Journal of Advanced Pharmacy Research

Section C: Drug Design, Delivery & Targeting

# Formulation and Evaluation of Baclofen Polymeric Nanoparticles for Transdermal Delivery *In-vitro* and *Ex-vivo* Optimization

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***Submitted on: 23-03-2021; Revised on: 12-04-2021; Accepted on: 16-04-2021***

**To cite this article:** Yussef, A. M.; Fayez, S, M.; Sakran, W. Formulation and Evaluation of Baclofen Polymeric Nanoparticles for Transdermal Delivery *In -vitro* and *Ex-vivo* Optimization. *J. Adv. Pharm. Res.* **2021**, *5* (2), 248-259. DOI: 10.21608/aprh.2021.68835.1125

## ABSTRACT

**Objectives**: Baclofen is a skeletal muscle relaxant with an anti-inflammatory effect. The current market products of baclofen are oral tablets and intrathecal injection which cause many undesirable systemic side effects. This study aimed to formulate topical formula of baclofen to decrease systemic side effects. Topical drug delivery systems formulated as nanoparticles (NPs) enhanced the low drug release and the low bioavailability of the traditional gels. This occurs by prolonging the contact time and increasing the permeability of the drug through the skin. **Methods**: In this study, formulae of the baclofen-loaded Eudragit® RL100 (ERL) NPs were prepared by nanoprecipitation method. Polyvinyl alcohol (PVA) was added as a stabilizer. The NPs were characterized by measuring their particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency percent (EE %) values. Their spherical morphology was confirmed using transmission electron microscopy (TEM). Viscosity was also measured. In vitro release and permeation studies were done to evaluate the release of the drug from the NPs gel and the permeability of the drug through the skin. The results were compared with formulated baclofen traditional gel. **Results:** ERL concentration and organic phase ratio were the main factors affecting the NPs formulation. A decrease in the particle size was observed with an increase in ERL% while the smallest particle size was observed with formulae containing organic phase (acetone: methanol) in the ratio of 1: 3. The highest EE% was observed with the highest ERL concentration and the same organic phase ratio (acetone: methanol 1:3). Formulae B3, B6, B9 were selected for their most promising results. Their particle size was 187.4 ±2.81, 126.3 ±1.47, 120.0 ±1.06 nm and their EE% was 78.9 ±0.30, 83.3 ±0.26, and 86.6 ±1.12, respectively. The percentages of the drug released from the selected formulae as well as the percentage of the permeated drug were significantly higher than that of the baclofen traditional gel. **Conclusion**: ERL NPs were capable of releasing baclofen and improving its permeability through the skin so it may be considered as a suitable promising alternative drug delivery system.

***Keywords:*** *Baclofen; Eudragit® RL100; Nanoparticles; Topical; Nanoprecipitation.*

## INTRODUCTION

Skeletal muscle relaxants are used to reduce spasms and relieve pain that impairs the function of daily living activities. They are often used in case of overuse stretch, vigorous exercise, lower back or neck pain, fibromyalgia, osteoarthritis, spinal spondylosis, spinal injuries, myofascial pain, and multiple sclerosis[1]. Oral therapy of skeletal muscle relaxants is effective but may cause several adverse effects such as gastric upset, sedation, fatigue, drowsiness, and headache in addition to withdrawal symptoms[2]. Baclofen is one of the oral skeletal muscle relaxants which has a narrow therapeutic index and a short biological half-life; roughly 2–4 hours[3]; in addition to the above-mentioned side effects. All these obstacles encourage us to search for an alternative dosage form for baclofen. From this point of view, designing a new topical formula containing ERL as a polymer for NPs formulation is considered to be a good solution. Our work aimed to formulate it as a transdermal drug delivery system (TDDS) which has many advantages over conventional modes of drug administration. It avoids hepatic first-pass metabolism, enhances the bioavailability, maintains a steady plasma profile for an extended period, decreases dose frequency, improves patient compliance, enhances therapeutic efficiency, and decreases the systemic side effects produced by the oral route of skeletal muscle relaxants[4]. Baclofen is a gamma-amino-butyric acid derivative, it's chemical structure is shown in **Figure 1**. Baclofen molecular weight is 213 g/mol which is appropriate for the transdermal delivery [5]. Baclofen boiling point is 364 C° and melting point is 372 to 376 °C [6]. Baclofen acts as a GABA agonist at GABA-B receptors in the brain and spinal cord, resulting in hyperpolarization of neurons expressing this receptor, most likely due to increased potassium ion conductance. It may also reduce pain in patients by inhibiting the release of substance P in the spinal cord, which makes it especially useful in treating muscle spasticity associated with spinal cord injury. In this study, we used ERL to formulate NPs to enhance the penetration of baclofen through the skin layers and overcoming the physiological barriers to deliver the drug to the site of action. Polymeric NPs are a good drug delivery system for water-insoluble drugs as they increase their aqueous solubility. ERL is a copolymer of ethyl acrylate, methyl methacrylate, and low content of methacrylic acid ester with quaternary ammonium groups. Although it is insoluble at physiological pH, its swelling properties make it a good media for dispersing the drug[7]. Eudragit polymers are non-toxic and show low chemical reactivity which makes them widely used as drug carriers. Their positive charges allow longer residence time on the skin surface[8]. Nanoprecipitation method was used as it is an easy and non-complex technique. PVA was used as a stabilizer to avoid aggregation and to provide stability to the NPs during the nanoprecipitation technique[9]. All formulae were characterized for their particle size, PDI, zeta potential, and EE %. The most promising formulae were subjected to morphology studies, viscosity, release, and ex-vivo permeation studies, also, to test its stability after storage for 6 and 12 months.

