0.23 nm). Nanoparticles were coated with chromium for better resolution [19].

Drug loading and entrapment

Using a method created by Shu-Ben *et al.*, the effectiveness of the drug loading and encapsulation of ASP-PLGA NPs was assessed [20]. The steps are described. After 30 min of phosphate buffer soaking, ASP-PLGA-NPs (10 mg) were centrifuged at 8000 rpm. By using HPLC at 235 nm, the clear supernatant was examined for the presence of ASP. Plotting the range of concentrations from 1 g/ml to 10 g/ml at 235 nm allowed for the calculation of the ASP standard curve. The following formula was used to determine the percentage of drug loading and the effectiveness of entrapment:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Amount of drug released from the lyophilized PLGA NPs}}{\text{Amount of drug initially taken to prepare the NPs}} X100$$

$$\text{Drug loading (\%)} = \frac{\text{Amount of drug found in the lyophilized NPs}}{\text{Amount of lyophilized NPs}} X100$$

***In vitro* drug diffusion study**

ASP-PLGA NPs were the subject of an *in vitro* drug diffusion investigation employing a cellophane membrane in a diffusion chamber made up of two compartments divided by a membrane, as stated by Das *et al.* [21]. In order to sustain contact with the maintenance membrane to be determined using PBS, a 0.5% ophthalmic solution (20 ml) using phosphate-buffered saline (PBS, pH 7.4) was supplied to the donor chamber while the receptor chamber was filled with 1 ml PBS. At certain intervals, samples (1 ml) of the receiver solution were obtained in order to calculate the amount of aspirin that diffused through the cellophane membrane. To maintain sink conditions, the same amount of buffer was introduced to the receiving chamber [22]. The HPLC technique was used to fig. out how much medication was released.

Transcorneal permeability

The transcorneal permeability of ASP-PLGA NPs was examined ex vivo in goat cornea. From the nearby slaughterhouse, the freshly sliced goat corneas were retrieved and kept in ice-cold PBS for future research. According to Gupta *et al.*, the experiment was conducted in a diffusion chamber made up of two chambers divided by a goat horn [23]. The donor chamber contained the ophthalmic solution (0.5%, 1 ml), and the receptor chamber had PBS (20 ml). In order to maintain sink conditions, samples were obtained at regular intervals and PBS was refilled with fresh media. HPLC was utilised to determine how much ASP diffused through the ocular membrane.

Stability studies

At storage temperatures between 2 and 8 °C, the physical stability of lyophilized nanoparticles was investigated. For six months, 20 mg of ASP-PLGA NPs were kept in amber glass vials and subjected to the aforementioned conditions. After zero, three, and six months, the lyophilized nanoparticles were examined for particle size and zeta potential. The Q1R2 ICH Guidelines, 2003 were followed for the stability studies [24].

Statistical analysis

All the statistical studies were done using SAS® Enterprise Guide 6.1 M1HF5 (6.100.0.4180) (64-bit) licensed to SVKM's NMIMS, Mumbai, India and installed on hp® Desktop with Intel® Core™ i3-4130T CPU @ 2.90 GHz. One-sample t-test was used at the 95% significance level with default settings [25].

RESULTS

Particle size, PDI, and zeta potential

The details of formulation approaches F1 to F8 are listed in table 1. The desired particle size and particle size distribution of ASP-PLGA NPs were obtained by optimizing various parameters such as the amount and rate of solvent addition, stirring rate, and amount of added glutaraldehyde to crosslink aspirin nanoparticles. It was found that the formulation F6 with protein: polymer ratio 1: 2 had a particle size of 160.9 nm, a PDI of 0.184 and a zeta potential of-14.1 mV, respectively.

Table 1: Formulations of ASP-PLGA-NPs

Formulation	Ratio of albumin: PLGA	Solvent used	Speed of agitation (rpm)
F1	0.5: 2	Tetrahydrofuran	1500
F2	1:2	Tetrahydrofuran	3000
F3	1:3	Tetrahydrofuran	2500
F4	1:2	Acetonitrile	3000
F5	1:3	Acetonitrile	3000
F6	1:2	Acetone	3000
F7	1:3	Acetone	3000
F8	1:2	Dichloromethane	3000

Scanning electron microscopy

SEM images of ASP-PLGA NPs showed spherical particles with a smooth surface (fig. 1). SEM images revealed even the uniformity in the size of the developed nanoparticle formulation.

Encapsulation efficiency

The ASP-PLGA NPs nanoparticles' encapsulation effectiveness varied from 24 to 60%, as indicated in table 2. Similar inclusion rates of between 40% and 60% were seen throughout all batches (table 2). For batches F4–F6, entrapment effectiveness and particle size were good. The highest capture efficiency with appropriate particle size was provided by batch F6, nevertheless. As a result, it was selected as the best method for further characterization. Results for particle size, PDI, and entrapment effectiveness are displayed in table 2. There was a significant difference between F6 and all other formulations except for F8 (outliers in terms of encapsulation efficiency, PDI, and percent drug loading, according to statistical

analysis (t-test on one sample, 95% significance) (data not shown). Particle size and zeta potential, however, did not significantly differ between the F6 formulation and other formulations. As a result, it was selected as the best method for further characterization.

