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Influence of nanoprecipitation method parameters on nanoparticles loaded with gatifloxacin for ocular drug delivery

A. Maaz[1], W. Abdelwahed[2], I.A. Tekko[3], S. Trefi[1]

[1](Department of Pharmaceutical Chemistry & Quality Control, Faculty of Pharmacy, Aleppo University, Syria) [2](Department of Pharmaceutical Technology, Faculty of Pharmacy, Aleppo University, Syria)
3(Department of biopharmaceutics and drug Formulation, Aleppo University, Syria)

ABSTRACT: *Poor ocular bioavailability of drugs (<1%) from conventional eye drops (i.e., solution, suspension, and ointments) is mainly due to the physiologic barriers of the eye. In general, ocular efficacy is closely related to ocular drug bioavailability, which may be enhanced by increasing corneal drug penetration and prolonging precorneal drug residence time. In our current work, we developed a colloidal system that is polycaprolactone (PCL) nanoparticles for Gatifloxacin ophthalmic delivery, to improve precorneal residence time and ocular penetration for enhanced drug bioavailability. In this research, we studied nanoparticles preparation procedures and the effect of process variables on its characters. Nanoparticles were prepared by nanoprecipitation technique and characterized for various properties such as particle size, polydispersity index PdI, zeta potential and Entrapment efficiency EE%. The developed nanosuspension showed a mean particle size in the range of 184 to 207nm, suitable for ophthalmic application with zeta potential range of -30 to -32 mV and Entrapment Efficiency EE% of 40%. These results demonstrated that, the developed nanosuspension was found to be applicable for sustained ocular drug delivery allowing minimizing dose repetition to reduce systemic side effects and enhance patient compliance.*

Keywords: Gatifloxacin, Nanoparticles, Nanoprecipitation, Ocular Delivery, Polycaprolacton

1. INTRODUCTION

Despite numerous scientific efforts in drug delivery field, efficient ocular drug delivery remains a challenge for pharmaceutical scientists. The unique structure of the eye restricts the entry of drug molecules at the required site of action. In ocular drug delivery system, ocular infections are treated by various topical drug applications in the form of solutions, suspensions and ointment. These conventional dosage forms suffer from the problems of poor ocular bioavailability due to the precorneal loss factors that include rapid tears turnover, nonproductive absorption, transient residence time in the cel-de-sac, and relative impermeability of the drugs to the corneal epithelial membrane [1, 2]. This poor ocular bioavailability imparts the need for frequent instillation to achieve the therapeutic effect, which may leads sometimes to undesirable side effects caused by systemic drug absorption. A polymeric nanoparticle formulation is one of the strategies currently used to improve drug absorption across biological membranes [3].

Hence, in our current work we followed recent applications of nanoparticulate systems in the field of ocular drug delivery, whereas utilizing nanoparticles for ocular disease treatment in a wide range of medical research fields has become a popular strategy in recent years [4-6]. Much of the published data suggests that in the case of ophthalmic drug delivery, an appropriate particle size, and a narrow size range, ensuring low irritation, adequate bioavailability and compatibility with ocular tissues, should be controlled for every drug loaded [7].

The preparation of Nanospheres (NS) by precipitation method has been studied extensively to produce high qualified NS [8], using biodegradable polymer polycaprolactone (PCL) which is an anionic polymer and one of the most commonly used polymers in ophthalmic drug formulations [9]. Nanprecipitation method is also called solvent displacement or interfacial deposition (Fig. 1), and it is one of the easiest preparation procedures of nanospheres. Additionally to its simplicity, this procedure is reproducible, fast and economic [10] but the encapsulation of water soluble compounds by this method is still providing challenges to the pharmaceutics person [11, 12].

