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Development of surface modified nanoparticles of curcumin for topical treatment of diabetic retinopathy: *In vitro*, *ex vivo* and *in vivo* investigation

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ABSTRACT

Diabetic retinopathy (DR) is the worst microvascular complication of diabetes that results in blindness. Present treatment of DR is invasive and associated with pain and inflammation. There is a need to develop painless treatment. The surface-modified PLGA (50:50) nanoparticles of Curcumin, which is a peroxisome proliferator-activated receptor-gamma agonist, were prepared to target DR by topical administration. Nanoparticles were optimized by a 3^2 factorial design. The Concentration of PLGA and number of HPH cycles at fixed pressure were independent variables whereas particle size. PDI and % entrapment efficiency were the dependent variables. Batch F6 with particle size, PDI, and % EE 194.7 nm, 0.231, and 90% respectively was selected as the optimized batch. The optimized batch was further subjected to spray drying and evaluated for various parameters. Residual solvent analysis was carried out by Gas chromatography. *In vitro* drug release study showed a biphasic drug release pattern of 70.17 ± 1.38% cumulative drug release at the end of 10 h. The release profile of curcumin from nanoparticles appeared to fit best with the Higuchi model. XRD study confirmed the molecular dispersion of the drug. The formulation showed satisfactory results in sterility testing, histology and isotonicity testing. *In vivo* study on rats showed dose dependent reduction in vascular endothelial growth factor (VEGF) concentration in vitreous fluid. Based on available evidences it can be concluded that the prepared formulation possesses great potential to manage diabetic retinopathy.

1. Introduction

Diabetes mellitus (DM) is the most common chronic metabolic disorder associated with elevated blood glucose levels. It has equally affected both developed as well as developing countries [1]. Many surveys including World Health Organization have reported that around 366 million people had DM in 2011 and by 2030 this number is projected to be around 552 million. India is now acting as the 'capital for diabetes' with 65 million diabetic patients in 2016 [2]. The major concern with diabetes is that it progressively affects almost all parts of the body. Diabetes mellitus and associated complications are known as the most considerable cause of morbidity and mortality worldwide [3].

The eyes of the patients in case of uncontrolled hyperglycemia are affected significantly. DM affects the eyes of the patients progressively and if not treated properly, then it may result in poor vision and in some cases may lead to the blindness as well. Cornea and retina are the major sufferers of the impact of hyperglycemia. It has been reported that in comparison to the normal cornea, the diabetic cornea experiences four times more glucose in the tear film. Moreover; patients with diabetes are more prone for visual losses due to the impact of hyperglycemia on the retina. Furthermore; diabetic patients are more suitable to any ocular disease in comparison to a normal person [4].

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As per the survey by the National Eye Institute, at present in India 7.7 million people are suffering from DR, which may reach to 11 million by 2030 and 15 million by 2050. Diabetic retinopathy (DR) is one of the major complications of diabetes which leads to blindness. Studies done in southern India showed that the range of prevalence of DR is 12.2%–18.03% in the population with known DM. Retina is the light-sensitive tissue at the back of the eye. Diabetic retinopathy occurs when high glucose damages the tiny blood vessels inside the retina Diabetic retinopathy usually affects both eyes. Neovasculiraztion i.e. formation of new but weak capillaries in the eye because of VEGF (Vascular Endothelial Growth Factor) is main complication associated with diabetic retinopathy. As newly formed capillaries are weak, these blood vessels can break easily and leak blood into the vitreous gel and resulting in blindness.

At present, DR is treated with vitrectomy, intravetrous injections, and laser treatment. All these methods are invasive and associated with pain along with the probability of development of secondary infections, fear of loss of vision and high cost of medicines which ultimately leads to poor patient compliance. That is why here is a dire need to develop non-invasive treatment for betterment of diabetic retinopathy which will improve patient compliance.

In the last couple of years, PPAR gamma receptors have grabbed the attention of researchers for their usefulness in the management of major complications of the ocular site. PPAR-γ has proved its involvement in the control of DR by inhibiting the progress of diabetic retinopathy at a different stage. PPAR-γ agonist possesses antioxidant activity that can control and reduce the formation of AGEs formation which plays a crucial role in diabetic retinopathy and associated microvascular complications [5,6]. The study also reveals the role of PPAR-γ in angiogenesis through streptozotocin-induced diabetic retinopathy via inhibition of NF-KB [7,8]. Inhibition of NF-kB can be correlated with inflammation and retinal leakage as well as leukostasis [9]. Furthermore; the PPAR-γ agonist can also control DR by upregulation of members of the Bcl-2 family which prevents the complex process of apoptosis [10]. PPAR-γ is also reported to play a crucial role in the management of diabetic retinopathy by prevention or control of neovascularization due to down regulation of VEGF [11].

Curcumin which is (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadine, 3,5dione) polyphenol obtained from *Curcuma longa* has increased the attention of many ophthalmologists for treatment of ocular complications. It is PPAR-gamma agonist. It has the potential to treat major ocular complications like diabetic retinopathy through down regulation of VEGF, COX-2, and relief from oxidative stress through the PPAR-gamma pathway. Curcumin has the potential to prevent angiogenesis and neovascularization. It has proved significant role in neurodegenerative and inflammatory disorders [12–14]. Despite this finding, the usefulness of curcumin in retinopathy is limited because of poor solubility and less bioavailability. This problem can be resolved by preparing nanoparticles of curcumin.