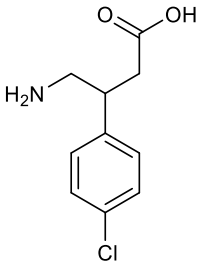


**Figure 1. Baclofen chemical structure [4-Amino-3-(4-chlorophenyl) butyric acid][5]**

## MATERIALS AND METHODS

### Materials

Baclofen was kindly supplied as a gift from Al Delta pharmaceutical company, Eudragit® RL100 was received as a gift sample from Evonik Germany, potassium dihydrogen phosphate, Sodium hydroxide, Acetone, Ethanol, and Methanol analytical grade was purchased from Adwic, El-Nasr Pharmaceutical Chemical Company, Egypt and polyvinyl alcohol and Hydroxy Propyl Methyl Cellulose from Alpha chemika India.

### Preformulation studies

#### *Baclofen Calibration curve*

A standard solution of baclofen in Phosphate Buffer (pH 7.4) was prepared and scanned using a UV-visible spectrophotometer (Shimadzu Tokyo, model 1800, Japan) to determine $\lambda_{max}$. Serial dilutions were prepared by transferring aliquots from the standard solution to obtain different concentrations from 100 to 1000 µg/ml. The absorbance of these concentrations was measured at the determined $\lambda_{max.}$ The standard calibration curve was made by plotting the mean absorbance of each sample against the concentration to obtain a straight line[10].

#### *Baclofen solubility study*

In 10 ml stoppered volumetric conical flasks, an excess amount of the drug was added to distilled water, phosphate buffer (ph7.4), Methanol, Ethanol, Acetone, 1% Acetic acid, and 0.1 M NaOH. The flasks were shaken mechanically for 72 hours at 37 ± 0.5 C° and

**Table 1. Formulation variables used in the preparation of baclofen-loaded ERL NPs**

| Formula code | Baclofen % | PVA% | ERL% | Methanol: Acetone Ratio |
|---|---|---|---|---|
| B1 | 1 | 1 | 2 | 1:1 |
| B2 | 1 | 1 | 2 | 1:3 |
| B3 | 1 | 1 | 2 | 3:1 |
| B4 | 1 | 1 | 4 | 1:1 |
| B5 | 1 | 1 | 4 | 1:3 |
| B6 | 1 | 1 | 4 | 3:1 |
| B7 | 1 | 1 | 6 | 1:1 |
| B8 | 1 | 1 | 6 | 1:3 |
| B9 | 1 | 1 | 6 | 3:1 |

speed 50 rpm. The solutions were filtered using (0.22 µm pore size filter paper). Absorbance was measured at 274 nm and the concentration of each solvent was determined[11].

**Preparation of baclofen-loaded ERL NPs**

The NPs were prepared by nanoprecipitation method developed by Fessi et al. The polymer selected and the drug must be insoluble in the aqueous phase[12]. Different concentrations of ERL were dissolved in a mixture of acetone and methanol with different ratios by sonication. The drug was dissolved in 0.1M NaOH by stirring at 60 C° using a magnetic stirrer (AccuPlate™ Analog hot plate stirrer Labnet international .Inc.). The drug solution was added to the organic one, then the formed organic phase mixture was added slowly using a syringe to an aqueous phase containing 1% PVA while stirring at 850 rpm[13]. PVA acts as a hydrophilic surfactant. The NPs were formed immediately by precipitating ERL carrying the drug particles. A milky solution was formed. The solution was stirred at 850 rpm at 50 C° for 3 hours to allow the evaporation of the organic solvents. Blank NPs were prepared by the same technique. Different variables applied in these formulae are presented in **Table 1.**

**Physicochemical characterization of the NPs**

***Particle size, polydispersity index, and zeta potential measurement***

Particle size, PDI, and zeta potential of all the baclofen-loaded ERL NPs formulae were measured using Malvern Zetasizer model ZEN3600 using dynamic light scattering. The PDI values reflect the uniformity and the distribution of the NPs. Zeta potential was measured by the electrophoretic light scattering method. All the measurements were carried out using diluted samples with filtered water at 25 ±0.5 C° and mean values were calculated from three consecutive measurements. Studying the impact of the drug either on particle size or zeta potential was done.

**Determination of the drug entrapment efficiency percent**

The NPs EE% corresponds to the amount of drug that can be incorporated into the NPs. It was indirectly determined by measuring the concentration of the free drug in the aqueous phase of the NPs dispersion using the centrifugation method. Three ml of the suspension was added to the centrifugal tube and the NPs were separated from the free drug by centrifugation at 5000 rpm for 30 minutes[14]. The clear supernatant containing the free drug was analyzed using UV spectrophotometer at $\lambda_{max}$ 274nm and the drug concentration was measured using the drug calibration curve. The EE% was calculated using the following equation.

$$EE\% = \frac{W\ initial\ drug - W\ free\ drug\ in\ supernatant}{W\ initial\ drug} \times 100$$

[Eq.1]

**Morphology of baclofen-loaded ERL NPs**

The morphology of the NPs of the selected formulae B3, B6, and B9 was examined using a transmission electron microscope (TEM) JEOL, JEM-2100, and Japan. One drop of the diluted sample was stained with 2% (W/V) phosphotungestic acid, placed on copper micro grids and left to dry at room temperature then it was viewed under the microscope lens.

**Preparation of the gel**

***NPs gel formulation***

The gel was formed using hydroxyl propyl methylcellulose (HPMC) by the hot water dissolution method. One-third of the nanosuspension was heated to 70 Cº, then HPMC was added to the heated suspension with stirring at 1200 rpm. The suspension was stirred until all HPMC particles were evenly dispersed. The rest of the nanosuspension was then added with continuous stirring to cool down the dispersion. As the dispersion temperature decreased the particles of HPMC became

hydrated and completely dissolved[15]. The gel was stored in the refrigerator for 24 hours to remove the bubbles and allow swelling of the gelling agent.

