RESEARCH ARTICLE

Formulation and Evaluation of Montelukast Sodium Nanoparticles for Transdermal Delivery

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ABSTRACT

Montelukast sodium is a potent leukotriene receptor antagonist of important role in the pathogenesis of asthma routinely used as prophylaxis to reduce asthmatic attack, being highly labile molecules strongly influenced by the environmental factor, which confined its formulation as liquid dosage form which makes their administration to infants and elderly with swallowing difficulties are challenging, driven by these facts, the transdermal route has been suggested as a suitable alternative to the oral route.

Montelukast sodium nanoparticles were formulated by facile nanoprecipitation method using Eudragit L100 as polymer and PVA, PVPK30, and tween 80 as a stabilizer. The effect of polymer concentration, stabilizer concentration, and stabilizer type on the nanoparticles' physical properties such as particle size, zeta potential, entrapment efficiency, and in-vitro release have been investigated. Furthermore morphological, and compatibility study was conducted; additionally, photostability and ex vivo permeation study through abdominal rat skin was performed, and the effect of using solid polymeric microneedles as permeation enhancer on drug flux was assessed.

Results revealed the suitability of the nanoprecipitation method to prepare polymeric nanoparticles. The measured particle size ranged between 178.6 ± 3.3 and 1273 ± 7.54 with relatively high entrapment efficiency and spherical, regular shape. Among the prepared formulations, F2 achieved complete and sustained release extended for 5 hours; based on its small particle size and release pattern, it was selected for further evaluation. The compatibility study excludes any interaction between montelukast and other components. Furthermore, the reduced amount of the montelukast cis isomer is proof of photostability enhancement which can be attributed to the drug embedment in the polymer matrix; the permeation study showed a significant increase in the amount of drug permeated through rat skin upon its treatment by solid microneedles compared with limited permeation of nanoparticles through bare skin.

Transdermal delivery of montelukast sodium was made feasible with better physicochemical properties and enhanced permeation by exploiting the merits of polymeric nanoparticles and microneedles simultaneously.

Keywords: Microneedles, Montelukast sodium, Polymeric nanoparticles.

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INTRODUCTION

Transdermal drug delivery is a way to achieve the systemic effect by applying drugs topically; the drug proceeds to penetrate the epidermis passing through its different layers without localization reaching the dermal circulation being accessible for systemic absorption. Besides being patient-friendly, transdermal drug delivery impart further merits such as escaping the first-pass metabolism, minimizing variation in drug blood concentration by controlled delivery over time, added to that, suitability as an alternative route whenever oral dosing is impermissible.

Despite these remarkable features, the impermeable nature of skin serves as a physiological barrier for the entrance of drug molecules, which should be handled firstly to optimize drug delivery via the skin.[1]

With the imperative need to pass the skin barrier without affecting its integrity and in conjunction with the advances in the nanotechnology field, many colloidal carriers with nano-size scales have been emanated in an attempt to increase systemic absorption through the skin. Therapeutic agent association with colloidal nanoparticles has shown to be beneficial to provide controlled release and protection of

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sensitive molecules against degradation and enhance skin permeability.[2]

Increasing attention has been paid to polymeric nanoparticles for their characteristic abilities in controlling drug release, entrapment of hydrophilic and hydrophobic drugs, and higher stability in comparison with lipid carriers, in addition to the ease of preparation by several feasible methods.[3]

Unfortunately, their transdermal field contribution is limited by the incapability to penetrate intact stratum corneum as it is localized in hair follicles forming depot for dermal drug delivery in a sustained manner.[4]

Montelukast sodium is a potent, selective antagonist for leukotriene receptors, an inflammatory mediator of solid implication in asthma's pathogenesis. By blocking this effect, Montelukast sodium prevents the worsening of asthma symptoms and conserves normal lung function. Besides its bronchoprotective effect, also Montelukast sodium has been used for the treatment of allergic rhinitis,[5] with all these pharmacological benefits. Another aspect regarding the physicochemical properties of montelukast sodium is highly recognized, as it is hygroscopic and highly affected by light and temperature; hence it is formulated as solid dosage forms only to reduce the effect of moisture and light, which indicate the reason of the unavailability of liquid dosage form yet, this fact imposes a challenge in administration of Montelukast sodium to infants and elderly population with swallowing difficulty.[6]

Driven by the need to enhance montelukast sodium stability and improve patient compliance, different approaches at formulation or route of administration level have been reported, including but not limited to fast-dissolving oral films, buccal patches, complexation with cyclodextrin, and various formulations at nano-scale level.[6]

Delivery of montelukast sodium via transdermal route has not been addressed widely, which is anticipated result bearing in mind the lipophilic nature (logP8.79) and high molecular weight (608.2 gm/mol) of montelukast sodium added this problem to the previously mentioned stability issue make the montelukast sodium ineligible candidate for transdermal transport. This necessitates appealing to the permeation enhancement techniques.