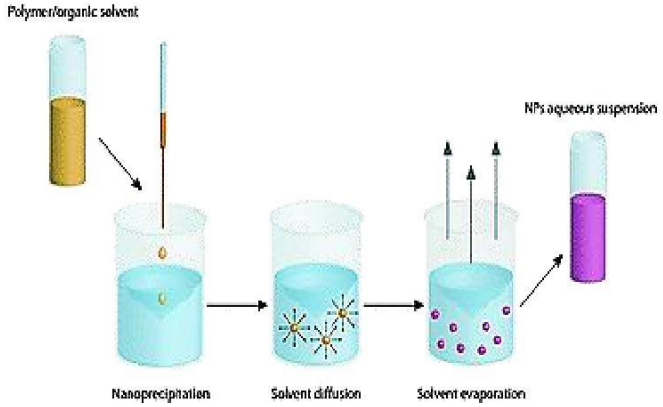

Fig. 1: schematic representation of nanoprecipitation method

Gatifloxacin (GFX) is a new 8-methoxy fluoroquinolone, a broad spectrum antibiotic, which like other members of that family, inhibits the bacterial enzymes DNA gyrase and topoisomerase IV. GFX exhibits enhanced activity against Aerobic Gram-Positive and negative Bacteria, such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Moraxella catarrhalis*, and *Legionella spp*. [13, 14]. Gatifloxacin like other fluoroquinolones is Diprotic molecules contain acidic and basic groups (Fig. 2), and depending on the pH of the aqueous environment, they can occur in four protonated forms: cationic, zwitterionic, neutral, and anionic. The equilibrium between the forms can be shifted by pH changes [14]. Solubility of GFX is pH dependent, with maximum aqueous solubility (40-60 mg/ml) occurring in a pH range of 2-5 [15]. This water solubility makes it diffuses during nanoparticles preparation into external aqueous phase causing low encapsulating percentage.

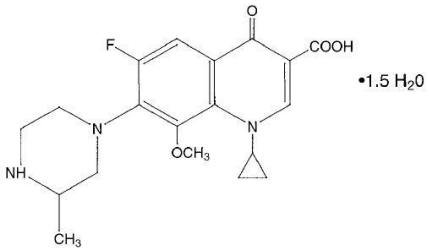

Fig.2. chemical structure of Gatifloxacin

The present work, an attempt has been made to prepare gatifloxacin nanoparticles formulation by nanoprecipitation method to be applicable for ocular drug delivery to improve precorneal residence time and ocular bioavailability. Both particle size distribution and polydispersity can influence the nanoparticulate drug delivery. A particle size below 250nm [2,16], and polydispersity index PdI near 0.2533 [2,17] were considered optimum for ocular administration. In this work many formulation variables have been studied for their effect on nanosphere characterizations such as: type of polymer used, aqueous to organic phase ratio, type and concentration of stabilizing agent, pH of aqueous phase and the amount of drug used in the formulation. All of these formulations were optimized and evaluated by measuring mean size, poly dispersity index PdI, zeta potential value and entrapment efficiency EE% for its best ocular application.

2. MATERIAL AND METHODS

2.1 Materials

Gatifloxacin was kindly provided as free sample by Delta for pharmaceutical industries (Syria). The polymers Polycaprolactone (PCL) with molecular weight of 45 000 was supplied by Sigma–Aldrich Chemicals (France) and Poly lactic acid (PLA) with molecular weight of 75000-120,000 g/mole was supplied by Sigma–Aldrich Chemicals (France). The stabilizing agent Poly vinyl alcohol (PVA) with molecular weight of 31000

and 89000-98000 g/mole; 99% hydrolyzed were purchased from Carl-Roth Chemicals (Germany) and Sigma-Aldrich chemical (France) respectively. Poloxamer®188 from Sigma-Aldrich chemical (France). Potassium dihydrogen Phosphate salt was supplied by Merck chemicals (Germany).

Analytical grade Acetone and Ethanol absolute were provided from Panreac products (Spain). HPLC grade acetonitrile were provided from Scharlau products (Spain).

2.1 Methods

2.2.1 Preparation of Nanoparticles

Gatifloxacin nanoparticles were prepared using the nanoprecipitation method [18].The formulation plan is shown in Table 1. Briefly, an aqueous solution of Gatifloxacin at different concentrations was prepared, in which stabilizing agent PVA with or without hydrophilic surfactant was added too. Specified amount of PCL polymer was dissolved in the organic phase. The organic solution was then added drop wise at the rate of (1 ml\min) into the drug and surfactant aqueous solution under continuous magnetic stirring at 50 rpm for 10 min at room temperature. The aqueous phase immediately turned into milky bluish opalescence due to the formation of the nanoparticles suspension. The formed nanoparticles were solidified then by evaporating the organic solvent at 45 ◦C and 100rpm for 30-45 minutes under reduced pressure using a Rotavapor (Rotavapor R-215, Buchi, Switzerland). Finally, the nanosuspension was concentrated to final volume of 10 ml by removal of water under the same conditions.

2.2.1.1 Optimization of polymer used

The selecting of polymer used was depended on the polymer that have good affinity with the dug and gives smaller particles size, smaller PDI and better zeta potential value. Two polymers are tested for these characters Poly Lactic Acid (PLA) and Polycaprolactone (PCL) with maximum amount of the polymer used as recommended for this method [13] and was optimized for all formulas (0.5% w/v of organic phase).