***Baclofen traditional gel formulation***

Baclofen was dissolved in phosphate buffer (pH 7.4) using a magnetic stirrer at 1200 rpm and 60 C° then 1% HPMC was added to the solution with the same method mentioned in NPs gel formulation.

**Viscosity measurement**

The viscosity of the selected NPs gel formulae B3, B6, B9, and baclofen gel ($B_0$)was measured to analyze the effect of NPs formulation and different polymer concentrations on the viscosity. The measurement was carried out by Brookfield RVDVE230 Medium-range viscometer. The spindle was immersed in the gel and the viscometer measured the additional torque required for the spindle to overcome viscous resistance and regain constant speed. The spindle was adjusted at 4 rpm speed and the study was carried out at 25 ± 0.5 C°.

***In-vitro* drug release study**

The drug release of the selected formulae B3, B6, B9, and the baclofen gel formula ($B_0$) was studied using USP dissolution apparatus (Distek dissolution system 2500i) using the membrane diffusion method. One and a half gm of the gel (equivalent to 15 mg baclofen) was placed in the Dialysis tubing membrane (Spectra Por®, MWCO 12–14 kDa, Sigma–Aldrich, USA) and tied at both sides then it was attached to the rotating paddle. The study was carried out at temp. 37±1 Cº and the rotating paddle was rotated at 100 rpm speed[9]. The dissolution medium contained 200ml phosphate buffer (pH 7.4). Samples of 5 ml of the phosphate buffer were withdrawn every 15 min time interval for 3 hours and replaced with fresh buffer solution. The withdrawn sample was analyzed using a UV spectrophotometer at $\lambda_{max}$ 274nm to measure the concentration of baclofen released using its calibration curve. The release study was carried out in triplicates and the average percentages were plotted against time. For a better analysis of the release data, release efficiency (RE), release rate (RR), and $t_{50\%}$ (release time for 50% fraction of the drug) were calculated. RE is calculated according to the following equation.

$$RE\% = \frac{\int_0^t y.dt}{y_{100}.t} \times 100 \quad \text{[Eq.2]}$$

Where y is the amount of drug dissolved at time t. The release efficiency indicates the rate and extent of drug release[14].

**Kinetics of drug release**

The data from the drug release study was fitted to different kinetic models to be analyzed. Zero-order kinetic model as the cumulative amount of the drug was plotted versus time (Eq. 3), first-order model where log cumulative of drug remaining was plotted versus time (Eq. 4), highuchi′s model where cumulative percentage of drug released was plotted versus square root of time (Eq. 5) and krosmeyer peppas model where cumulative amount of drug was plotted versus time (Eq. 6)[16]. The reaction constant (K) and regression constant ($r^2$) were also calculated for all models.

$Q = k_0 t$ [Eq.3]

$\log Q = \log Q0 - K1t/2.303$ [Eq.2]

$Q = K_H\sqrt{t}$ [Eq.5]

$Mt/M\infty = K.t^n$ [Eq.6]

**Ex-vivo drug permeation study**

The skin permeation studies are known for their values for studying the rate and mechanism of percutaneous absorption of drugs. Also, they are predictive of the in vivo performance of the prepared formulae. The permeation of the drug through excised rabbit skin membrane was studied for the selected formulae and compared to the baclofen gel. Approval for the study on rabbit skin was obtained from the animal ethics committee, Faculty of Pharmacy, Helwan University (Ref, 0014A—16). The dorsal side of the rabbits was shaved using an electric clipper to avoid any cuts of the skin. Rabbits were sacrificed and the processed skin was separated. The fatty acids and connective tissue layers were removed by rubbing with alcohol swap. The cleaned skin was washed with distilled water followed by phosphate buffer (pH 7.4) and cut into 2.5 cm diameter circular patches then it was stored in freezer at -20 C° till the time of the study. The permeation study was performed using the dissolution apparatus. The rabbit skin was fitted to a double open-ended glass test tube and the test tube was attached to the shaft of the rotating paddle. The study was carried out at 37±1 Cº and the rotating paddle was rotated at 100 rpm speed. The dissolution medium was 200 ml phosphate buffer (pH 7.4). One and a half gm of the gel (equivalent to 15 mg baclofen) was applied on the surface of the skin to the stratum cornea and the dermal side facing the dissolution medium. Five ml samples were withdrawn and replaced with fresh phosphate buffer every 15 minutes time interval for 3 hours. The withdrawn sample was analyzed using the UV spectrophotometer at $\lambda_{max}$ 274 nm to measure the concentration of baclofen permeated using its calibration curve. The cumulative amount of drug permeated per unit area (µg/cm$^2$) was plotted against time (min). The permeation rate parameters; flux (Jmax), permeation constant (Kp), and the enhancement ratio (ER) were calculated from the following equations[17,18]:

$$\text{Jmax} = \frac{Amount\ of\ permeated\ drug}{\text{Time}\times\text{Area of membrane}} \quad \text{[Eq7]}$$

$$Kp = \frac{\text{J max}}{Initial\ drug\ concentration} \quad \text{[Eq.8]}$$

$$Er = \frac{\text{J max}\ of\ formula}{Jmax\ of\ control} \quad \text{[Eq.9]}$$

Permeation efficiency (PE) and t50% were also calculated. The data from the permeation study was fitted to different kinetic models to find the best fitting model according to the $r^2$ value.