Recently, more focus has been paid to microneedles, a physical permeation enhancer of micron-scale composed from tiny projections arranged in arrays supported by a baseplate that can bypass stratum corneum. Once applied to the skin, temporal micro conduits are created through which different varieties of drugs such as compounds of high molecular weight, hormones, nanoparticles can be delivered to the systemic circulation in a minimally invasive manner utilizing different strategies like a poke with a patch, coat, and poke, dissolving and hollow microneedles.[7]

In this study, and through a combined approach, we tried to exploit the benefits of polymeric nanoparticles and microneedles simultaneously by preparing montelukast sodium as polymeric nanoparticles of enhanced stability and delivering them transdermally with the aid of microneedles.

MATERIALS AND METHODS

Materials

Montelukast sodium was purchased from Hyper Chem, China. Eudragit® L 100 supplied from Samara'a drug industry. Iraq. Methanol from Sigma-Aldrich, Germany. Polyvinyl Alcohol (PVA), cold from Central drug house, India. Polyvinyl Pyrrolidone K30 (PVP K30) from Provizer Pharma, India. Tween 80 from Riedel De Haen AG, Germany. Acetic acid, sodium acetate, phosphate-buffered saline (pH 7.4) from Fisher Scientific, UK Polymeric microneedles Micropoint, Singapore. Dialysis membrane 8-14 kDa HiMedia Lab Pvt. Ltd India.

Preparation of Montelukast Sodium Polymeric Nanoparticles

Montelukast sodium polymeric nanoparticles were prepared by the nanoprecipitation method. An accurately weighed amount of montelukast sodium and polymer were dissolved simultaneously in 7 mL methanol, which is completely miscible with water. Subsequently, the resulted drug-polymer solution, which represents the organic phase, was injected by 27 G syringe needles positioned directly into the continuous aqueous phase (35 mL acetate buffer pH 4.5) containing previously dissolved stabilizer at a rate of 0.5 mL/min with continuous stirring of 750 rpm at 25°C. The aqueous phase color converted into milky opalescence due to nanoparticles formation; the residual organic solvent was removed by evaporation at 40°C for 30 minutes on a magnetic stirrer, the obtained dispersion washed with deionized water by centrifugation at 3000 rpm for 10 minutes and subsequently lyophilized and stored for further use.[8] The detailed experimental procedure is shown in Figure 1.

The glassware was wrapped with aluminum foil. The preparation was done under subdued light to protect the drug from photodegradation; the rate of organic phase addition, the temperature of preparation, and stirring speed were kept constant.

Different formulas of polymeric nanoparticles were prepared, as shown in Table 1.

Characterization of Montelukast Sodium Polymeric Nanoparticles

Particle Size, Polydispersity Index, and Zeta potential Analysis

Average particle size and polydispersity index were measured in triplicate for all prepared formulas using particle size

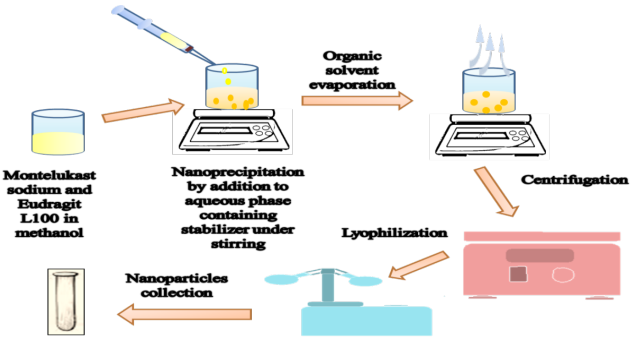

Figure 1: Preparation of montelukast sodium polymeric nanoparticles

Table 1: Preparative Characteristics of Polymeric Nanoparticles Formulas

Formula code	*Polymer*	*Montelukast sodium (w/v%)*	*polymer (w/v%)*	*Stabilizer(w/v%)*		
				PVA.	*Tween80*	*PVP*
F1	Eudragit L100	0.7%	0.35%	0.5	-	-
F2	Eudragit L100	0.7%	0.35%	1	-	-
F3	Eudragit L100	0.7%	0.35%	2	-	-
F4	Eudragit L100	0.7%	0.175%	1	-	-
F5	Eudragit L100	0.7%	0.7%	1	-	-
F6	Eudragit L100	0.7%	0.35%	-	1	-
F7	Eudragit L100	0.7%	0.35%	-	-	1

analyzer, which determines variation in light scattering at room temperature and 90° as scattering angle, polymeric nanoparticles dispersion diluted appropriately, shake well, and measured immediately.[8]

Zeta potential was measured by zeta sizer instrument, which measures electrophoretic mobility, which then converted into zeta potential; the samples were prepared for measurement as the previously mentioned method in measuring particle size was done in triplicate.[9]