Table 1: Various parameters evaluated for nanoparticles characterizations*

Parameters			Value			
OP	Solvent (OP:AP ratio)	Acetone	1:2	3:5	2:5	1:5
		Ethanol absolute	1:2			
	Polymer (%w/v)	PLA	0.5			
		PCL	0.5			
	Flow rate ml/min		1	2		
AP	Non-solvent pH	Water pH	5.43			
		Buffer pH	9.3	8.3	7.3	2.9
	SA	PVA-LMW (%w/v)	0.1	0.2	0.4	
		PVA-HMW (%w/v)	0.1			
	GFX (mg)		10	20	30	40
	Surfactant	Poloxamer®188 (%w/v)	0.35			

*Abbreviations indicate: OP; Organic phase, AP; Aqueous phase, PLA; Poly lactic acid, PCL; Polycaprolactone, SA; Stabilizing agent, PVA-LMW; Poly vinyl alcohol with low molecular weight, PVA-HMW; Poly vinyl alcohol with high molecular weight, GFX; Active ingredient Gatifloxacin.

2.2.1.2 Optimization of stabilizing agent molecular weight and quantity

The stabilizing agent used in all formulas was poly vinyl alcohol (PVA) with two molecular weights; Low Mol. wt. (LMW) 31000 g/mole and High Mol. wt. (HMW) 89000-98000 g/mole, with different amounts for selected one (0.1% - 0.2% - 0.4% w/v% of aqueous phase). These two parameters are studied to evaluate their effects on mean size, PdI, zeta potential values of prepared nanoparticles and by observing the absence or presence of aggregates for best stability.

2.2.1.3 Optimization of organic phase

Two types of organic phase as polymer solvent were tested: acetone and absolute ethanol. The criteria for selecting the best solvent are high solubility of the polymer in the solvent and ease of evaporation and removal.

2.2.1.4 Determination of organic to aqueous phase volume ratio effect

Changing the organic to aqueous phase ratio was studied as follows: (3:5, 2:5 and 1:5). All other constituents of the formulation were unchanged. This parameter is studied to evaluate its effect on particles mean size and PdI.

2.2.1.5 Determination of organic phase flow rate effect

The addition of organic phase into aqueous one during procedure was completed by using two kinds of syringes to apply two different flow rates; 5cc syringe (2ml/min) and 1cc syringe (1ml/min). This parameter's effect was evaluated by measuring the mean particles size and PdI.

2.2.1.6 Determination of aqueous phase pH effect

As long as Gatifloxacin solubility in aqueous phase is pH dependent, this parameter was studied with different pH values; distilled water with pH 5.43 and phosphate buffer with pH 2.9, 7.3, 8.3 and 9.3. All other constituents of the formulation were unchanged. The effect of pH value was evaluated by measuring the mean size, PdI, zeta potential, and the entrapment efficiency.

2.2.1.7 Determination of Gatifloxacin amount effect

Four amounts of Gatifloxacin were tested (10, 20, 30, and 40 mg), all other constituents were unchanged. The effect of Gatifloxacin quantity was evaluated by measuring the particles mean size, PdI, zeta potential, and the entrapment efficiency.

2.2.1.8 Determination of hydrophilic surfactant addition effect

Poloxamer® 188 was the surfactant which was tested with its recommended quantity 0.35% w/v of aqueous phase in the optimized formula. The effect of Poloxamer® 188 addition was evaluated by measuring the particles mean size, PdI, zeta potential, and the entrapment efficiency.

2.2.2 Evaluation of Gatifloxacin Nanoparticles

2.2.2.1 Particle mean size analysis and zeta-potential determination

The mean size (Z-average) of the GFX-loaded nanospheres and polydispersity index (PDI) was determined by dynamic light-scattering particle size analyser (DLS), using a Malvern Zetasizer Nanoseries (Nano-ZS, Malvern Instruments, Malvern, UK). The size distribution analysis was performed at a scattering angle of 173º and at temperature of 25◦C. Zeta potential (mV) values are measured with the same instrument with electrophoretic light scattering technique by calculating Smoluchowski's equation from electrophoretic mobility of nanoparticles. Nanoparticles samples were prepared by taking 1ml from nanosuspension, which was diluted into 10mL of double distilled water, and sonicated for 1min by sonicator (Wise clean-ultrasonic cleaner-wuc-ao6h, Korea). For each sample, the mean diameter/PDI/zeta potential ± standard deviation of three determinations were calculated.