**Stability study**

The selected formula was kept in air-tight glass vials and stored at temperatures ranging from 2-5 C° for 6 and 12 months. The samples were analyzed for their particle size and zeta potential to assess the physical stability of the prepared NPs. The samples were visually inspected for their stability[12].

**Statistical analysis**

All data were presented as mean ± standard deviation (n=3). They were analyzed by one-way ANOVA followed by Tukey Kramer's post-test using Instat 3 software. If the P-value was less than or equal to 0.05, the difference between means was considered significant.

## RESULTS AND DISCUSSION

**Pre-formulation studies**

***Baclofen calibration curve***

The UV scanning of baclofen in phosphate buffer (PB) pH 7.4 revealed that the wavelength of maximum absorbance (λmax) was found to be 274 nm at pH 7.4 as shown in **Figure 2**.The calibration curve was constructed by plotting absorbance versus the concentration of baclofen. A linear relationship obeying Beer Lambert's law was obtained within the used concentrations with $r^2$ value of 0.9997 as shown in **Figure 3.**

***Baclofen solubility study***

Baclofen is slightly soluble in water and insoluble in organic solvents (methanol, ethanol, and acetone). It shows high solubility in dilute acids and alkalis (1%aceticacid and 0.1 M NaOH) as shown in **Table 2**.

**Preparation of baclofen-loaded ERL NPs**

The nanoprecipitation method is the most suitable technique for preparing NPs containing water-insoluble drugs such as baclofen. It is a safe method as it avoids the use of toxic solvents and surfactants. This method depends on adding water-miscible solvents like acetone and methanol which diffuse rapidly in the water giving a chance for ERL to precipitate as NPs carrying the drug particles[9,19]. The addition of PVA is important for the stabilization of the NPs and the prevention of their agglomeration by reducing the surface tension between water and solvents[20]. By applying this technique, the NPs of ERL loading Baclofen were successfully formed.

**Physicochemical characterization of the NPs**

***Particle size, polydispersity index, and zeta potential measurements***

The particle size and size distribution are important parameters for the uniformity and accurate administration of the formula. The size of NPs was affected by two factors; polymer concentration and organic phase composition. The particle size of all formulae ranged from 120 nm to 319 nm. It decreased proportionally with increasing ERL concentration[14]. Formulae (B7, B8 & B9) containing the highest ERL concentration (6%) showed the lowest particle size. The composition of the organic phase also affected the particle size as the solubility of the drug changed according to the type and concentrations of organic solvents used. Formulae containing methanol and acetone with ratio (3:1) showed the smallest particle size while formulae containing methanol and acetone with ratio (1:3) showed the largest particle size with the same ERL concentration. This may be attributed to the solubility of baclofen which is higher in methanol than in acetone as shown in **Table 2**. All the samples had good PDI values ranged from 0.268 to 0.512 which reflected the uniformity of NPs and good size distribution, with no NPs aggregates. PDI values higher than 0.7 indicate heterogeneous distribution[21]. Zeta potential is an important indicator for the stability of the suspension, as the zeta potential value increases, the stability increases due to the strong electrostatic repulsion between particles which prevents aggregation and allows good separation between the particles and from surfaces[22]. The samples had high zeta potential values ranging from +30.4 mV to +45.2 mV. This indicated the stability of the formulae as it is reported that zeta potential values of 30 mV represents full electrostatic stability.[23] The positive charges in the medium were due to the unbounded amino group ERL[24]. By comparing the particle size of formulae B1, B4, and B7 with their plain formulae, we found that the drug had an impact on increasing the size of NPs. The plain formulae particle sizes were 84.79 nm, 85.39 nm, and 102.2 nm respectively. By comparing zeta potential, we found that all formulae either plain or medicated had the same positive charges and this indicated that the drug didn't affect the native charges of the polymer. All the values of particle size, PDI, and zeta potential are illustrated in **Table 3**.

**Table 2. Baclofen solubility study data**

| Solvent | Solubility mg/ml (n=3 ±SD) |
|---|---|
| 0.1M NaOH | 42.7 ±0.32 |
| 1% Acetic acid | 38.6 ±0.91 |
| Phosphate buffer 7.4 | 14.8 ±0.90 |
| Water | 5.4 ±0.60 |
| Ethanol | 4.5 ±0.25 |
| Methanol | 3.7 ±0.59 |
| Acetone | 1.3 ±1.04 |

**Table 3. Particle size, PDI, zeta potential, and EE% measurements of Baclofen-ERL NPs**

| Formula code | *Particle Size (nm) | *PDI | *Zeta potential (mV) | *EE% |
|---|---|---|---|---|
| B1 | 211.3±9.16 | 0.268±0.01 | +35.3±1.78 | 78.8±0.66 |
| B2 | 319.9±2.29 | 0.512±0.12 | +45.2±5.59 | 72.9±3.11 |
| B3 | 187.4±2.81 | 0.408±0.02 | +34.7±2.54 | 78.9±0.30 |
| B4 | 142.2±2.72 | 0.464±0.03 | +30.4±1.57 | 83.3±1.27 |
| B5 | 281.0±9.54 | 0.418±0.05 | +38.5±0.77 | 81.8±0.60 |
| B6 | 126.3±1.47 | 0.239±0.002 | +33.0±1.179 | 83.3±0.26 |
| B7 | 124.0±0.50 | 0.273±0.005 | +40.4±0.46 | 85.5±0.56 |
| B8 | 130.0±1.65 | 0.421±0.02 | +39.0±0.37 | 83.1±0.10 |
| B9 | 120.0±1.06 | 0.280±0.007 | +37.9±1.61 | 86.6±1.12 |