Henceforth; by considering the involvement of PPAR-γ agonist in the management of diabetic retinopathy, current research involves the development of surface-modified nanoparticles of curcumin by using biodegradable polymer PLGA. Surface modification was achieved by using Polysorbate 80. Nanoparticles were optimized by using 3^2 factorial designs and optimized nanoparticles were evaluated for various parameters viz. particle size, PDI, entrapment efficiency, yield, drug loading, *in vitro* drug release and ocular irritation study (*ex vivo*). Effectiveness for diabetic retinopathy was further investigated on Wistar Rats by measurement of VEGF level with the help of ELISA.

2. Material and method

2.1. Materials

Curcumin was obtained as a gift sample from Reve Pharma Pvt. Ltd., Nasik, India. PLGA 50:50 (RESOMER®) was gifted by Evonik Pvt. Ltd. Mumbai, India. Acetone, Polysorbate 80, Potassium dihydrogen orthophosphate, and dipotassium hydrogen phosphate was obtained from SDFCL, Mumbai. Polyvinyl alcohol was procured from Thomas baker, Mumbai, India. All other solvents and materials used were of analytical grade.

2.2. Analytical method development

To determine the concentration of curcumin in various samples obtained in the proposed investigation, the UV visible spectrophotometric method was developed and validated as per ICH Q2 (R1) requirements. The absorbance was measured at 429 nm. At an early stage, a stock solution of curcumin of 100 μg/ml was prepared by using ethanol and phosphate buffer (pH 7.4) in 1:1 proportion as per the method described by Majumder KK et al. [15].

2.3. Fabrication of nanoparticles

For the fabrication of surface modified nanoparticles of curcumin, a single emulsion solvent evaporation method was used. It was associated with the preparation of organic and aqueous phase separately. Curcumin (15 mg) and PLGA 50:50 was dissolved in ethanol and acetone respectively which was the organic phase. The aqueous phase involves polyvinyl alcohol (1%) and tween 80 (0.45%) dissolved in water. The Aqueous phase was placed under the probe sonicator and drop by drop organic phase was added to obtain the O/W emulsion with a smaller droplet size. Further, to reach a smaller size, the prepared system was subjected to High Pressure Homogenizer (ShearJet HL 60, Dyhydromatic) at fixed 15 kpsi pressure with variable cycles. Each batch was analyzed for particle size and PDI by using a zeta sizer (SZ-100 Ver 2.40, HORIBA). In the lateral stage, all batches were subjected to solvent evaporation by using a magnetic stirrer for 12 h. The obtained suspension was filtered (membrane filter) and subjected to cold centrifugation at 4 °C, 15, 000 rpm for 1200 s. Entrapment efficiency was determined by using clear supernatant obtained after the centrifugation [16].

2.4. Experimental design

For optimization of curcumin-loaded surface-modified PLGA nanoparticles, 3^2 factorial design was used. This design was applied to check the impact of independent variables viz. PLGA concentration (X1) and HPH cycles (X2) on responses as Particle size (Y1), PDI (Y2), and percent entrapment efficiency (Y3). Throughout the process pressure of HPH was kept constant (15 kpsi). Independent variables were investigated at three levels as shown in Table 1.

Where; −1: low level; 0: middle level; +1 high level.

The resulting data were fitted into Design-Expert software v13 and analyzed statistically using analysis of variance (ANOVA). Based on optimization, a batch that was found to be optimized, the sediment of the same batch was washed with distilled water and subjected to spray drying. Spray-dried nanoparticles were further evaluated for various parameters.

2.5. Characterization of nanoparticles

2.5.1. Residual solvent analysis

In order to ensure that nanoparticles are free from organic solvents

Table 1
Coded levels translation in actual units.

Independent variables	Coded levels		
	−1	0	+1
Concentration of PLGA 50:50 (mg): X1	50	75	100
Number of HPH cycles: X2	5	4	6

(methanol and DCM), the residual solvent analysis was carried out by using gas-chromatography headspace analysis. N–N–N-Dimethylformamide was used as the solvent for sample preparation.

2.5.2. Particle size and PDI

The particle size of nanoparticles plays a pivotal role in their movement towards the posterior segment of the eye. It affects cellular uptake, distribution, and pharmacokinetics of nanoparticles significantly. Smaller particles are more compatible with the eye site. PDI is can be correlated with stability of nanoparticles [17]. Henceforth; estimation of particle size and PDI is one of the crucial aspects in nano-particulate system. Particle size and PDI was measured by using Zetasizer.

2.5.3. entrapment efficiency

Entrapment efficiency (EE) is an important factor to consider when evaluating nano formulation. Due to a lack of functional techniques to identify nanocarriers, proper EE assessment requires a clean separation of nanocarriers from free drugs which is known as indirect method for estimation of the entrapped drugs.

Entrapment efficiency was carried out by using an estimation of the free amount of drug in supernatant obtained after cold centrifugation. Free drug concentration was determined by using a UV- spectrophotometer at 429 nm [18].

Percent entrapment efficiency was calculated by using the following formula;

$$E.E.\ (\%) = \frac{Total\ amount\ of\ the\ drug - Amount\ of\ the\ free\ drug}{Total\ amount\ of\ the\ drug} \times 100$$

2.5.4. Zeta potential

Zeta potential and particle size together govern the stability of nanoparticles. Zeta potential measures the charge on a particle and its magnitude. Many of researchers have claimed that particles with zeta potential ±20 mV are more stable. Moreover; zeta potential also affects the *in-vivo* uptake of nanoparticles. Therefore; zeta potentials of the optimized batch were determined by using Zetasizer (Nano ZS 90-Malvern).