In vitro drug diffusion

Fig. 2 displays the *in vitro* diffusion patterns of developed formulations. When ASP-PLGA NP diffusion profiles are compared to those of ASP drug solutions containing the same quantity of ASP, it is shown that nanoparticles release the maximum amount of drug (71.4%) after 4 h while ASP-PLGA NPs release the maximum amount of drug (80%) after 1 h.

In vitro transcorneal permeability

Studies on *in vitro* transcorneal permeation using the newly created formulation revealed comparable and sustained permeation (66.2%) through the goat cornea after 6 h in comparison to that of the drug solution, indicating maximal drug release (61, 4%) after 3 h as shown in fig. 3.

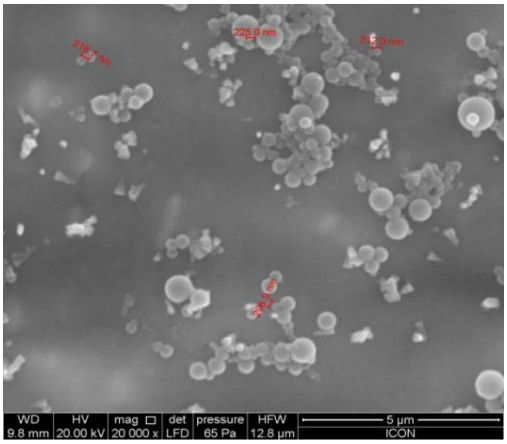

Fig. 1: SEM image of optimized ASP-loaded nanoparticles (F6)

Table 2: Evaluation of ASP-PLGA-NPs

ASP-PLGA NPs formulation	F1	F2	F3	F4	F5	F6	F7	F8
Particle size (nm)#@	380.0±9.9	227.3±12.2	459.6±10.4	124.5±6.5	194.8±18.6	160.7±9.2	182.2±15.2	662.1±27.7
Polydispersity index (PDI) and	0.416±0.042	0.286±0.014	0.404±0.02	0.179±0.016	0.274±0.012	0.184±0.016	0.288±0.004	0.342±0.082
% drug loading and	9.45±0.6	11.23±0.8	10.58±0.24	12.44±0.22	12.76±0.27	16.94±0.7	14.28±0.5	9.48±0.25
% Encapsulation efficiency*	46.0±4.09	47.66±3.61	43.01±2.36	53.11±2.42	51.11±2.34	60.06±4.20	56.64±4.10	23.89±1.64
Zeta potential	-30.6±0.02	-18.8±0.32	-14.1±0.03	-4.6±0.05	-12.2±0.20	-14.1±0.02	-18.2±0.06	-42.9±0.64

[a]Results are mean±standard deviation (SD), #n =3; @statistically not significant: one-sample t test, p≤0.05; and statistically significant: one-sample t-test, p>0.05; *statistically significant after deleting outlier F8: one-sample t-test, p>0.05.

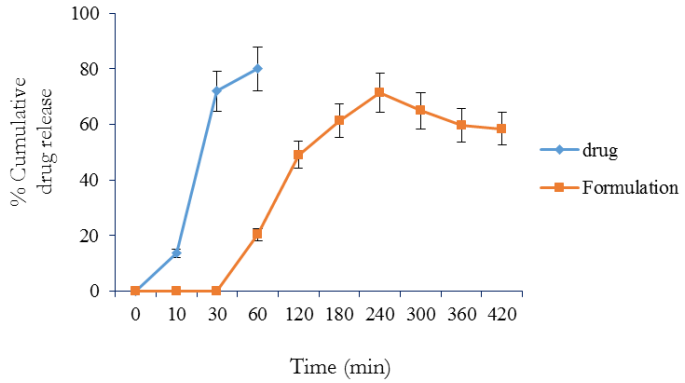

Fig. 2: *In vitro* drug release of ASP-PLGA-NPs and ASP drug solution (n=3, Error bar indicates SD)

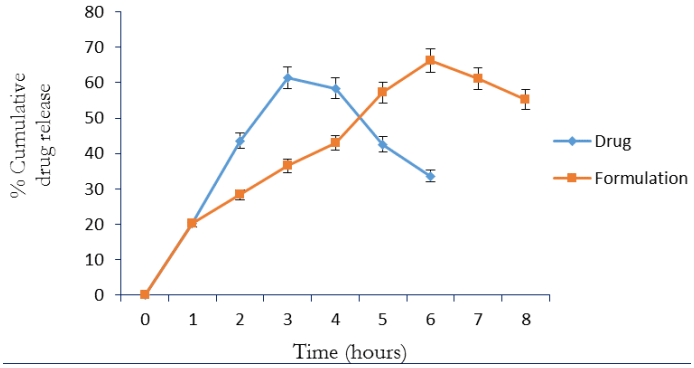

Fig. 3: Trans-corneal permeability of ASP-PLGA-NPs and ASP drug solution (n=3, Error bar indicates SD)