**Each value represents the mean ± SD (n=3)*

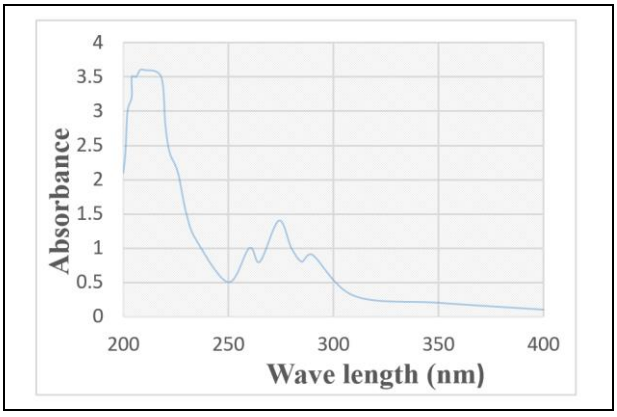


**Figure 2. Baclofen UV spectrum.**

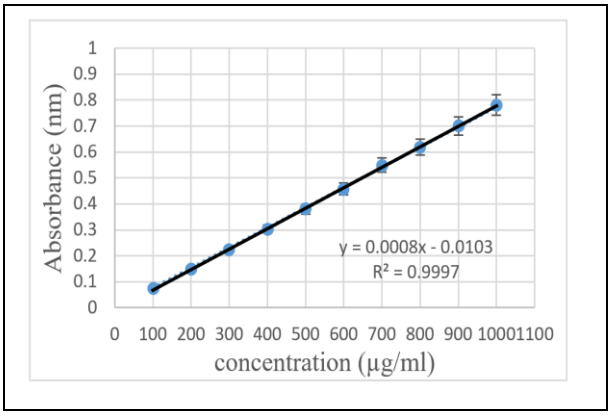


**Figure 3. Baclofen calibration curve in phosphate buffer (pH7.4) using UV spectrophotometer at $\lambda_{max}$ 274nm (n=3 ±SD)**

**Determination of the drug entrapment efficiency percent**

All formulae showed high EE% ranged from 72.9% to 86.6% as shown in **Table 3**. ERL has been stated to have a high ability to entrap poorly water-soluble drugs due to the slow diffusion of the drug to the aqueous phase[24]. The EE% of the drug was affected by both polymer concentration and organic phase

composition. Increasing ERL concentration leads to an increase in the viscosity of the organic phase and so decreases the movement of drug molecules from the organic phase to the aqueous phase[23]. A slight increase in the EE% was observed with the ratio of Methanol: Acetone 3: 1 due to the higher boiling point of methanol (64.7 C°) than acetone (56 C°) which makes the evaporation of methanol takes longer time than acetone. Based on what stated before, the diffusion of the drug to the aqueous phase was decreased as long as the organic solvent was present in the medium as droplets and was increased as soon as the solvent was removed[12,25]. The diffusion of the drug to the aqueous phase decreased at a higher ratio of methanol causing an increase in its EE%.

**Selection of the best formulae**

All formulae showed a small particle size with suitable PDI and high EE%. Formulae (B3, B6 &B9) were selected for further evaluation, as they were composed of the most effective organic phase ratio (Methanol: acetone 3:1) with different ERL concentrations. Each formula was selected as it showed the smallest p. size and the highest EE% when compared with a group of formulae containing a similar amount of ERL {group I (B1, B2 &B3), group II (B4, B5 &B6), and group III (B7, B8 & B9). By selection of these three formulae, we could study the effect of increasing ERL concentration on the morphology of NPs, the viscosity of the gel formed, the release profile, and the ex-vivo permeation studies.

**Morphology of baclofen-loaded ERL NPs**

The morphology of the NPs was observed using TEM. The photographs as shown in **Figure 4** revealed that the NPs had spherical shapes without perforation or imperfections on the surface of the NPs. These uniform surface properties were helpful in the topical delivery of the drug. The mean particle size diameter measured by TEM was 61.46 ± 15.62 nm for B3, 63.58 ±14.26 nm for B6, and 60.91 ± 12.14 nm for B9. These mean diameters were smaller than those obtained by the zeta sizer (187.4, 126.3 &120 nm for B3, B6& B9, respectively). These difference may be due to that, zeta sizer measures the hydrodynamic diameters of NPs while TEM measures the size of dried particles [26]

**Preparation of the NPs gel**

Different percentages of the gelling agent HPMC were used to reach the desired viscosity, spreadability, and homogeneity of the gel. 0.75%w/w, 1%w/w and 1.25%w/w of HPMC were used. 0.75%w/w showed insufficient viscosity, 1%w/w and 1.25%w/w showed accepted viscosity and based on other factors such as the Spreadability and the visual aspects, 1%w/w HPMC was selected as the ideal percentage for the gel formulation.

**Viscosity measurement**

Viscosity measurement is an important parameter in gel formulation and application, as it influences the product's physical form, appearance, texture, and flow behavior. The viscosity measurement of the selected formulae B3, B6, B9, and baclofen gel was 26190 ±10, 35546 ±11.54, 42093 ±15.27, and 12240 ±20 c.P respectively. The presence of ERL polymer in the NPs gel had a remarkable effect on increasing the viscosity of the NPs gel compared to the baclofen gel. ERL has long molecular chains that enhance intermolecular attractions which contribute to the resistance to the flow and so increase viscosity[27]. An increase in the viscosity values was observed with the increase in ERL concentration[19].