2.5.5. Drug loading efficiency and percent yield

Spray dried nanoparticles were subjected for evaluation of drug loading efficiency and % yield. The following formula was used for the calculation of drug loading efficiency as given by Tao et al. [19].

$$DL\ (\%) = \frac{Total\ amount\ of\ the\ drug - Amount\ of\ the\ free\ drug}{\text{Weight of spray dried nanoparticles}} \times 100$$

Percent yield was calculated as the total weight of spray dried product with respect to initial weight of drug and polymer [20].

The percent yield was calculated as:

$$\%\ Yield = \frac{Total\ weight\ of\ spary\ dried\ nanoparticles}{Drug + polymer\ weight + dispersing\ agent} \times 100$$

2.5.6. In vitro drug release and release kinetics

Optimized nanoparticles of curcumin were subjected to *in vitro* drug release by using the method given by Kesarla et al. (2016). The dialysis membrane was soaked overnight into diffusion medium-phosphate buffer (PB) pH 7.4. One end of the membrane was tied and a 1 ml formulation was added in to the sac. Another end of the membrane was closed tightly and suspended in a beaker having PB 7.4 (100 ml). Beaker was placed on magnetic a stirrer at 50 rpm and at 37 ± 0.5 °C. After a specific time interval, a 5 ml sample was withdrawn and analyzed spectrophotometrically at 238 nm. After each sample withdrawal equal quantity was replaced by the medium. Percent cumulative drug release data was calculated. Further; data obtained from the above study was subjected to drug release kinetics [21].

2.5.7. Shape and surface morphology

The shape and surface morphology of fabricated nanoparticles plays a conclusive role in ocular tolerance and patient compliance. A smooth surface is a key to maintaining minimum sensitization by exogenous particles. Based on this, fabricated nanoparticles were subjected to morphological analysis by scanning electron microscopy (ZEISS 505/506).

2.5.8. X-ray diffraction studies (XRD)

An X-ray diffraction study was performed to check the nature of the drug and fabricated nanoparticles. Plane drug and fabricated nanoparticles were analyzed by XRD instrument by using a Cu-Ka line (radiation source) operated at a voltage of 40 kV and current of 35 mA. All samples were measured in the 2Θ angle range between 1 and 50°.

2.5.9. Ocular irritation potential

The ocular irritation potential of optimized nano-particles was evaluated by a histological study of the goat eye cornea. The eyes of freshly sacrificed goat were taken from the local slaughterhouse. Corneas were removed from the eyes and incubated at 37 °C for 5 h in formulation, Sodium dodecyl sulfate solution 0.1% w/w (positive control) and saline solution (negative control). After incubation, corneas were washed with saline solution and immediately fixed in 8%, w/w formalin. Dehydrated tissue in an alcohol gradient was placed in melted paraffin and solidified to form a block. Cross-sections were cut, stained with hematoxylin, and eosin and observed under motic microscope [22].

2.5.10. Isotonicity testing

Isotonicity testing was performed by using own blood sample. A drop of the formulation was mixed with a drop of blood on the glass slide. A thin smear was formed and a few drops of Wright's stain were added over to it. After 2 min of staining, the excess quantity of stain was washed off with distilled water. Similarly, another slide was prepared by mixing 0.9% sodium chloride with blood. Both slides were observed under a microscope at 40× magnification [23].

2.5.11. Sterility testing

Prior to *in vivo* study, optimized nanoparticles were sterilized by UV irradiation. Nanoparticles were exposed to UV radiation for 1.5 h [24] and then dispersed in sterile water aseptically. Further sterility testing was carried out as per the procedure given in Indian Pharmacopoeia in an alternate thioglycollate medium and soyabean casein digest medium.

2.5.12. In vivo study

The effectiveness of fabricated nanoparticles in diabetic retinopathy was investigated by measurement of vascular endothelial growth factor (VEGF) level in diabetes-induced Wistar rats. Increased level VEGF plays an important role in the overall progress of diabetic retinopathy. The experimental protocol was approved by Animal Ethical Committee. The experiment was designed and conducted in accordance with the guidelines laid by CPCSEA, New Delhi. The animals were acclimatized and uphold on a normal food, water and libitum for a week. Randomly animals were divided into 5 groups as each group contained 6 rats

Table 2
Treatment group.

Group n = 6	Treatment and dose
Vehicle control	5 ml saline solution intraperitoneal injection
Diabetes control	STZ 65 mg/kg intraperitoneal injection
Vehicle treatment	STZ 65 mg/kg intraperitoneal injection + treated with drop of saline water topically
Formulation treatment-1	STZ 65 mg/kg intraperitoneal injection + treated with drop of nanoparticles (concentration 4 mg/ml)
Formulation treatment-2	STZ 65 mg/kg intraperitoneal injection + treated with drop of nanoparticles (concentration 8 mg/ml)

(Table 2).

Hyperglycemia was induced by intraperitoneal injection of 65 mg/kg STZ and verified for the same after 3 days (glucose level more than 250 mg/dl). After a week of diabetes, treatment was initiated. The non-diabetic control rats were treated with distilled water while STZ-treated animals were treated with one drop of nano formulation. After 4 weeks of continuous treatment, VEGF protein concentrations in the retina (vitreous fluid) from each group were determined [25].