Drug Content Determination of Polymeric Nanoparticles

The amount of Montelukast sodium in each formula was determined, 1mL of polymeric nanoparticle dispersion was placed in a volumetric flask, and the volume was completed to 10 mL by addition of methanol; after that, the diluted sample was sonicated for 1hr to guarantee complete dissolution of nanoparticles and filtered by 0.45 µm syringe filter, further dilution by methanol was performed, the exact amount of drug was determined spectrophotometrically by measuring the UV absorbance at 345 nm which is the confirmed λ_{max}, the experiment was done in triplicate.[9]

Determination of Entrapment Efficiency

Entrapment efficiency was measured to detect the amount of drug that incorporated inside the polymeric nanoparticles precisely, 1-mL from the polymeric nanoparticles dispersion was placed inside Amicon ultrafilter with a molecular weight cut off (MWCO 3kDa) and centrifuged for 15 minutes at 3000 rpm. The polymeric nanoparticles resuspended with deionized water, and another round of centrifugation was done to remove the loosely bounded drug. After that, 0.1 mL from the filtrate was diluted with deionized water, and the amount of free drug was determined spectrophotometrically by measuring the UV absorbance at 345 nm. The entrapment efficiency was calculated by applying the following equation:

$$\%EE = A_{(total)} \_ A_{(free)} / A_{(total)} \times 100 \quad ...(Eq.1)$$

Where:

% EE: is entrapment efficiency percentage, $A_{(total)}$ total amount of drug determined through measurement of drug content, and $A_{(free)}$ is a free amount of the drug that passes through Amicon ultrafilter, the measurements were done in triplicate.[11]

In-vitro Release of Montelukast Sodium from Polymeric Nanoparticles

The release behavior of Montelukast sodium from polymeric nanoparticles was studied , three mL of polymeric nanoparticles dispersion which is equivalent to 4.2 mg of Montelukast sodium were placed inside cellulose membrane sac of molecular weight cut off 8-14 kDa that was pre-soaked with dissolution medium for 8hours, the open ends of the sac were tied closely to prevent any leakage, then the sac was place in a beaker containing 200 mL of phosphate buffer pH7.4 with 0.5%tween 80 as a dissolution medium which placed over a magnetic stirrer rotated at 50rpm along with maintaining the temperature at 37 ± 0.1°C, at predetermined interval of 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 hours, aliquots of 2mL were withdrawn from the dissolution medium and immediately replenished with fresh dissolution medium to maintain sink condition, the collected aliquots were filtered by 0.45 µm syringe filter and subsequently analyzed spectrophotometrically at 345 nm which is the confirmed λ_{max} of Montelukast sodium, the in-vitro release study was carried out in triplicate.[12]

Morphological Characterization of Polymeric Nanoparticles

The selected formula's size and morphology were investigated using transmission electron microscopy; the drop of the sample was loaded on a carbon-coated copper grid and allowed to stand at room temperature for 90 seconds to form a thin film. The grid was allowed to dry in the air thoroughly; after that, the samples were observed, and photomicrographs were taken at different appropriate magnifications.[13]

X-ray Powder Diffractometry

Powder x-ray diffraction (XRD) is used to identify the crystalline pattern of the drug and to detect the physical transformation that concomitantly occurred during formulation; accordingly, the test was overlooked for the pure drug, physical mixtures in a ratio of 1:1 for a drug with polymer and the optimized nanoparticles formula, the study was confirmed by powder x-ray diffractometer at continuous scan range of 2θ = 10 - 50, the operating voltage was 30 kV and the current 20 mA.[14]

Differential Scanning Calorimetry (DSC)

The differential scanning calorimetry (DCS) is a thermal analysis carried out to study the thermal properties, physical changes of various substances and to confirm the presence of any interaction between drug and excipients, the test was performed for pure drug, physical mixtures in a ratio of 1:1 for a drug with polymer, and the optimized nanoparticles formula.

Five mg from each sample were placed inside an aluminum pan and sealed, then heated at a heating rate of 10°C/min with a

temperature range of 30°C to 300°C in DSC instrument under a nitrogen atmosphere.[14]

Photo Stability Study of Montelukast Sodium Nanoparticles

A total of 5 mL of the optimized polymeric nanoparticle dispersion was placed inside a clear flask and subjected to direct sunlight for 6 hours. At the end of 6 hours, the sample analyzed by high-performance liquid chromatography (HPLC) to identify the significant degradation products, the method used for the analysis of montelukast sodium and its degradation products concurrently was as it is ascertained in the United State Pharmacopeia, the mobile phase was gradient mixture of 0.2% trifluoroacetic acid (solution A), methanol and acetonitrile (solution B), the column used as stationary phase (4.6 mm x 10 cm, 3 µm), the UV detector was 255 nm, the flow rate 1.5 mL/min.[15,16]

