Formulation and characterization of Flurbiprofen nanoparticles loaded microneedles

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Keywords: Flurbiprofen, Nanoprecipitation method, PVA, PVP, Microneedle, SEM.

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Abstract

Flurbiprofen (FBP) is a NSAID used in treatment of inflammation, migraine, rheumatic diseases, sore throat and primary dysmenorrhea. FBP is 99% protein bound with rapid oxidative metabolism .The elimination is mainly through urine and the elimination half-life about 3.5 hours. Due to its poor solubility, FBP is a great candidate for formulation of nanoparticles. Poor bioavailability results in frequent dosing and poor patient compliance. Hence an approach has been made to develop FBP nanoparticles loaded microneedle patch for transdermal delivery with faster release and patient compliance.

FBP nanoparticles were prepared by using nanoprecipitation method. The particles sizes and zeta potential was measured using zeta potential analyzer. The particles morphology was also determined using SEM. The in-vitro release of FBP from the nanoparticles was carried out in phosphate buffer PH (7.4) containing 10% ethanol to simulate in vivo release in the skin. Microneedle patch of PVA and PVP was prepared using Polydimethylsiloxane (PDMS) micromolds. The ratio of PVA to PVP of matrix solution was optimized to attained maximum needle strength. The optimized strip was evaluated for ex-vivo skin permeation.

FBP nanoparticles particle size was in nano size ranged from (9.9 to 158 nm) with positive zeta potential. The drug entrapment efficiency was varied with the drug polymer ratio from 53% -

85%. The SEM showed uniform shape and regular distribution of particle sizes. The in-vitro drug release study of nanoparticles exhibited an immediate release of FBP. The outcomes shown that as the ratio of PVP-K30 increased in the polymeric solution blend the needle fraction force decrease. The histopathological study of treated skin showed almost comparable cellular integrity as related to skin treated with PBS (pH 7.4) as control. In addition the skin permeation study show the microneedles permeate more efficiently than simple ordinary patches of the drug through the skin by 4.1 folds.The microneedle patches loaded with FBP nanoparticles were successfully prepared and evaluated .

Keywords: Flurbiprofen, Nanoprecipitation method, PVA, PVP, Microneedle, SEM.

تشكيل الابر المجهرية المحملة بالجسيمات النانويه لدواء الفلوربايبروفين

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الكلمات المفتاحية: فلوربايبروفين، طريقة الترسيب النانوي، PVP،PVA ، ابر مجهرية، SEM

الخلاصة

الهدف: فلوربايبروفين هو دواء لاستيرودي مضاد للالتهاب يستخدم لعلاج الالتهاب، الشقيقة، امراض الروماتيز،احتقان البلعوم وعسر الطمث الاولي.99% من الفلوربايبروفين مرتبط بالبروتين مع ايض تأكسدي سريع.يطرح بصورة رئيسية عن طريق البول ويكون عمر النصف الحيوي له حوالي ثلاث ساعات ونصف . بسبب ذوبانيته الغير كافية ، فلوربايبروفين مرشح رائع لايصال الدواء عبر الجلد حيث ان ضعف التوافر الحيوي ينتج جرعات متكررة وقلة التزام المريض بالعلاج لهذا السبب ، تم اعتماد نهج لتطوير الابر المجهرية المحملة بالجسيمات النانويه لدواء الفلوربايبروفين لايصال الدواء عبر الجلد والحصول على تحرر افضل للدواء والتزام افضل للمريض بالعلاج .طرق العمل:تم تحضير الجسيمات النانوية للفلوربايبروفين باستعمال طريقة الترسيب النانوي.تم قياس احجام الجسيمات

جهد الزيتا باستخدام محلل جهد الزيتا.تم تعيين شكل الجسيمات باستخدام المجهر الالكتروني الماسح .تم تنفيذ التحرر المختبري للفلوربايبروفين من الجسيمات النانوية في محلول منظم فوسفاتي ذي درجة حامضية (7.4) ويحتوي على 10% من الايثانول ل تحفيز تحرر الدواء في داخل الجلد.تم تحضير رقعة الابرة المجهرية المتكونة من ال PVA و PVP باستخدام قوالب صغيرة من (Polydimethylsiloxane (PDMS .تم تحسين نسبة ال PVA الى ال PVP للخليط للحصول على اقصى قوة للابرة. تم اختبار الشريط المحسن لاجل التحلل المختبري، تحرر الدواء، تغلل الدواء خارج الجلد ودراسة التشريح المرضي..تراوح حجم الجسيمات النانوية لدواء الفلوربايبروفين من 9.9 الى 158 نانومتر مع جهد زيتا موجب.تغيرت كفاءة احتباس الدواء مع نسبة الدواء الى البوليمر من 53% الى 85%.اظهرت نتائج المجهر الالكتروني الماسح شكل موحد و توزيع منتظم لاحجام الجسيمات .اظهرت دراسة تحرر الدواء المختبري للجسيمات النانوية ,تحرر فوري ل دواء الفلوربايبروفين . اظهرت النتائج عند زيادة نسبة ال 30PVP-K في مزيج محلول البوليمر ف ان قوة كسر الابرة تقل . لم تظهر نتائج التشريح المرضي للجلد المعالج اي علامة من علامات الالتهاب وهي قابلة للمقارنة الخلوية للجلد المعالج ب محلول منظم فوسفاتي ذي درجة حامضية (7.4) .بالاضافة الى ذلك اظهرت دراسة نفاذية الجلد للدواء بان الابر المجهرية اكثر كفاءة في النفاذ عبر الجلد من الرقع العادية والبسيطة بمقدار 4.1 .تم تحضير واختبار رقع الابر المجهرية المحملة بالجسيمات النانوية لدواء الفلوربايبروفين .تقترح النتائج الواعدة للبحث دراسات اضافية للتحرر الداخلي عبر الجلد من اجل تقديرالتوافر الحيوي.