Analysis of VEGF level: Rats were sacrificed using Pentobarbitone sodium (overdose). The vitreous fluid from both eyes was isolated and kept in a deep freezer. VEGF concentration in the vitreous fluid was then determined by using Rat ELISA Kit (R&D Systems, Inc. Minneapolis) and analyzed on ELISA.

3. Results and discussion

3.1. Fabrication of nanoparticles

Nine batches of curcumin-loaded PLGA NPs were prepared by using the single emulsion solvent evaporation method. The optimized batch was further subjected to spray drying.

3.2. Experimental design

For optimization of curcumin-loaded nanoparticles, two factors three level design was used. The two independent variables viz. PLGA concentration (X1) and HPH cycles (X2) at a fixed pressure of 15 kpsi with their coded and actual units are shown in Table 3.

Responses particle size (Y1), PDI (Y2), and Entrapment efficiency (% EE) (Y3) were found to be in the range of 136.7 ± 6 to 283.9 ± 8 nm, 0.210 ± 0.03 to 0.301 ± 0.02 and 85 ± 2 to 94 ± 1 respectively (Table 3). Software suggested that the best-fitted model is quadratic for Y1, Y3, and liner for Y2. As the value of probability is less than 0.05 in each model, results of ANOVA and regression analysis confirmed that the suggested models were significant for operational parameters (Table 4).

$$Y1 = +164.33 + 45.88\,X1 * + 29.43 * X2 - 0.4250 * X1\,X2 + 44.25 * X1\,2 + 3.60 * X2\,2 \quad (1)$$

$$Y2 = +0.2528 + 0.0383 * X1 + 0.0017 * X2 \quad (2)$$

$$Y3 = +88.94 + 3.17 * X1 + 1.42 * X2 + 0.1250 * X1\,X2 + 0.8333 * X1\,2 - 0.4167 * X2\,2 \quad (3)$$

Equation 1 indicates the positive impact of PLGA concentration and HPH cycles on particle size (Y1) i.e. as the PLGA concentration and HPH cycles increases, there is an increase in particle size as well. A high concentration of PLGA increases the viscosity of the system and ultimately reduces the impact of applied shear. In this case, with the used HPH, we found that, with increasing cycles there is an increase in particle size which might be due to the development of static charges as reported by the manufacturer. Equation 2 showed that the positive impact of X1 and X2 on PDI. From equation 3, it can be concluded that as the concentration of polymer and number of HPH cycles increases, the % EE also increases. With increasing concentration of PLGA and number of HPH cycles, the maximum amount of drug gets entrapped in polymeric nano-droplets. In each response the impact of PLGA was comparatively more significant than HPH cycles. Impact of X1 and X2 on responses can be interpreted by using 3D response plots as shown in Fig. 1.

For the selection of optimized batch desirability, a search approach was used. Less particle size, PDI below 0.3, and better entrapment efficiency were the selection criteria for the optimized batch. We also considered the impact of concentration of PLGA on drug release while performing the optimization. Based on the selection criteria, batch F6 with 75 mg PLGA and 6 HPH cycles having desirability 1 was selected as an optimized batch.

Table 3
Design parameters, formulation composition, experimental conditions and characterization of nanoparticles.

Run	FC	Coded levels of variables		Particle size (nm) (Y1)	PDI (Y2)	%EE (Y3)
		Factor X_1 (PLGA)	Factor X_2 (No of cycles)			
1	F1	50 (−1)	4 (−1)	136.7 ± 6	0.210 ± 0.03	85 ± 2
2	F2	50 (−1)	5 (0)	156.7 ± 10	0.220 ± 0.05	86.5 ± 1
3	F3	50 (−1)	6 (+1)	201.9 ± 8	0.239 ± 0.07	87.5 ± 2
4	F4	75 (0)	4 (−1)	146.9 ± 6	0.261 ± 0.05	87 ± 1.5
5	F5	75 (0)	5 (0)	158.5 ± 5	0.223 ± 0.03	89 ± 2
6	F6	75 (0)	6 (+1)	194.7 ± 11	0.231 ± 0.06	90 ± 2
7	F7	100 (+1)	4 (−1)	220.4 ± 7	0.290 ± 0.08	91 ± 3
8	F8	100 (+1)	5 (0)	266.3 ± 9	0.300 ± 0.05	93 ± 2
9	F9	100 (+1)	6 (+1)	283.9 ± 8	0.301 ± 0.02	94 ± 1

3.3. Characterization of fabricated nanoparticles (optimized nanoparticles)

3.3.1. Residual solvent analysis

Fabricated NPs were subjected to residual solvent analysis in order to ensure that the selected method is enough to make the system free from the used organic solvent. The gas chromatography method was used to check the presence of residual solvents in spray-dried NPs. No residual solvent was observed in the sample. Results are shown in Fig. 2.

3.3.2. Particle size and PDI

Spray-dried nanoparticles were again subjected to determination of particle size and PDI. Particle size was found to be 198.4 nm with PDI 0.276. A slight increase in particle size was observed after spray drying which might be due to air drying of material which prevents the shrinking. The particle size was less than 200 nm indicating its suitability for ocular administration. Ophthalmic formulation should not contain particles greater than 10 μm which otherwise leads activation of defence mechanism of eye and can also lead to scratching and ultimately injury to eye. The PDI (Polydispersity Index) is used to identify the degree of non-uniformity of particle size distribution. PDI of nanoparticles should be less than 0.3 [26]. Results of the optimized batch further support the stability of fabricated nanoparticles and suitability for ocular administration which favours the traveling of nanoparticles rapidly deep into eye towards the posterior segment.