**In-vitro drug release study**

The amount of drug released from the NPs through the dialysis tube to the release medium (phosphate buffer, pH 7.4) was measured by UV spectrophotometer at $\lambda_{max}$ 274nm. All the formulae showed uniform release of the drug without burst effect. This was explained as the unbounded drug adsorbed on the surface of the NPs did not release at first[16,9]. **Figure 5** illustrates the release profile of baclofen from the selected NPs formulae (B3, B6, and B9) and the baclofen gel formula ($B_0$). The release profile was constructed for up to 180 min. There was a significant difference ($P<0.001$) when the drug released from each of the selected formulae was compared with the drug released from the baclofen gel. The release from NPs formulae was much higher than that of the baclofen gel formula. This proved that formulating the drug in the form of polymeric NPs enhanced the release of the drug. A significant difference ($P<0.001$) was observed when comparing the percentage of drug released from the NPs formulae B3, B6 andB9 with each other. They released 93.56%, 96.3%, and 98 % of the drug, respectively. This could be explained as the quaternary ammonium groups were increased by increasing ERL concentration leading to an increase in the EE% of the drug which enhanced the release of the drug from the NPs. This result was in agreement with Ram A. et al. and Pignatello R. et al. [28,29]. In addition to increasing the permeability of the polymer to water and the porosity of the matrix due to increasing the content of quaternary ammonium group and so increasing the drug release[14]. For more precise comparison, evaluation of the release profile was not only based on the percentage of drug released. RE, RR, and t50% were also evaluated as shown in **Table 4**. RE is more accurate as it reflects both the rate and the extent of drug release. The slow release of the drug from the NPs is due to the strong ionic interaction between baclofen and ERL[30]. This explained why RE of formula B9 (6% ERL) was significantly ($P<0.001$) lower than both formulae B3 (2% ERL) and B6 (4%ERL).

**Table 4. Release data of baclofen from polymeric NPs and traditional gel**

| | B3 | B6 | B9 | Traditional gel ($B_0$) |
|---|---|---|---|---|
| ***Release efficiency%** | 66.7 ±0.10 | 66.5 ±0.35 | 59.6 ±0.30 | 29.46 ±0.32 |
| ***Release rate** | 0.93 ±0.01 | 0.71 ±0.03 | 0.72 ±0.01 | 0.29 ±0.03 |
| ***$t_{50\%}$** | 70.18 ±0.23 | 72.01 ±0.37 | 83.39 ±0.48 | 157.06 ±0.31 |
| ***% of Drug released** | 93.56 ±0.35 | 96.3 ±0.30 | 98.53 ±0.32 | 55.53 ±0.40 |

** Each value represents the mean ± SD (n=3)*

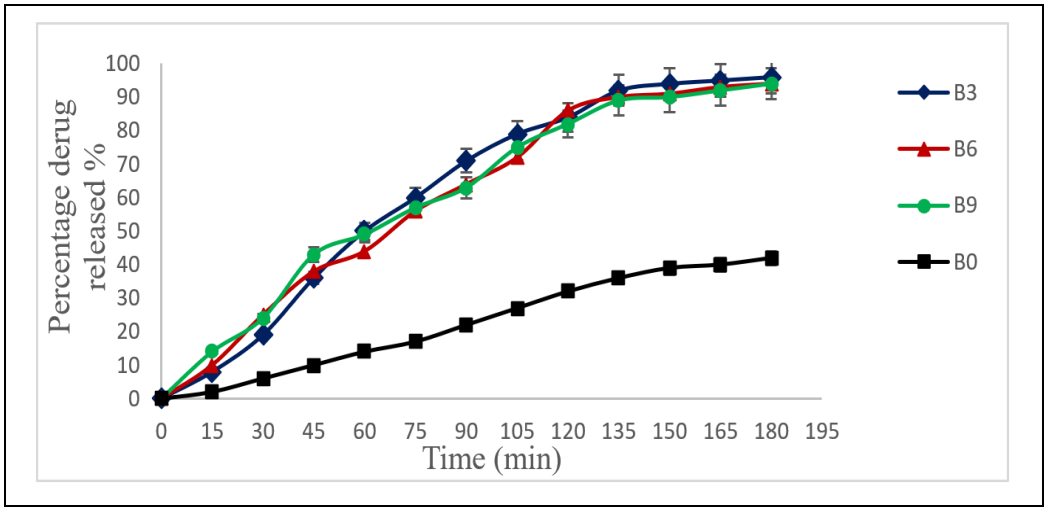


**Figure 5. Comparing release profile of baclofen from NPs with different concentration (B3, B6 and B9) with traditional gel formula.**

There was a significant difference (P<0.001) in RR between all the NPs formulae and the baclofen gel formula. Both, formulae B3 and B6 recorded a suitable t50%. The baclofen gel formula showed a slow rate of release and a very low RE% and this was attributed to the poor water solubility of the drug. It also recorded a very high t50%.

**Kinetics of drug release**

To determine accurately the model of drug release, the drug release data were applied into different kinetic models, namely, zero order, first order, and Higuchi diffusion-controlled mechanism. The release of baclofen from all the selected nanoparticles formulae obeyed Higuchi's model of matrix diffusion according to the highest linearity values ($R^2$) as shown in **Table 5**. This was confirmed by Fatma A. Abobakr et al. who stated that the diffusion mechanism of systems containing NPs followed three steps. The first step began with swelling of the matrix due to penetration of water inside the system followed by converting the NPs into a rubbery matrix. This was enhanced in our study by the presence of quaternary ammonium groups in ERL polymer as mentioned above in the release study. The third step enhanced the diffusion of the drug from the rubbery matrix[31]. Further analysis by krosmeyer peppas model was done to differentiate between Fickian and non-Fickian diffusion. The three formulae showed non-Fickian diffusion with n values greater than 0.45[19]. These confirmed that the drug released from the ERL NPs could be released by several phenomena [12]. Based on several previous studies, the release was enhanced by diffusion and dissolution phenomena[28,32]. The release of baclofen gel ($B_o$) obeyed zero-order kinetic model.