1. Introduction

Nanotechnology provides non-invasive methods to enhance skin permeability for several medications which leads to the improvement of formulation, drug delivery, dose minimizing, enhancing of drug release and protecting drug from enzymatic breakdown [1].Many problems are associated with delivering drug orally such as GIT upset, first pass metabolism and less patient adherence which results in less effective drug release. The delivery of drugs via skin overcomes all of those issues providing more convenient release [2]. The challenge in the delivery of the drugs through the skin is the ability to cross the stratum corneum which represents the primary barrier that leads to delay the permeability of transdermal drug and minimize its bioavailability [3].Several techniques have been developed to improve the transdermal drug permeation and absorption such as ultrasound, iontophoresis, electroporation, microneedles and nanocarriers [4]. Transdermal drug delivery system offers many advantages over other routes through increasing the duration of action with uniform plasma levels which allows a better release, bioavailability and the reduction of the number of doses [5].

Microneedle mediated transdermal drug delivery has been widely employed as a novel

transdermal drug delivery system .It consists from needle-like projections for the perforation of stratum corneum to create channels that enhance the permeability of molecules with large size (>500 Da), proteins and nanoparticles via skin [6]. Microneedles are able to penetrate the epidermis without nerve endings stimulation which allows painless application and a better patient compliance [7]. Microneedle drug loaded nanoparticles provide simple, effective and non-invasive technique compared to hypodermic needles and syringes which leads to target drug delivery with better release of the drug especially drugs used in chemotherapy. A site-specific targeting is achieved as a result the death of healthy cells was prevented [6]. Nanoparticles have been widely investigated in the drug delivery for the improvement of solubility for poorly soluble drug (Class II) as a result of enhancing their bioavailability [3] FBP is a NSAID used in treatment of inflammation, migraine, rheumatic diseases, sore throat and primary dysmenorrhea [8]. FBP is 99% protein bound with a rapid oxidative metabolism .The elimination is mainly through urine and the elimination half-life about 3.5 hours [(9)].Due to its poor solubility, FBP is a great candidate for nanoparticle preparation .It has a molecular weight of 244.26 Da and log P (octanol/water, pH 7.4) is 3.80 [10].

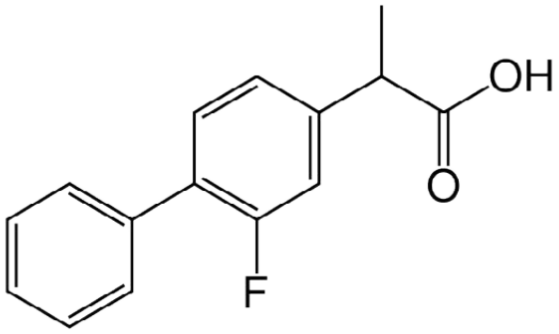


Figure (1): Chemical structure of FBP [(3)]

The objective of this study was to formulate and evaluate FBP nanoparticles with the enhancement of its solubility and improvement of patient compliance.

2. Materials and Methods

Chemicals

FBP was supplied from (ProvizerPharmaIndia), Polyvinyl Alcohol (PVA), cold (Central drug house, India), Polyvinyl Pyrrolidone K30 (PVP K30) and CMC (Provizer Pharma, India), Poloxamer 188 (HiMedia Lab. Ltd. India) and Ethanol (Sigma-Aldrich, Germany). All other chemicals used in this study were analytical grade.

Preparation of Flurbiprofen nanoparticles

Nine formulas (F1-F9) of FBP nanoparticles were prepared in a previous research by using nanoprecipitation method [11].

Table (1) represents the compositions of the prepared formulas of FBP nanoparticles.

Table (1):- FBP formulas components(11).

Formula Code	FBP Conc. mg/ml	volume injected ml	Poloxamer 188 Conc.%	PVP Conc.%	PVA Conc.%	Solution volume ml
F-1	10	5	0.05			50
F-2	10	5	0.1			50
F-3	10	5	0.2			50
F-4	10	5		0.05		50
F-5	10	5		0.1		50
F-6	10	5		0.2		50
F-7	10	5			0.05	50
F-8	10	5			0.1	50
F-9	10	5			0.2	50

Production of microneedle patches.