Microneedles Application and EX-Vivo Permeation Study

Polymeric microneedles composed from PVA were used to pierce freshly extracted abdominal rat skin, the application process performed by microneedle spring applicator, thereafter, Ex vivo permeation study through microneedle-treated abdominal rat skin was conducted using Franz diffusion cell, the receptor compartment filled with 60 mL of phosphate buffer of pH7.4 containing 0.5% tween 80 and thermostated at 37 ± 1°C, the rat skin was fixed as a barrier between the donor and receptor compartments in a way that the stratum corneum side faced the donor chamber, the sides of the Franz cell tightly closed by Teflon tape , polymeric nanoparticles equivalent to 4.2 mg Montelukast sodium re dispersed in deionized water and added to donor compartment, the donor chamber and the sampling arm were wrapped with Parafilm M® to prevent evaporation of permeation medium, a magnetic bar was previously added to the receptor media to ensure continuous agitation of about 50 rpm, the study continued for 8 hours, at predetermined intervals 1 mL sample was withdrawn and immediately replenished with fresh receptor media to maintain the sink condition, the withdrawn sample filtered and then analyzed spectrophotometrically at 345 nm, the cumulative amount of drug permeated through rat skin was quantified.

As a control, the experiment was repeated using abdominal rat skin untreated with microneedles, and the permeation study was conducted keeping the same experiment conditions; parameters as steady-state flux and permeation coefficient were also calculated.[17]

Statistical Analysis

The results of the experiments are given as a mean of triplicate samples ± standard deviation and were analyzed according to the one-way analysis of variance (ANOVA) at the level of significance ($p < 0.05$).

RESULTS AND DISCUSSION

Effect of Formulation Variables on Physical Properties of Nanoparticles

The successful preparation of nanoparticles requires careful selection of different variables that have a crucial role in forming and stabilizing polymeric nanoparticles. In our study, montelukast sodium is a weakly acidic drug of two pKa 2.8 and 5.7. It exhibits a pH-dependent solubility pattern of increasing solubility above its pKa; simultaneously, it is freely soluble in water and methanol,[16,5] regarding the polymer selected to encapsulate the drug. Eudragit L100 is a well-known anionic pH-sensitive polymer that releases drug loading above pH6 and recently gaining increasing attention as a drug carrier for dermal diseases; it is freely soluble in methanol and practically insoluble in water.[18]

Based on these facts, the formation of montelukast sodium polymeric nanoparticles prepared by a facile nanoprecipitation method has been prominent instantaneously by turning the dispersion into milky opalescence color, which indicates the correctness in the selection of different preparation parameters.

Various parameters involved in the preparation were studied to optimize the nanoparticle particle size, polydispersity index, zeta potential, and entrapment efficiency; the obtained results are tabulated in Table 2.

The particle size ranged between 178.6 ± 3.3 and 1273 ± 7.54. Simultaneously, the polydispersity index value lies between 0.12 ± 0.014 and 0.68 ± 0.03. Figure 2 interprets the impact of the stabilizer concentration, polymer concentration, and stabilizer type on particle size and polydispersity index.

To investigate the effect of stabilizer concentration on particle size , PVA used as stabilizer in F1,F2, and F3 with three different concentration of 0.5,1, and 2% w/v of aqueous phase, it was evident that significant decrease in particle size happened with the increasing the concentration of PVA ($p < 0.05$), PVA is widely used as stabilizer as it can perform steric stabilization by adsorption at the nanoparticle surface and shielding the formed particles with thick layer that prevent their coalescence to reduce their interfacial energy, additionally, with increasing the concentration of stabilizer the viscosity of the external

Table 2: Preparative characteristics of polymeric nanoparticles formulas

Formula code	*Particle size (nm)*	*Polydispersity index*	*Zeta potential*	*Entrapment efficiency%*
F1	519.4 ± 11.4	0.271 ± 0.023	-36.78 ± 7.19	91.3% ± 2.65
F2	192.7 ± 12.15	0.152 ± 0.014	-20.26 ± 4.36	93.9% ± 0.96
F3	236.7 ± 19.53	0.14 ± 0.026	-13.15 ± 1.54	95.4% ± 3.43
F4	1273 ± 7.54	0.68 ± 0.03	-18.66 ± 3.26	88.7% ± 6.88
F5	540.1 ± 7.85	0.337 ± 0.054	-22.77 ± 3.85	97.2% ± 2.41
F6	835 ± 87.39	0.52 ± 0.026	-17.28 ± 2.006	85.6% ± 5.5
F7	178.6 ± 3.3	0.12 ± 0.014	-21.14 ± 4.283	90.7% ± 6.71