**Ex-vivo drug permeation study**

The amount of drug permeated through the excised skin to the release media phosphate buffer (pH 7.4) was measured by UV spectrophotometer at $\lambda_{max}$ 274nm. **Figure 6** illustrates the permeation profile of baclofen through the excised skin from the selected NPs formulae (B3, B6, and B9) and the baclofen gel formula

**Table 5. In-vitro drug release kinetics of baclofen from ERL NPs formulae and traditional baclofen gel**

| Formula code | Regression Coefficient ($r^2$) | | | | |
|---|---|---|---|---|---|
| | Zero Order | 1st Order | Higuchi's | Korsmeyer's peppas | |
| | | | | $r^2$ | N |
| **B3** | 0.9351 | 0.7858 | 0.9752 | 0.9662 | 1.231 |
| **B6** | 0.9722 | 0.8499 | 0.9769 | 0.9841 | 1.139 |
| **B9** | 0.9676 | 0.8495 | 0.9717 | 0.9785 | 1.501 |
| **Traditional gel ($B_0$)** | 0.9844 | 0.9721 | 0.9313 | 0.9782 | 0.986 |

**Table 6. Permeation data from the baclofen-loaded ERL NPs gel and traditional baclofen gel**

| | B3 | B6 | B9 | $B_0$ |
|---|---|---|---|---|
| ***Permeation efficiency%** | 66.5 ±0.53 | 64.6 ±0.61 | 65.2 ±0.44 | 33.6 ±0.10 |
| ***$t_{50\%}$ (min)** | 71.73 ±0.61 | 72.94 ±0.35 | 70.01 ±0.27 | 179.07 ±0.73 |
| ***% of Drug permeated** | 96.5 ±0.40 | 94.6 ±0.47 | 94.7 ±0.45 | 55.6 ±0.38 |
| ***flux (µg/cm$^2$/h)** | 90.83 ±0.34 | 89.11 ±0.44 | 89.2 ±0.43 | 39.98 ±0.48 |
| ***Permeability coefficient Kp (cm$^2$/h)** | 0.06 ±5×10$^{-4}$ | 0.059 ±3×10$^{-4}$ | 0.059 ±3×10$^{-4}$ | 0.026 ±3×10$^{-4}$ |
| **Enhancement ratio (ER)** | 2.29 | 2.26 | 2.24 | - |

**Each value represents the mean ± SD (n=3)*

**Table 7. Ex-vivo permeation data fitted to different kinetic models**

| Formula code | Regression Coefficient ($r^2$) | | | | |
|---|---|---|---|---|---|
| | Zero Order | 1st Order | Higuchi's | Korsmeyer's peppas | |
| | | | | $r^2$ | n |
| **B3** | 0.9753 | 0.821 | 0.9942 | 0.9949 | 1.289 |
| **B6** | 0.9899 | 0.8492 | 0.9903 | 0.9821 | 1.018 |
| **B9** | 0.9814 | 0.8662 | 0.9904 | 0.9792 | 0.872 |
| **Traditional gel ($B_0$)** | 0.9964 | 0.8783 | 0.964 | 0.9948 | 1.285 |

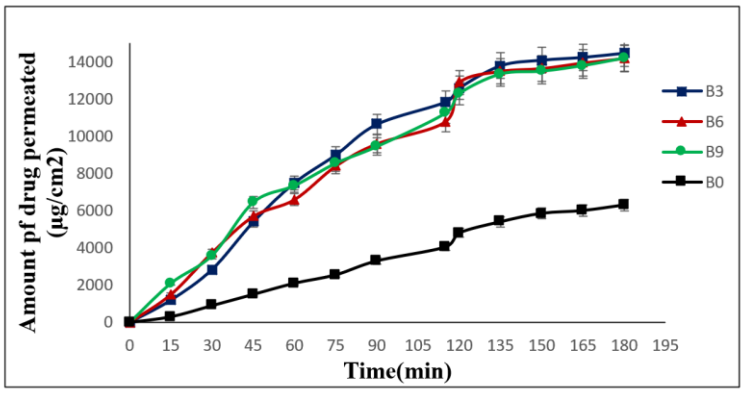


**Figure 6. Comparing permeation profile of baclofen from NPs with different concentration (B3, B6 &B9) with traditional gel formula ($B_0$) (n=3 ±SD)**

**Table 8. Particle size and zeta potential analysis at different time through stability study**

| Formula code | *Particles size (nm) | | | *Zeta potential (mv) | | |
|---|---|---|---|---|---|---|
| | 0 month | 6 month | 12 month | 0 month | 6 month | 12 month |
| **B3** | 187.4 ±2.81 | 191.7 ±1.82 | 204.7 ±1.311 | +34.7 ±2.54 | +15 ±0.15 | +9.17 ±0.59 |
| **B6** | 126.3 ±1.47 | 140 ±0.93 | 152.3 ±2.25 | +33.0 ±1.18 | +23.76 ±0.42 | +18.3 ±1.07 |
| **B9** | 120 ±1.06 | 136.9 ±1.68 | 158.3 ±1.99 | +37.9 ±1.62 | +16.03 ±0.45 | +14.8 ±1.23 |