Stability studies

As indicated in table 3, there were no appreciable changes in the measured parameters for the formulations kept for six months at 2-8 °C. After six months of storage at-8 °C, the ASP-PLGA NPs' particle size increased somewhat, from 162 nm to 172.6 nm. Zeta potential readings were adjusted from-15.3 mV to-13.9 mV, albeit very significantly. The ASP-PLGA NPs were stable at 2-8 °C for six months, according to the stability study results. The findings indicate that ASP-PLGA NPs maintain their particle size even after extensive storage (six months). These findings conflict with earlier research by Tang and Singh [22], who claimed that aspirin caused PLGA to degrade in an extremely short amount of time. No other drug has been associated with the rapid degradation of PLGA that has been documented in the literature.

Table 3: Stability study data of ASP-PLGA-NP's at 2-8 °C

Parameters	Month 0	Month 3	Month 6
Particle size (nm)[@]	162±6.16	166.5±10.24	172.6±10.44
Zeta potential [26][@]	-15.3±1.54	-14.8±2.06	-13.9 mV±1.71

[a]Results are mean±standard deviation (SD), #n =3; [@]statistically not significant: one-sample t test, p=0.05

DISCUSSION

The literature shows that the particle size is decisively influenced by these parameters [27]. Increasing the PLGA concentration increased the particle size and agglomerates of free PLGA could be observed, possibly due to differences in the saturation solubility of PLGA in various solvents. Since the components used in the formulation had low solubility in dichloromethane, a higher particle size was observed [28].

From the literature it can be concluded that rough particles produce less stable emulsion droplets compared to smooth spherical particles due to reduced contact between particle and interface, resulting in lower adsorption energy of the rough particles [29]. Therefore, spherical nanoparticles contribute to better formulation stability by reducing antiparticle friction due to the smooth surface [30].

The ASP-PLGA NPs nanoparticles' encapsulation effectiveness varied from 24 to 60%. The entrapment for different formulations revealed a broad range from 24 to 60%. The solubility of the drug in the aqueous phase, the rate at which the solvent precipitates in the organic phase, which in turn depends on the solvent's high and low vapour pressures, and the viscosity of the internal phase are all factors in drug encapsulation. But because of its high vapour pressure, it has been discovered that employing acetone as a solvent causes more entrapment [31]. Acetone quickly diffused and evaporated in water because of its high vapour pressure, which left little time for aspirin molecules to disperse in the aqueous phase and led to a high acetone entrapment. This caused the polymer to precipitate quickly. Vineeth *et al.* reported a similar outcome [32].

The main chain ester bonds of the PLGA copolymer are broken down into oligomers, then monomers, through hydrolysis or biodegradation. This has been demonstrated for several drug kinds and proteins with various polymer ratios both *in vivo* and *in vitro* [33, 34].

The improved batch had a two-stage release pattern, as depicted in fig. 2: an initial burst release followed by a steady release phase (extended-release). Drug type, drug concentration, polymer hydrophobicity, and drug diffusion from the polymer matrix were all factors in the initial drug burst. The presence of water in the matrix causes the polymer to hydrolyze into soluble oligomeric and monomeric components, which causes the delayed release phase. As a result, a pathway is opened up for the drug to be released through diffusion and erosion until the entire polymer has broken down [35].

When compared to the medication solution, the transcorneal permeability investigation of the ASP-PLGA NPs revealed a persistent penetration profile. This might be because the nanoparticles are more soluble and have smaller particle sizes. Additionally, aspirin has an inherent high permeability property because it is a member of BCS Class II, which facilitates the increased pro-fleshing of ASP-PLGA-NPs.

The findings indicate that ASP-PLGA NPs maintain their particle size even after extensive storage (six months). These findings conflict with earlier research by Tang and Singh who claimed that aspirin caused PLGA to degrade in an extremely short amount of time [36]. No other drug has been associated with the rapid degradation of PLGA that has been documented in the literature. Precise PLGA degradation profile in the aspirin-loaded PLGA gel system.

CONCLUSION

In the present study, the ASP-PLGA NPs were prepared using the solvent evaporation method as an ideal preparation technique for improved inclusion efficiency, zeta potential, and particle size. *In vitro* release studies and the transcorneal permeation studies performed showed the maximum drug release in 4 h and 6 h, respectively, which means that this formulation can give sustained release at the desired site of action compared to the drug that reached a peak concentration of at the end of 1 h or 3 h. The formulation showed acceptable stability when stored at 2-8 °C. It can be concluded that ASP-PLGA-NPs can be used successfully for ocular administration and treatment of eye diseases.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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