2.2.2.2 Encapsulation Efficiency Determination

The amount of drug loaded into the nanoparticles was evaluated through direct way by calculating the amount of drug that was found inside the particles obtained and comparing it with the practical total amount used to prepare the nanoparticles (T).Total Gatifloxacin concentration (T) was determined after dissolution of 1 ml of nanoparticles suspension in 4 ml acetone. After dissolving the polymer, 4 ml of Hydrochloric acid (HCL) 0.1N was then added, and the volume was completed to 10 ml with mobile phase used for high performance

liquid chromatography (HPLC) analysis method, and the mixture was mixed carefully for 30 min by magnetic stirring and sonicated for 5min. thereafter the amount of drug in the water phase was detected applying HPLC analysis. Drug-loaded nanoparticles were separated from the aqueous medium containing non-associated Gatifloxacin by ultracentrifugation (CP 80WX Himac preparative ultracentrifuge, Hitachi, Japan). Samples were centrifuged at 45 000 rpm for 30 min at 4◦C, and loaded Gatifloxacin in the sediment was determined under the same condition of the total (T). The encapsulation efficiency of nanoparticles was determined according to Lakshmana Rao et al. [19].

HPLC separation was performed with Agilent Liquid Chromatographer (LC-1260 Infinity, Agilent, Germany) achieved on RP-C18 column (EC 150/4.5 Nucleodur 100-5 C18ec, Macherey-Nagel, Germany) with mobile phase consisting of acetonitrile and 0.05M phosphate buffer in the ratio of 25:75 v/v in isocratic mode was used. The HPLC system was operated at flow rate is 0.8ml/min and the detection wavelength is 293nm and the. All the measurement were performed at 25◦C. The encapsulation efficiency was determined and calculated as follows:

$$\textbf{Entrapment efficiency}\ (\%) = \frac{\textbf{Mass of drug in nanoparticles}}{\textbf{Mass of drug used in formulations}} \times \mathbf{100}$$

3. RESULTS AND DISCUSSION

3.1 Characterization of GFX-encapsulated nanoparticles; Particle Size and PDI

3.1.1 Optimization of polymer used

Based on literature data, the three most commonly used polymers in ophthalmic drug formulations are poly (alkyl cyanoacrylates), polycaprolactone, and poly (lactic acid) /poly (lactic-co-glycolic acid) [2]. Two kinds of polymers were tested in the optimization of unloaded nanospheres, poly lactic acid (PLA) and polycaprolactone (PCL) Fig.3. The maximum amount of the polymer used as recommended for this method [20], and was optimized for all formulas (0.5% w/v of organic phase).

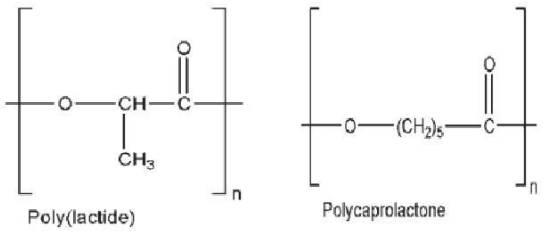

Fig.3. Chemical structure of used polymers PCL and PLA

The results are presented in Table 2. Obtained particles size was 136-146 nm and PdI of 0.109-0.154 for PLA and PCL respectively. It was clear that no significant differences were observed on particle size and PDI between both used polymers. Unloaded nanoparticles had a negative surface charge which can be attributed with the presence of end carboxyl groups of the polymer on nanoparticles surface [21]. Both used polymers are Aliphatic polyesters and have terminal carboxylic group which caused negative charge on the surface of nanospheres and so negative zeta potential values were obtained, with no significant differences also between both, -4.71 and -2.39 for PLA and PCL respectively.

Table 2: Effect of polymer type on the mean size of nanoparticles, PDI and zeta potential*

Formula	Polymer type	Mean size (nm) ± SD	PDI ± SD	Zeta potential ± SD
F1	PLA	136.7 ± 0.45	0.109 ± 0.003	-4.71 ± 0.19
F2	PCL	146.6 ± 1.442	0.154 ± 0.0179	-2.39 ± 0.1

*Data are the mean of three determinations ± SD.

With these results PCL was fixed in subsequent experiments because this polymer has a low commercial price that makes it a good candidate for large scale applications.