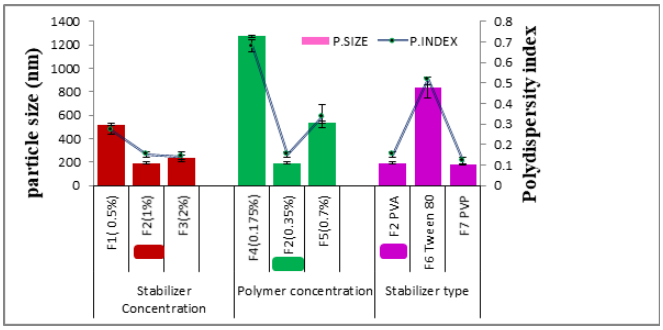

Figure 2: Particle size and polydispersity index of the prepared formulas

phase increase which hinder the collision of the particles, apparently the concentration of 0.5% PVA was inadequate to cover the surface of the particles accordingly there was a high tendency to agglomerate and forming large particles, by increasing the concentration of PVA to 1% particle size reduced but slight increase in particle size was recorded by further increase of stabilizer concentration to 2% which can be attributed to the increment of the external phase viscosity which is directly correlated with the stabilizer concentration, for polydispersity index which is a measure for particle size distribution ,it is clear that polydispersity index value decrease with increasing the stabilizer concentration which give a strong indication about homogeneity of the dispersion, the obtained results were in entire agreement with that previously reported by Sharma *et al*.[19]

With regards to the effect of polymer concentration on the particle size and polydispersity index, the different concentration of Eudragit L100 polymer was used in F4, F2, and F5, which is 0.175, 0.35 and 0.7% w/v of the organic phase. A significant change in particle size ($p < 0.05$) was noticed by increasing the polymer concentration, F4 with the lowest polymer concentration of 0.175%w/v exhibit the highest particle size measure, and the polydispersity index is indicative of the wide distribution of the particles, given the importance of the polymer in the formation of the nanoparticles. It can be said that the used polymer concentration inappropriate to initiate nucleation and particle growth. Accordingly, the drug and polymer present as an irregular agglomerate of considerable size, with increasing polymer concentration in F2 to 0.35% the particle size decrease which is sufficient to enclose the drug molecule and form uniform particles, in F5 with a concentration of 0.7%w/v the particle size was increased which is expected result as the higher concentration of the polymers is associated with increasing viscosity of the organic phase which restricts its diffusion towards the external phase, and accordingly, the particles size enlarged proportionally with polymer concentration, the results are in accordance with the previously published by G.Torres-Flores *et al*.[20]

The effect of varying stabilizer types on the prepared nanoparticles' physical properties was studied using three different stabilizers: PVA, PVPK30, and tween 80 in F2, F6, and F7 respectively, and their concentration were kept constant.

The results indicate a significant increase in the particle size ($p < 0.05$) and polydispersity index when tween 80 was used as a stabilizer, tween 80 is a nonionic surfactant with a high HLB value of 15 and possess high solubilizing capacity, more drug was solubilized by tween 80 and diffused toward the external aqueous phase in which it present as micellar form; consequently, the amount of tween 80 that present at the interface between the external and the internal phase was not enough to stabilize the particles, accordingly large agglomerates of polydisperse nature were formed, similar finding were reported by S.Ray *et al*.[21]

Concerning the zeta potential, which is the electrokinetic potential in colloidal systems and considered as a measure for system stability through determining the degree of repulsion between the particles. The obtained results were ranged from -36.78 ± 7.19 to -13.15 ± 1.54, all the prepared formulations having a negative charge which is related to ionization of the carboxyl group of the Eudragit L100 at pH4.5 of the external aqueous phase. Even though the higher zeta potential value is preferred to ensure system stabilization and prevent particles agglomeration after storage, but taking into account that the used stabilizer impart stabilization through steric stabilization rather than electrostatic can explain the stability of the prepared system regardless of zeta potential values, added to that, the decreasing in zeta potential value refer to coverage of the particle surface by the adsorbed stabilizer.[22]

The prepared formulations exhibit relatively high entrapment efficiency ranged between 97.2% ± 2.41 and 85.6% ± 5.5. An insignificant difference was noticed in entrapment efficiency results $p > 0.05$. A high encapsulation efficiency can be ascribed to the poor solubility of the drug in the used external phase, added to the high solubility of the drug in the organic solvent; as a result, less amount of drug directed toward the external aqueous phase.[23]

The effect of different formulation variables on the release behavior from the nanoparticles was studied. Figure 3 depicts the results.

A significant decrease in the amount of drug released ($p < 0.05$) was noticed with increasing the concentration of stabilizers. F1 with the lowest stabilizer concentration 0.5%w/v exhibits complete drug release with 1 hour, while F2 with 1%w/v PVA, the release extended to 5hours, and in F3 with the highest concentration of PVA 75% of the loaded drug was released after8hours from commencing the test.