3.3.3. Zeta potential

Determination of zeta potential is one of the factors that can be correlated with the stability of colloidal dispersion. It provides information on charges and the magnitude of particles. It should be ±20 mV and above which maintains the particle in Brownian motion. The zeta potential of the optimized batch was found to be −23.3 mV (Fig. 3). Results clearly indicate the stability of prepared nanoparticles. Zeta potential and particles size together indicates the good stability of prepared colloidal system. Additionally; presence of tween 80 can lead to improved delivery efficiency by tissue interaction. Tween 80 also acts as a surfactant which helps to reduce the surface tension at the ocular site.

3.3.4. Drug loading efficiency and percent yield

Spray-dried nanoparticles were evaluated for drug loading efficiency and percent yield. Drug loading efficiency was found to be 8.43 and product yield was found to be 69%. Less yield is due to material loss during spray drying [27].

Table 4
Summary of results of regression analysis and ANOVA for measured responses.

Response	Model	F value	P-value	R^2	SS	DF	MS	Model Significance
Y1	Quadratic	32.23	0.0083	0.9817	21772.38	5	4354.8	Significant
Y2	Liner	16.26	0.0038	0.8442	0.0088	1	0.0044	Significant
Y3	Quadratic	913.46	<0.0001	0.9993	74.01	5	14.80	Significant

Y1: Particle size; Y2: PDI; Y3: %Entrapment efficiency; SS: Sum of Square; DF: Degree of freedom; MS: mean square.
Software suggested following polynomial equations for the dependent variables.

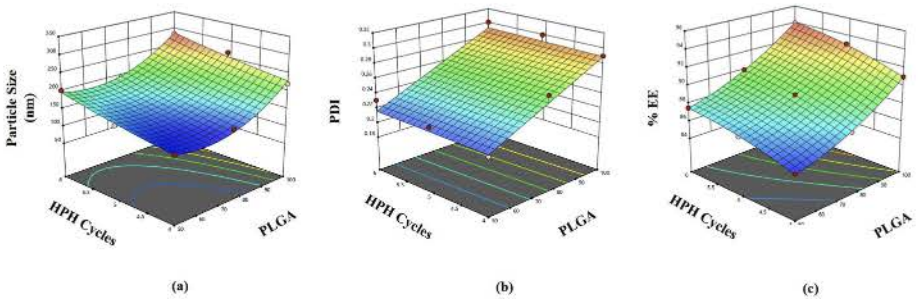

Fig. 1. Three dimensional response surface plot for a) Particle Size (Y1), b) PDI (Y2), %EE (Y3).

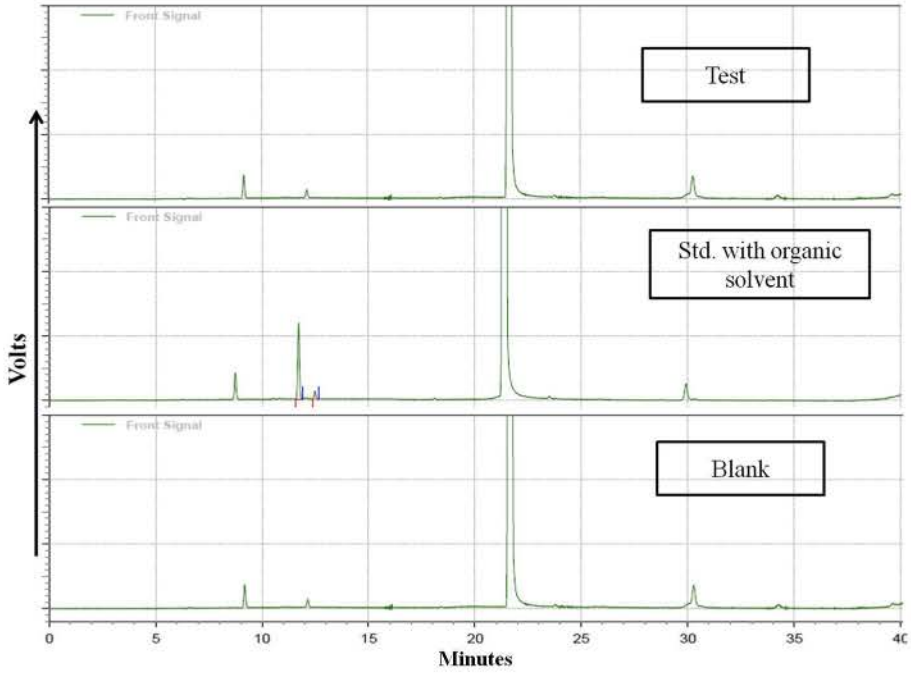

Fig. 2. Residual solvent analysis.