3.1.2 Optimization of stabilizing agent molecular weight and quantity

The stabilizing agent which used in all formulas is poly vinyl alcohol (PVA) with two molecular weights; Low Mol. wt. (LMW) 31000 g/mole and High Mol. wt. (HMW) 89000 g/mole, and with different amounts (0.1% - 0.2% and 0.4% w/v% of aqueous phase) for best stability. From the data obtained as presented in Table 3, it was very clear that the particle size is strongly influenced by both parameters, with size range of 146 and 233 nm and PdI of 0.154 and 0.278 for LMW PVA and HMW PVA respectively. After choosing LMW PVA due to its better results, three amounts 0.1% - 0.2% and 0.4% have been tested. The mean size for particles was 146, 243 and 302 nm respectively and size distribution became wider, where PdI reached to 0.246 with increasing particle size for highest amount of PVA. The results obtained here were found to be in accordance with a previously published study [8,22]. PVA (Fig.4.) is a swellable, hydrophilic macromolecule, although it is not required to ensure the formation of NP by nanoprecipitation, but the addition of PVA helps to preserve the nanoparticle suspensions from agglomeration over long storage periods. The particle size is strongly influenced with direct proportion by the PVA nature (molecular weight) and concentration [8], which could be due to high viscosity and interfacial tension of aqueous phase. It has been reported that PVA grades with high degrees of hydrolysis have low solubility in water. The solubility, viscosity, and surface tension of PVA depend on temperature, concentration, % hydrolysis and molecular weight of the material [22]. Therefore high molecular weight and high concentration of PVA led to larger particle size and wider size distribution. For this reason PVA with lower molecular weight was retained for the following experiments for best particles uniformity and greatest amount 0.4% (v/v) was selected for best stability.

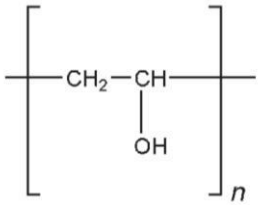

Fig.4. chemical structure of Polyvinyl Alcohol

Table 3: Effect of PVA molecular weight and quantity on the mean size of nanoparticles, PdI and zeta potential (Z.P.)*

Formula	PVA MW	PVA (%w/v)	Mean size (nm) ± SD	PdI ± SD	Z.P. ± SD
F3	High	0.1%	233.9 ± 0.737	0.278 ± 0.027	-8.14 ± 0.312
F2	Low	0.1%	146.6 ± 1.442	0.154 ± 0.0179	-2.39 ± 0.1
F5	Low	0.2%	243 ± 3.637	0.069 ± 0.008	-7.8 ± 0.142
F7	Low	0.4%	302.4 ± 1.504	0.246 ± 0.008	-14.7 ± 0.1

*Data are the mean of three determinations ± SD.

3.1.3 Optimization of organic phase

Acetone and absolute ethanol were examined as polymer solvent which both are miscible in water and easy to remove by evaporation, but absolute ethanol was not suitable for dissolving PCL polymer with such molecular weight (40,000) easily. Due to this reason, acetone was employed as organic phase for later preparations.

3.1.4 Determination of organic to aqueous phase volume ratio effect

We experimented three different Ratio of non-aqueous to aqueous phase (3:5 / 2:5 and 1:5) to obtain low particle size. The results are shown in Table 4 nanoparticles of average size of 362, 243 and 214 nm were obtained respectively. No significant variations were noticed between last two proportions, but mean particle size increased with increasing organic phase portion. Over that size of distribution also became wider, whereas polydispersity index PdI was 0.227, 0.069 and 0.099 respectively. As some researches pointed out, may changing the solvent/non-solvent volume ratio was not a determinant factor for nanoparticle formation and their final characteristics, provided that the final mixture itself did not become a solvent for the polymer [2]. But in our experiments higher proportion of organic phase led to increase particle size which is corresponding with other researches [21]. It could be attributed to an increase in the time required to evaporate the organic phase. No significant differences were noticed on zeta potential values by this parameter as long as the amount of used polymer and PVA were unchanged. From previous results we expected that 1:2 organic to aqueous phase ratio is the best proportion which induced smaller particle size and narrower size distribution and it was retained for the following experiments.

Table 4: Effect of organic to aqueous phase volume ratio on the mean size of nanoparticles, PdI and zeta potential*

Formula	OP:AP ratio**	Mean size (nm) ± SD	PdI ± SD	Zeta potential ± SD
F4	3:5	362.7±4.158	0.227±0.017	-13±0.854
F5	2:5	243±3.637	0.069±0.008	-7.8±0.142
F6	1:5	214±1.29	0.099±0.036	-11 ±0.854

*Data are the mean of three determinations ± SD.
** OP:AP ratio indicates organic to aqueous phase volume ratio.

3.1.5 Determination of organic phase flow rate effect

At organic phase dropping into aqueous one step, we changed used injector syringe from 5cc into 1cc, in order to reduce the flow rate form 2ml/min to 1ml/min. This led to significantly decreasing in particles size from 302 to 211nm and smaller size distribution from 0.246 to 0.084 as shown in Table 5. May these results due to changing the rate of particle formation stages which determine the particle size as reported in previous researches [20], a high nucleation rate and low growth rate is the key factor for smaller and uniform particles formation. As for zeta potential values it has been noticed from all former results that smaller particles lead to smaller numerical zeta potential value. The increase in nanoparticle size may possibly have influenced the surface charge of the PCL nanoparticles [21]. Smaller flow rate was fixed for later experiments for smaller particles size results.