The release pattern from F1 occurred rapidly as the formula with the lowest stabilizer concentration tends to present as the clusters of polymers and stabilizers rather than uniform nanoparticles. While Eudragit L100 exhibits pH-dependent solubility, and its swelling and erosion are directly proportional to the increment of pH[24] accordingly, complete drug release was observed with 1-hour due to the polymer's rapid dissolution is a direct consequence of imperfect stabilization of the particles.

F2 and F3 showed sustained release profile, which can be attributed to the proper stabilization of nanoparticles; faster release occurred from F2, which can be explained in term of

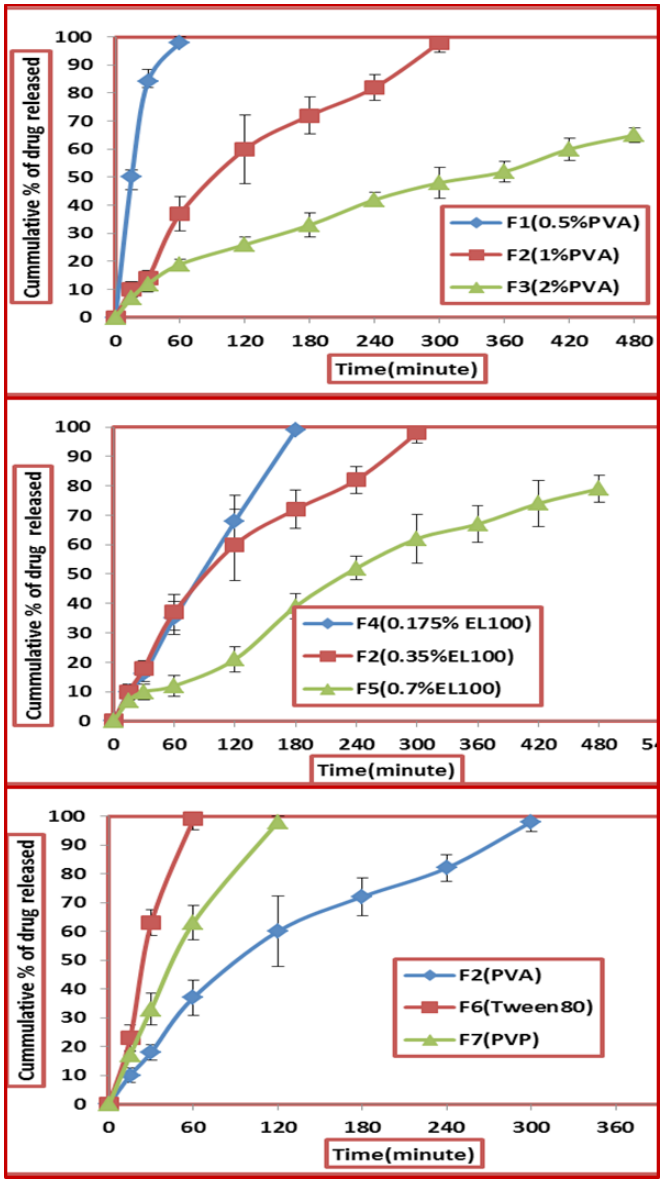

Figure 3: Effect of Various Preparation Parameters on the cumulative drug release, results are mean ± SD for n=3 tests

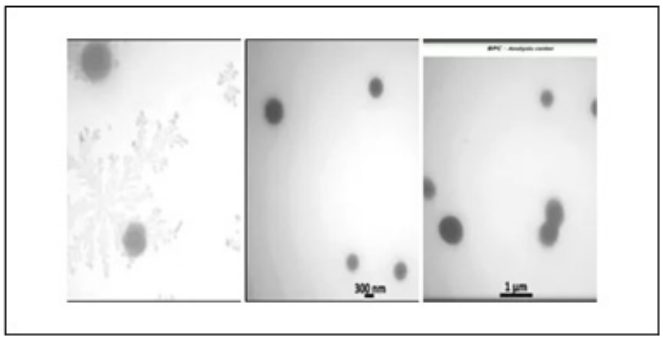

Figure 4: TEM Images of Montelukast Sodium Polymeric Nanoparticles

its smaller particle size with higher surface area subjected to dissolution medium.

Contrary to expectation, F3 with the highest stabilizer concentration retard the release significantly; it can be argued that PVA at higher concentration can absorb a large amount of dissolution media and swell to form a thick hydrogel layer adsorbed tightly at the surface of nanoparticles, creating a barrier for drug diffusion out of the polymeric matrix, besides impeding the fast erosion of the pH-sensitive polymer.[25]

The effect of polymer concentration on the drug release from nanoparticles was studied by comparing the release profile of formulas F4, F2, and F5; the results indicated remarkable prolonged-release upon increasing the polymer concentration, as the formula F5 prepared with the highest Eudragit L100 concentration of 0.7%, released about 80% of its loading after 8 hours with a significant difference ($p < 0.05$) than other formulations.