3.3.5. *In-vitro drug release*

Fabricated nanoparticles were subjected to *in vitro* drug release study for 10 h. A characteristic biphasic drug release pattern was observed which is associated with initial burst release followed by controlled drug release. The optimized batch showed 70.17 ± 1.38% drug release at the end of 10 h [28]. Drug release behaviour is shown in Fig. 4. PLGA 50:50 contains lactic acid and glycolic acid in 50:50 proportions. Lactic acid is hydrophobic in nature, while glycolic acid in hydrophilic in nature. Drug releases from PLGA by diffusion as well as erosion on surface as well as on bulk side. Therefore; as the glycolic acid proportion increases, the drug release also increases because of its hydrophilic characteristics. PLGA 50:50 is known grade for rapid drug release. Many *in vivo* studies indicated that, biodegradation of PLGA takes place by non-enzyme mechanism as simple hydrolysis of ester linkage. It gets broken down in to lactic acid and glycolic acid and eliminated from the body by tricarboxylic acid cycle in the form of carbon dioxide and water. Therefore; this polymer is more preferable for long term use. No toxicity is reported by long term use of polymer.

Data obtained from *in vitro* the drug release study was further subjected to mathematical treatment to determine drug release kinetic profile. The release constant was calculated from the slope of the appropriate plots and the regression coefficient (R^2) was determined (Table 5). The drug release was best explained by Higuchi kinetic with the highest R^2 value indicates drug release takes place by both, diffusion as well as erosion which is characteristics of PLGA polymer.

3.3.6. *Surface morphology*

The shape and surface morphology of prepared nano-particles was

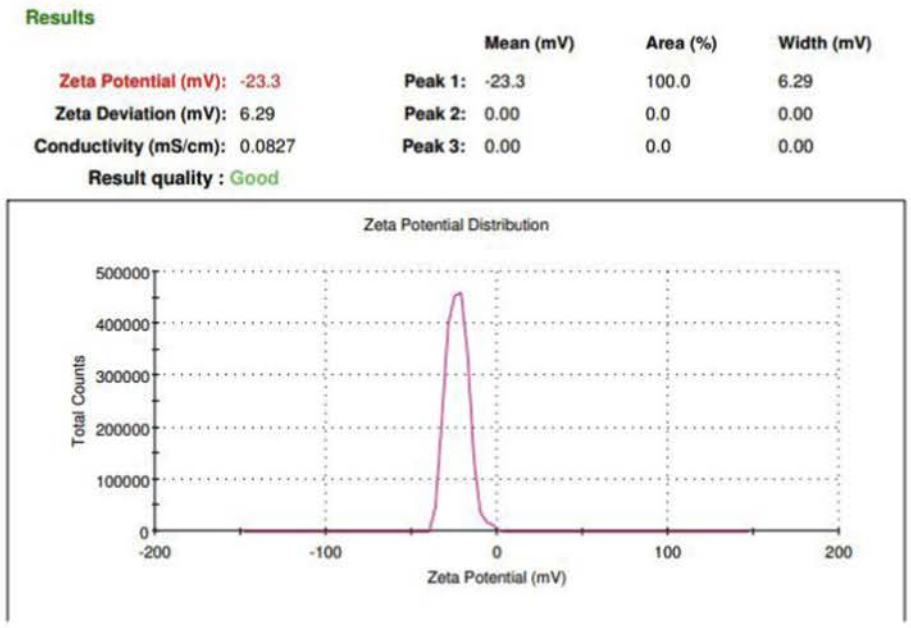

Fig. 3. Zeta potential of optimized batch.

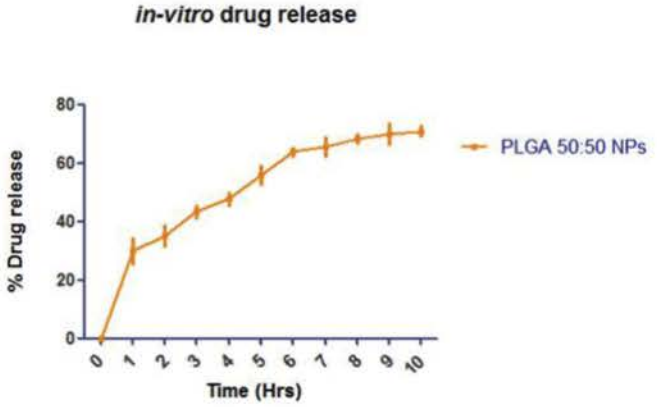

Fig. 4. *in vitro* drug release from nanoparticles (n = 3, mean ± SD).

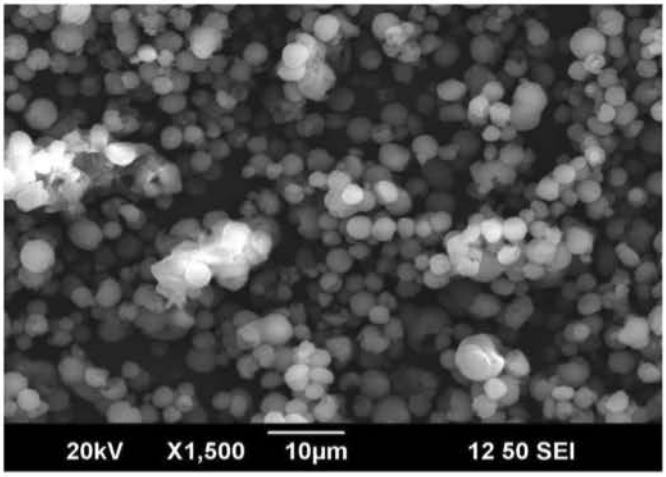

Fig. 5. SEM image of spray dried nanoparticles.

Table 5
Model fitting for release profile.