Table 5: Effect of organic phase flow rate on the mean size of nanoparticles, PdI and zeta potential*

Formula	Flow rate	Mean size (nm) ± SD	PdI ± SD	Z.P. ± SD
F7	2 ml/min	302.4 ± 1.504	0.246 ± 0.008	-14.7 ± 0.1
F8	1 ml/min	211.4±0.2	0.084±0.032	-7.08±0.61

*Data are the mean of three determinations ± SD.

3.1.6 Determination aqueous phase pH effect

After getting the best size of the particles with narrow size distribution the optimized blank formula has been loaded with Gatifloxacin and Entrapment efficiency has been examined.The calibration of peak area versus Gatifloxacin concentration was linear in the concentration range of (0.001–0.08 mg/ml), and shows a correlation coefficient of 0.9995 (Fig. 5). Injections, in triplicate, were done at each concentration for standards and samples. The analytical method was validated as usually required.

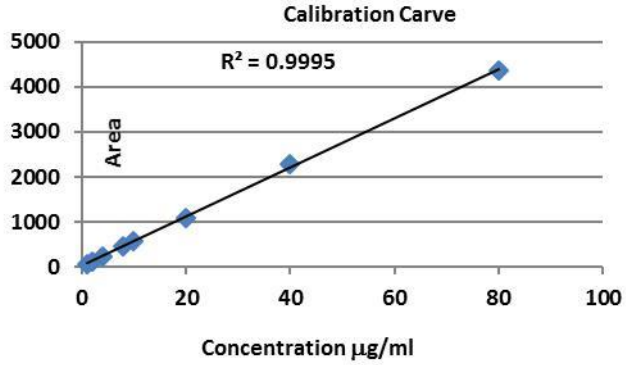

Fig. 5. Calibration carve of gatifloxacin standard solutions

Extra peaks in drug-loaded nanoparticles similar to pure drug spectra (Fig. 6a and 6b) showed the presence of drug in nanoparticles. The effect of pH aqueous phase of formulation (drug solvent) on the entrapment efficiency (EE %) besides other nanoparticles characterizations is represented in Table 6. Nanoparticles with a 1mg/ml loading of GFX were prepared as for previous study except that water pH in followed experiments replaced with phosphate buffer adjusted to different pH values of 2.9, 7.3, 8.3 and 9.3.

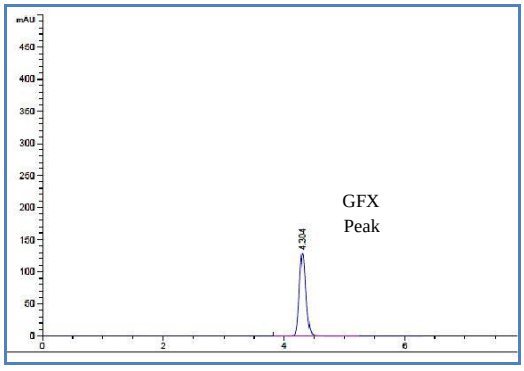

Fig. 6a: peak of standard solution of GFX

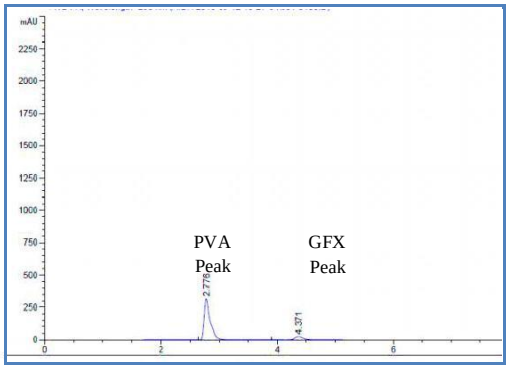

Fig. 6b: Peak of GFX loaded in nanoparticles

Because the lipophilicity of gatifloxacin is controversial [14], and its solubility is pH dependent with max aqueous solubility occurring in pH (2-5) [15], encapsulating efficiency of GFX has been reported to improve by adjusting pH of the aqueous phase by suppression of ionization and hence its solubility [14].

Obviously as shown in Fig. 7, there was an increase in drug encapsulation with increasing aqueous phase pH from 2.9 to 9.3 by 6.5 %, 12 %, 13.9 % and 23.5% respectively. It was therefore likely that Above GFX iso-electric point, the solubility of GFX and hence its hydrophobic nature increases as the pH of solution increases and this could enhance drug entrapment into nanoparticles [24]. The studies also illustrate that drug entrapment was profoundly higher for particles prepared in phosphate buffer pH 9.3 than those prepared in water pH 5.43 (Table 6).