The effect of the type of stabilizer on the drug release from the nanoparticles was studied. The release profile of the formulations F2, F6, and F7 stabilized with PVA, tween 80, and PVP were compared.

The faster release revealed from F6 stabilized by tween80 can be related to the surfactant activity of tween 80, enhancing the exposure of the polymeric matrix to the release medium and its consequent erosion. A similar finding has been reported previously by Mishra *et al*.,[26] F7 stabilized with PVP exhibited significantly faster release ($p < 0.05$) compared to F2 stabilized by PVA. This result can be accounted for small particle size of F7, besides the aforesaid gelatinization ability of PVA over the polymeric matrix, which eventually succeeded in sustaining the release of the drug despite the pH-sensitive nature of the used polymer.[25]

F2 was selected as the best formula for its small particle size, good entrapment efficiency, and ability to release drug loading completely in a sustained pattern within 5 hours; the selected formula was subjected for further evaluation.

Morphological Characterization of Polymeric Nanoparticles

The prepared nanoparticles were visualized under TEM at different magnifications. Figure 4 showed that the nanoparticles have a regular spherical shape, well stabilized by added stabilizer adsorbed at their surfaces; the investigated particles have a size of 198 nm, which is in agreement with the results obtained by the particle size analyzer.

X-ray Powder Diffractometry

The XRD study revealed that Montelukast sodium present as amorphous distinctive, stable form, the XRD pattern appears as diffused rather than separated sharp peaks, the results are in accordance with Patil-Gadhe *et al*.,[27] the diffractogram of physical mixture between montelukast sodium and Eudragit L100 confirmed the amorphous nature of both the drug and polymer, at the same time rule out any possible interaction between them, the diffraction pattern of the optimized nanoparticle formulations displayed new separated peaks at 14.8°, 20.4° and 23.5° related to the crystalline nature of mannitol which is used as a cryoprotectant during lyophilization process, similar findings have been reported previously by Zu, Yuangang *et al*. who prepared resveratrol as nanoparticles and used mannitol as a cryoprotectant in

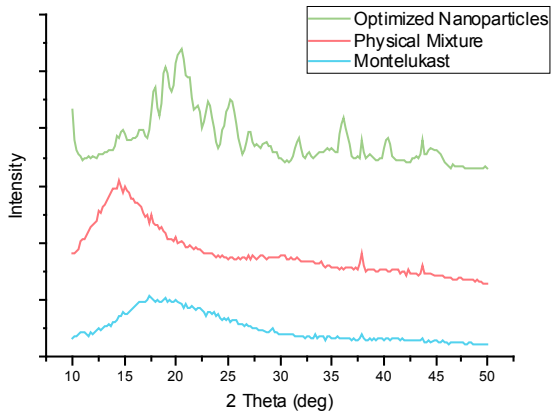

Figure 5: XRD Diffractogram of a)Montelukast sodium, b)Physical Mixture of Drug and Polymer, c) Lyophilized Nanoparticles

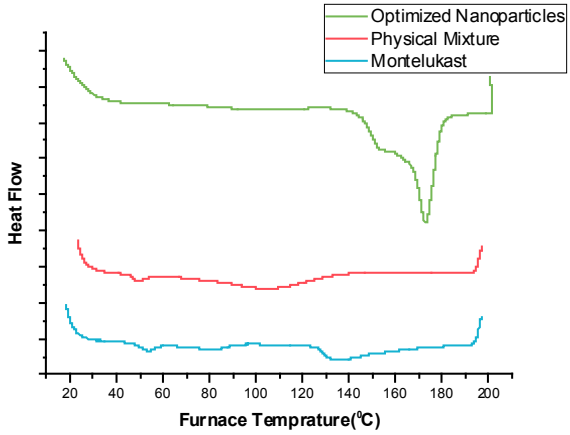

Figure 6: DSC Thermogram of a)Montelukast Sodium, b)Physical Mixture of Drug and Polymer, c) Lyophilized Nanoparticles

Lyophilization.[28]

The XRD study confirms the amorphous nature of montelukast sodium as well as exclude any interaction or recrystallization during the preparation process; the results are illustrated in Figure 5.

Differential Scanning Calorimetry (DSC)

The DSC thermogram of montelukast sodium showed broad endothermic peaks at 135.5°C and between 50–60°C; the first one refers to the drug's melting point, while the second one denotes the glass transition temperature of montelukast sodium, both peaks confirm the amorphous nature of the drug, and it is in line with previous results.[29,30] The thermogram of the physical mixture between the drug and polymer did not show the appearance of new peaks, which indicate high compatibility between the drug and polymer; furthermore, the thermogram of the optimized nanoparticles showed complete disappearance of montelukast sodium peaks which indicate complete entrapment of the drug inside the polymeric matrix, besides that, the new endothermic peak appeared at 170°C represents the melting point of the used cryoprotectant crystalline mannitol,[28] the results are depicted in Figure 6.