Coefficient of determination (R^2)				Best fit model
First order	Zero order	Higuchi	Hixon-Crowel cube root	
0.7578	0.8969	0.9654	0.9483	Higuchi

investigated by SEM. The result is shown in Fig. 5. Nanoparticles appeared spherical with no fracture. This morphology is particularly important for the ocular site in order to avoid irritation and scratching on the corneal surface. It also ensures uniform deposition of particles with minimum sensitization. Moreover; non-spherical as well as fractured particles may leads to scratching effect to cornea and leads to irritation. Hence; as prepared particles were found to be spherical with smooth surface, it can be used for further *in vivo* investigation [18].

3.3.7. X-ray diffraction study

Curcumin and drug loaded nanoparticles were subjected to an X-ray diffraction study and diffractograms were recorded. The diffractogram of the drug showed characteristic peaks as shown in Fig. 6. Sharp peaks of the drug disappeared in the XRD of nanoparticles (Fig. 7). Results confirm the molecular dispersion of the drug [20].

3.3.8. Ocular irritation potential (ex vivo)

The ocular itchiness capability of prepared nanoparticles was evaluated by a goat eye corneal histology. The cornea framework was well conserved in saline solution and cornea-treated with the formulation. Even so, the corneal structure was seriously compromised by the control group (SDS treated). The Corneal structure of positive control was invaded. There was no hemorrhage or necrosis observed with the formulation of the treated cornea. Therefore, the prepared formulation was found to be suitable for further *in vivo* investigations. Results are shown in Fig. 8.

3.3.9. Isotonicity testing

Isotonicity is an important characteristic of ophthalmic formulation which has to be maintained to prevent any tissue damage or irritation to the eye. It refers to the osmotic pressure exerted by salts in an aqueous solution. The ophthalmic formulation must possess osmotic pressure within the range of 290–310 mOsmol/kg [29].

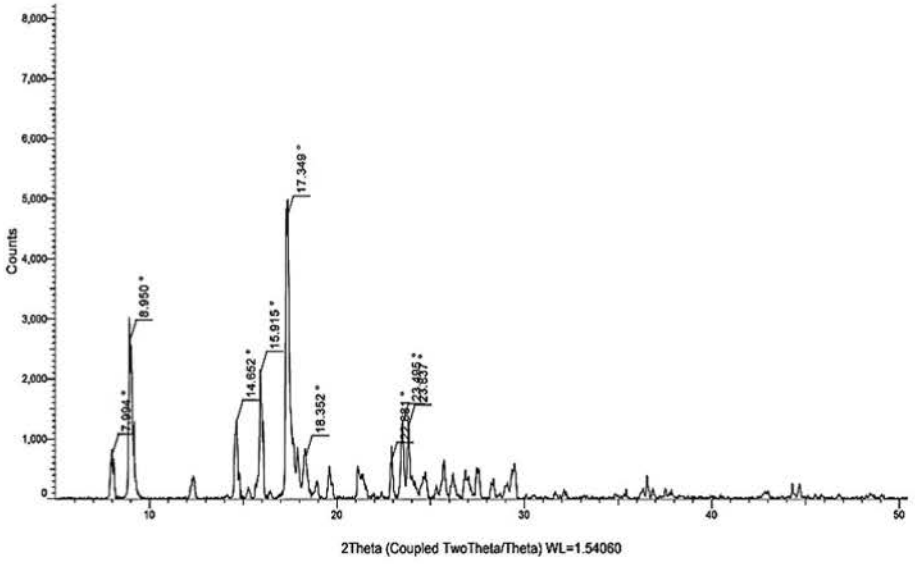

Fig. 6. XRD of curcumin.

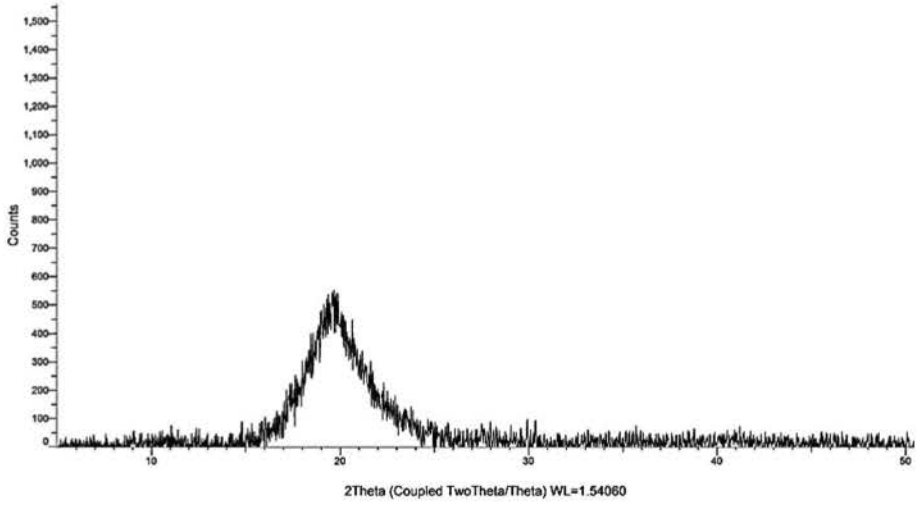

Fig. 7. XRD of drug loaded nanoparticles.