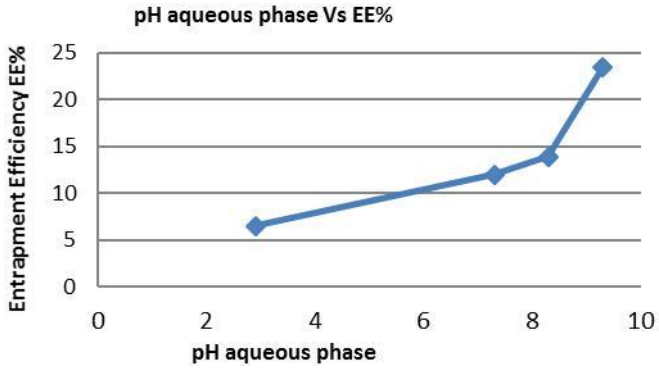

Fig.7. the correlation between pH aqueos phase and EE%

Whereas, the greater ionization degree of gatifloxacin in water pH 5.4 as compared to in phosphate buffer pH 9.3 at most probably contributed destabilization of particles hence decreasing Entrapment efficiency. The lower of GFX solubility at the higher pH of 9.3 makes it adsorbed and/or dispersed into the polymeric matrix system of PCL nanoparticles. It readily precipitated in aqueous medium and gets encapsulated by the PCL matrix preventing its diffusion in external phase. Some researches illustrated that drug adsorbed on the surface of particles and may have also contributed to an improved nanosuspension stability [25]. It was interesting to note as shown in Table 6 that Particles size was affected by increasing drug encapsulating efficiency displayed from 154nm to 185nm, may this due to the increasing of drug content of the nanoparticles. Gatifloxacin loaded nanoparticles have a higher surface charge (>-20) in comparison with blank formulas and this makes them discrete and prevents agglomeration. On the contrary, in case of drug absence, all formulas have lower surface charge (>-15) and may this make them aggregate. Extremely positive or negative zeta potential values cause larger repulsive forces, whereas repulsion between particles with similar electric charge prevents aggregation of the particles and thus ensures easy redispersion, so it is always favorable. High negative zeta potential values in all drug-loaded formulations may be due to same explanation that has been mentioned before. Fluoroquinolone molecules including GFX have wide range of electrostatic potential from negative to positive demonstrates the presence of dipole–dipole intermolecular interactions [14]. So may negative electrostatic potential on the surface of a drug molecule adsorbed into PCL matrix resulted to displayed zeta potential values. May this also explains the increase in surface charge with increasing the amount of drug that is loaded within particles. From all of the above the higher pH value 9.3 was fixed for followed formulas.

Table 6: Effect of aqueous phase pH on the mean size of nanoparticles, PdI and Z.P. and EE%*

Formula	pH	Mean size (nm) ± SD	PdI ± SD	Z.P. ± SD	EE%
F9	Water 5.43	154.8±1.79	0.138±0.018	-22.5±0.55	9.6%
F10	Buffer 9.3	185.9±2.574	0.058±0.017	-24.7±1.65	23.53%
F11	Buffer 8.3	187.8±1.153	0.073±0.01	-28.3±0.551	13.9%
F12	Buffer 7.3	169.7±0.757	0.122±0.032	-22.6±1.4	12%
F13	Buffer 2.9	188.8±1.153	0.236±0.005	-26.5±1.99	6.5%

*Data are the mean of three determinations ± SD

3.1.7 Determination of Gatifloxacin amount effect

In order to establish the maximum amount of drug that could be incorporated into nanoparticles at such conditions, the initial approach involved increasing the theoretical loading of Gatifloxacin in the formulation by 1, 2, 3 and 4 mg/ml and the particles obtained have been evaluated for its characterizations as before. The results are shown in Table 7.

Table 7: Effect of gatifloxacin amount on the mean size of nanoparticles, PdI and Z.P. and EE%*

Formula	GFX amount (mg)	Mean size (nm) ± SD	PdI ± SD	Z.P. ± SD	EE%
F10	10	185.9±2.574	0.058±0.017	-24.7±1.65	23.53%
F14	20	184.6±1.277	0.192±0.001	-30.1±2.78	33.5%
F15	30	182.8±1.493	0.198±0.009	-30.2±1.18	13.43%

*Data are the mean of three determinations ± SD.

It was clear that there were corresponding increasing in EE% from 23.5 to 33.5% for 1 and 2 mg/ml respectively; however the corresponding drug entrapment decreased to 13.4% for 3mg/ml (Fig. 8). Relatively, 4mg/ml was over saturated solution and the drug was not able to dissolve completely by this concentration, so it was excluded from application.