**Each value represents the mean ± SD (n=3)*

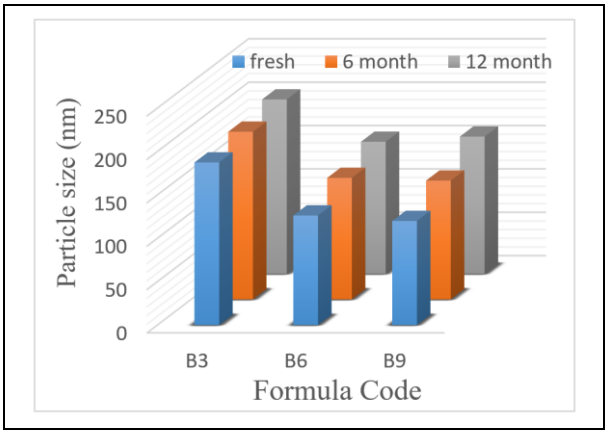


**Figure 7. Effect of storage on particle size of the selected NPs formulae after 6 and 12 months.**

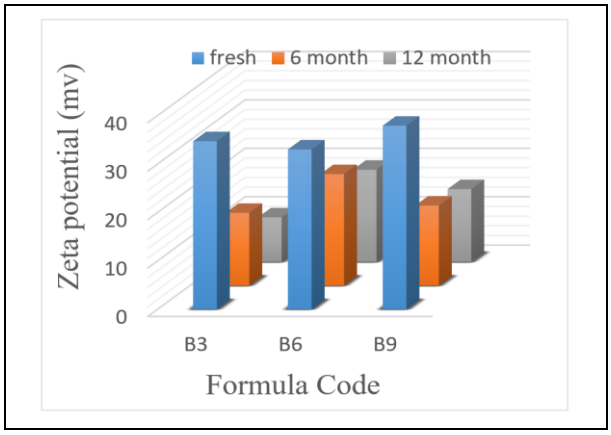


**Figure 8. Effect of storage on zeta potential values for the selected NPs formulae after 6 and 12 months.**

($B_0$). There was a significant difference ($P < 0.001$) in the amount of drug permeated from all the selected NPs formulae B3, B6, and B9 and the baclofen gel formula $B_0$. A non-significant difference ($P > 0.05$) was observed when comparing the amount of drug permeated from formulae B6 and B9. The amount of drug permeated from formula B3 was significantly higher than both formulae B6 and B9 ($P < 0.001$). This was explained as at high ERL concentration, the drug permeation decreased significantly due to the strong ionic binding of the drug to the polymer so the drug was retained in the formula after application which results in reduced partition into the skin[33]. For comparison, flux (Jmax), ER, and Kp were calculated and results are illustrated in **Table.6**. There was a significant difference in the flux ($P < 0.05$) of the selected formulae B3, B6, and B9 compared to the baclofen gel $B_0$. The increase in the flux expressed in terms of ER which was 2.2 indicating more than two times folds increase in the permeation of the baclofen gel. This enhancement in the drug permeation rate was referred to the small size of the NPs which offers several benefits such as; high solubility, high surface area, and high diffusion rate[34]. The permeation study data were fitted into different kinetic models and $r^2$ values were calculated, the results are shown in **Table 7**. The most fitted models were Hughie's and Korsmeyer's peppas and further analysis to calculate n values ($n > 0.45$) showed non -fickian diffusion. Baclofen traditional gel formula ($B_0$) obeyed zero-order kinetic model.

**Stability study**

The selected formulae were visually inspected every 6 months for any changes in color and consistency. No changes were observed in the visible properties. The size and stability of the NPs were analyzed by comparing their particle size and zeta potential measurements to the freshly prepared samples. The zeta potential values were still positive but decreased significantly ($p<0.001$) due to the development of counter charges in solution [16] while a significant increase ($p<0.001$) in the particle size of NPs was observed[35]. This decrease in the zeta potential explained the increase in particle size which was due to the agglomeration of NPs [32]. The results are illustrated in **Table 8** and **Figures 7 and 8**.

## CONCLUSION

Baclofen-loaded ERL NPs were successfully formulated using the nanoprecipitation method. The NPs were characterized by measuring their particle size, PDI,

zeta potential, and EE%. Formulae B3, B6, and B9 were selected for further evaluation, as they were composed of the most effective organic phase ratio (Methanol: acetone 3:1) with different ERL concentrations. The selection of this organic phase was based on the drug solubility which was higher in methanol than acetone. These formulae showed the smallest p. size and the highest EE% when compared to other groups of formulae containing a similar amount of ERL. These three formulae were selected to study the effect of increasing ERL concentration on the morphology of NPs, the viscosity of the gel formed, the release profile, and the ex-vivo permeation studies. All NPs showed a spherical uniform shape. Viscosity and release were increased by increasing ERL concentration while permeation was decreased. Viscosity was increased due to increasing the intermolecular attraction of ERL while the release was increased, as the quaternary ammonium groups were increased causing an increase in the EE% of the drug which enhanced the release of the drug from NPs. The amount of drug permeated from the NPs formulae decreased by increasing ERL concentration. This was attributed to the strong ionic binding of the drug to the polymer so the drug was retained in the formula after application which results in reducing partition into the skin. Conclusively, in-vitro and ex-vivo studies showed that the NPs formulae had a significantly higher percentage of drug release and permeation than the baclofen gel. The cationic character of ERL and the small size of the NPs were responsible for accelerating the release and enhancing the permeation of baclofen. The stability studies showed that the formulae were stable over the period of the study. This study suggests that the baclofen-loaded ERL NPs are stable and provide promising results for the transdermal delivery of baclofen and may be considered a suitable promising alternative drug delivery system for baclofen.

**Acknowledgment**

We are thankful to Al Delta Pharmaceutical Company for providing a free sample of Baclofen. We are very grateful to Evonik Germany for providing us with the free sample of Eudragit® RL100. Great thanks to the National Nutrition Institute (NNI) and Faculty of Pharmacy October 6th University for providing their laboratories and facilities to complete the research work.

**Conflict of interest**

The authors declare that there is no conflict of interest regarding the publication of this paper.

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