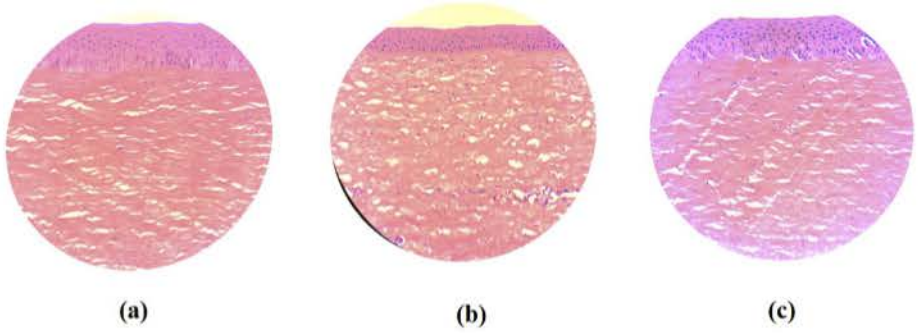

Fig. 8. Histological section of goat eye cornea a) negative control: untreated cornea, b) test specimen: formulation treated cornea, c) positive control: SDS treated cornea.

The Isotonicity of prepared nanodispersion was checked by using a blood sample. The Structure of RBC was well retained in formulation treated blood sample as well as 0.9% sodium chloride solution treated solution. No rupture was observed. Results are shown in Fig. 9. Therefore; it can be concluded that the prepared formulation is isotonic and safe for its intended use [23].

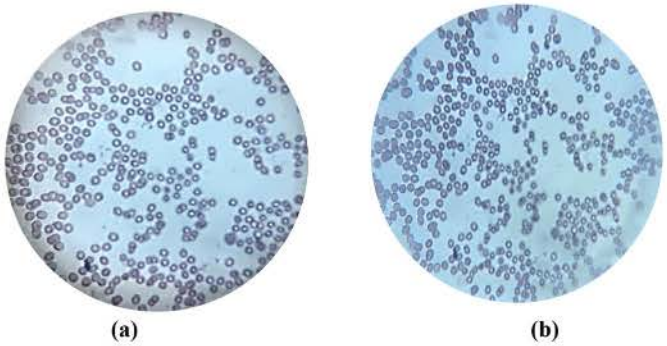

Fig. 9. Isotonicity image a) RBC treated with 0.9% NaCl b) RBC treated with formulation.

3.3.10. *In vivo evaluation*

The effectiveness of prepared formulation containing PPAR-γ agonist in diabetic retinopathy by down-regulation of VEGF was studied in STZ-induced diabetic rats by ELISA. A reduction in VEGF level was observed in the entire treatment group in comparison to the untreated group (Fig. 10). The used concentration of curcumin was 4 mg/ml and 8 mg/ml which was found an effective concentration on ARPE-19 cells by Bucolo et al. [30]. A group of rats treated with a simple vehicle showed a marked increase in VEGF level and also discovered a dose-dependent decrease in VEGF level after 4 weeks of therapy. Results are shown in Fig. 10. As many of evidence has already stated that VEGF increased level is the main contributor to neovascularization and further cascade of diabetic retinopathy, here our evidence shows the impact of this formulation in control of VEGF level and ultimately progress of DR. Results indicates the ability of fabricated nanoparticles in management of diabetic retinopathy via topical administration. Additionally; as the study proves the down regulation of VEGF by a PPAR-γ agonist, there is also hope to cure diabetic retinopathy by some other possible mechanisms which are associated with PPAR-γ receptors like inhibition of formation of AGE, decrease in angiogenesis, inflammation, retinal leakage and retinal leukostais as well as protection from apoptosis and oxidative stress on RPE. These results indicates that curcumin loaded surface modified PLGA nano particles possesses great potential to treat DR.

4. Conclusion

Diabetic retinopathy (DR) is known to be one of the worst complication of diabetes which leads to blindness. Current treatment of DR includes vitrectomy, intravetrous injection, and laser burns which are invasive, painful and associated with fear of loss of vision. The present research showed the significance of the involvement of PPAR-γ receptors in the management of Diabetic retinopathy. Developed nanoparticles of curcumin showed the remarkable reduction in vitreous VEGF levels in rats. This downregulation of VEGF in comparison to the untreated group proved the involvement of PPAR-γ in diabetic retinopathy management as well as the movement of nanoparticles towards the posterior segment eye. Our evidence supports the use of prepared surface-modified nanoparticles in the treatment of diabetic retinopathy. It overcomes the limitations of the current treatment of DR. Advantages of prepared nanoparticles involved ease of administration, painless system, pocket friendliness and self-administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

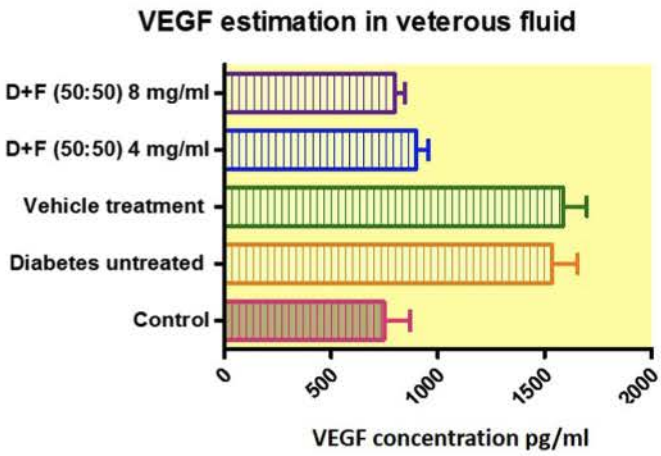

Fig. 10. Effect of treatment on VEGF level.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jddst.2022.103835.

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