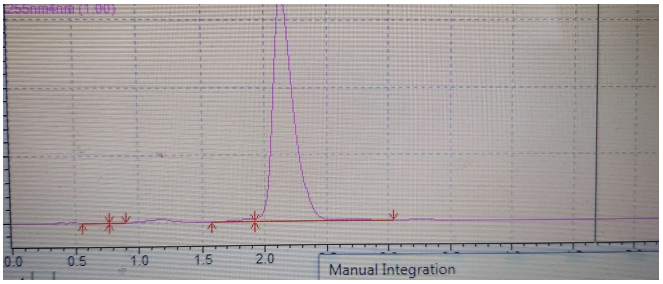

Figure 7: Montelukast sodium and its degradation products

Photo Stability Study of Montelukast Sodium Nanoparticles

The photodegradation of montelukast into its cis isomer is a well-defined problem that affects the stability of the drug; accordingly, to ensure the competence of the nanoparticles in reducing photodegradation and to approve the efficiency of the photoprotection measures in preserving the drug during the preparation process, the major degradation products upon light exposure was assessed quantitatively, the results indicated high stability of montelukast sodium, where the percentage of montelukast sodium was 97.9% with relative retention time at 2.1 minutes, while the % of the significant detected degradation products cis isomer and Montelukast S-oxide was 0.6 and 0.35% with relative retention time at 1.9 and 0.95 minutes respectively, the results are illustrated in Figure 7.

Previous reports showed rapid degradation upon subjecting to daylight with rapid formation cis isomer, as after 1hour 30% of cis isomer was formed,[16] the higher attained stability is related to the entrapment of the drug inside the polymeric shell, which acting as a shield around the drug molecules reducing the chance of its interaction with the detrimental light, correspondingly, Tagliari *et al.* reported photostability enhancement of nifedipine when it is loaded in polymeric nanoparticles.[31]

Microneedles Application and *Ex-vivo* Permeation Study

The permeation study of montelukast sodium nanoparticles was conducted through bare skin and the skin treated with polymeric microneedles; thereafter, the microneedles' penetration-enhancing effect was assessed.

It was found that after 8 hours of commencing the study, more than 90% of the loaded drug with nanoparticles permeated through the skin previously treated with microneedles. On the other side, at the end of the same period, the amount permeated through bare skin was not exceeded 14% which indicates a significant difference ($p < 0.05$) in the cumulative amount of the drug permeated. The steady-state flux in both cases was calculated from the permeation profile represented in Figure 8. It was 156.2 ± 0.43μg/cm^2.hr and 25.171 ± 1.118 μg/cm^2.hr for polymeric nanoparticles permeated through skin treated with microneedles and bare skin, respectively. Besides, the permeation coefficient was found to be 0.111 ± 0.00031 cm/hr and 0.0179 ± 0.0008 cm/hr, respectively, a significant increase ($p < 0.05$) in a steady-state flux of nanoparticles was noticed through the skin treated with microneedles.

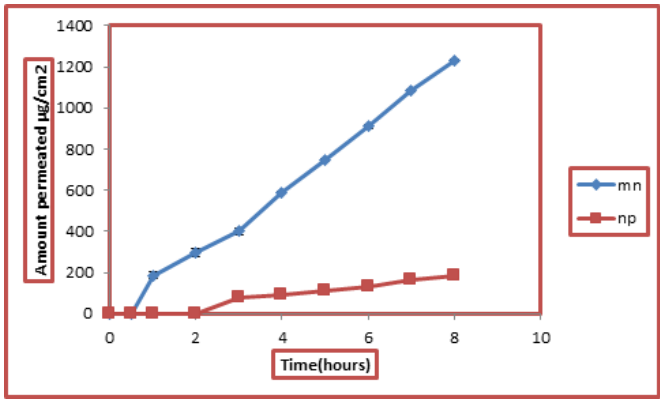

Figure 8: Permeation profile of nanoparticles through skin treated with microneedles and bare skin

The permeation is enhanced by 6.2 fold, which indicates the evident contribution of microneedles in increasing permeation, as microneedles capable of creating microchannels larger than the diameter of nanoparticles facilitate their transport through the skin. At the same time, results reflect the poor permeation of nanoparticles through bare skin in which the trans appendageal is the accredited pathway for their transport which hove a poor contribution in transdermal absorption. Similarly, Ramadan *et al*. have been reported improvement in permeation of lamivudine polymeric nanoparticles with the aid of microneedles.[13]

CONCLUSION

Montelukast sodium was successfully delivered as polymeric nanoparticles through a transdermal route with enhanced stability evading the shortcoming associated with its complicated physicochemical properties. Microneedles treatment significantly enhances drug permeation through the skin; we highlight this route as an alternative to the oral route of administration, targeting the children population with high compliance.

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