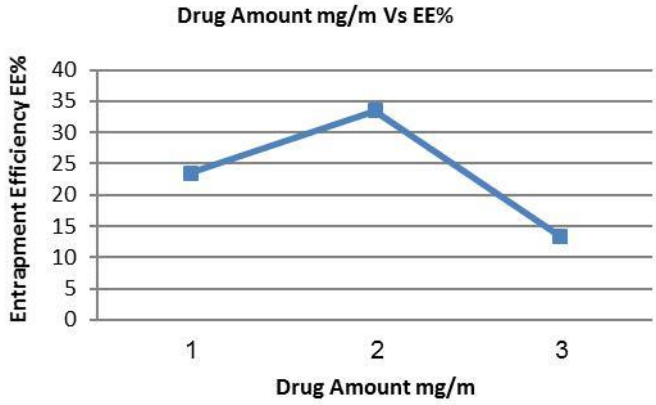

Fig.8. the correlation between drug amount mg/ml and EE%

There are limited physical interactions between the drug and the polymer matrix, then the majority of the drug will likely be localized on the surface of the particles due to the high ionic strength of the drug. In such an arrangement, the drug molecules on the particle surface can be easily washed away in aqueous solutions [26], thus resulting in decreasing entrapment efficiency with increasing the amount of loaded drug. In our experiments we didn't reach to high drug incorporation efficiency (Max 40%) may be this attributed to the water soluble nature of Gatifloxacin, this led to its rapid partitioning into the aqueous phase and hence decreased entrapment into the nanoparticles during polymer deposition.

This finding are corresponding with previous researchers which attributed the decreased drug entrapment with increasing theoretical drug loadings to an enhanced drug leakage into the aqueous phase at high loadings [21], which may also apply for our study. Another reason for the decreasing drug entrapment with increasing theoretical drug loadings could be the decrease in nanoparticle recovery which may also lead to an enhanced drug loss.

3.1.8 Determination of hydrophilic surfactant addition effect

Drug encapsulating efficiency was significantly affected by addition of non-ionic surfactant poloxamer188 with amount 3.5% w/v of aqueous phase with higher EE% 40% in our work, which may be due to decreased partitioning of GFX into the outer aqueous phase and better dispersion obtained by adding a hydrophilic surfactant. Also as shown in Table 8, Poloxamer188 addition significantly has influence on the mean particle size of the nanoparticles [21]. The best Zeta Potential values were observed for F16 with -32 value, and may this as mentioned before caused by increased drug adsorption and/or dispersion into PCL nanospheres. For all the above results, presumably F16 seems to be our optimized formula for appropriate mean size nanospheres and best PdI, zeta potential and entrapment efficiency values.

Table 8: Effect of poloxamer®407 addition on the mean size of nanoparticles, PDI and Z.P. and EE%*

Formula	Poloxamer®407 amount (%w/v)	Mean size (nm) ± SD	PDI ± SD	Z.P. ± SD	EE%
F16	3.5%	207.8 ± 0.115	0.071 ± 0.018	-32.5 ± 4.4	40.1%

*Data are the mean of three determinations ± SD

From all previous experiments, we can summarize optimal parameters for best nanoparticles characterizations which are suitable for ocular drug delivery Table 9.

Table 9: Optimizedparameters for best nanoparticles characterizations

Parameters			Value
Organic phase OP	Solvent (OP:AP ratio)	Acetone	1:2
	Polymer (%w/v)	PCL	0.5
	Flow rate ml/min		1
Aqueous phase AP	Non-solvent pH	Buffer pH	9.3
	Stabilizing agent	PVA-LMW (%w/v)	0.4
	Gatifloxacin (mg)		20
	Surfactant	Poloxamer®188 (%w/v)	0.35

4. CONCLUSION

In our current work, we have prepared PCL nanoparticles of Gatifloxacin using nanoprecipitation technique. Hence, Nanoprecipitation is a simple, fast and reproducible method which is widely used for the preparation of both nanospheres and nanocapsules and its superior advantage is obtaining small particles size (about 200nm) and narrow size distribution (lower PDI values). The optimized Gatifloxacin loaded PCL nanoparticles formulations (F14 and F16) were in nano size range (<210nm) with lower PDI (<0.2), high zeta potential value (-30 to -32 mV) and adequate encapsulating efficiency exhibiting a homogenous, stable and effective nanosuspension. Gatifloxacin loaded nanoparticles (DNPs) prepared could fulfill the criteria for both bioavailability and sustained dose maintenance for longer period when compared with conventional dosage forms, and may have potential topical ocular application, which is in favour of sustaining drainage of drugs from conjunctiva sac of the eye.

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