In this approach, microneedle mold contained 225 pyramidal microneedles (array size 15×15) measuring 500 μm in height, 200 μm in base diameter and needle pitch 1500 μm located in a ~ 4 cm^2 area. This mold purchased from Micropoinr Technologies Pte Ltd (Figure 2).

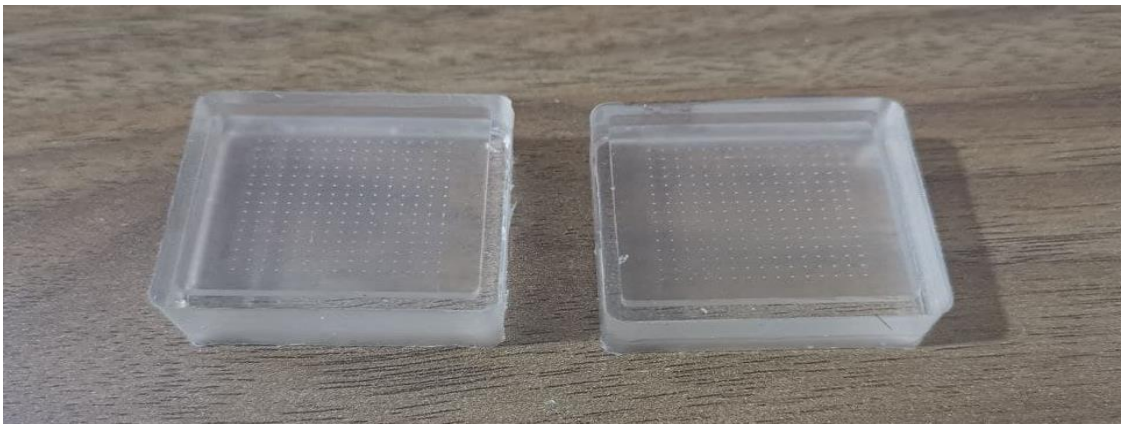


Figure 2: Microneedle mold

Preparation of microneedle matrix material

Different polymers used as a matrix material (polyvinyl alcohol (PVA) and polyvinylpyrrolidone-K30 (PVP-K30), Poloxamer 188 and carboxymethyl cellulose (CMC)) each one alone and in different ratios were utilized to prepare 14 microneedle batches (Table 4). To make a polymeric solution, 4 g of each polymer and in combination in different ratio without and with plasticizer (glycerin) were dissolved in 20 mL DW to form 20% polymeric solution and mixed thoroughly using spatula and heated at 60 ºC for 2hr. Then, these polymeric solution was kept in a sealed glass bottle overnight, to be used later to prepare the microneedles.

Drug loading into the microneedle matrix

FBP nanodispersion was concentrated at 40 ºC for about 1 hr. to increase FBP-NP concentration in the nanodispertion to about 50 mg/ml. Then the nanodispersion is mixed with the microneedle matrix to prepare a matrix containing 12.5 mg/ml of FBP-NP. Nearly 4 mL of the prepared polymeric nanodispersion were introduced to microneedle silicon mold. Then the casted mold was sonicated for 2hr and kept overnight in vacuumed desiccator to facilitate the drying

process [12]. Same procedure performed by casting raw FBP dispersion to prepare microneedle containing raw FBP instead of FBP nanoparticles to study the effect of nanoparticle of the microneedle characteristics. After complete drying the patch was cut to smaller patches with same dimension of MN patches that results from PDMS mold.

Table 2: Composition of Microneedles Formulas

Formulas	FBP-NP	FBP	Glyn %	PVA (gm)	PVP (gm)	CMC (gm)	Poloxamer 188 (gm)	Transdermal Patch Type
MN1	50	-	10	4				Microneedle
MN2	50	-	10		4			Microneedle
MN3	50	-	10			4		Microneedle
MN4	50	-	10				4	Microneedle
MN5	50	-	10	3	1			Microneedle
MN6	50	-	10	2	2			Microneedle
MN7	50	50	10	2	2			ordinary

Characterization of microneedles patches

Morphology of microneedle patches

Scanning electron microscope (SEM) was used to observe the morphologies and dimensions of the prepared MN patches including the heights, widths, lengths and interspacing of the polymeric MN patches and compared the outcomes with those of the master mold.

Drug content

Three microneedle patches (2cm×2cm) from each formulation batch; these strips were placed in 50 mL (25mL phosphate buffer pH 7.4 + 25 mL ethanol) solutions individually and kept on magnetic stirrer for 3hr. Then the solution was filtered and appropriately diluted using suitable solvent (buffer). Finally calculate FBP concentration by measuring the absorbance of drug at its λ_{max} [13].

Appearance

The prepared microneedle patches were checked visually for any defects. Only patches with no deformities in the needles and patch were further evaluated.

Mechanical properties of microneedles

Mechanical failure of microneedles was regarded as a result of axial loading. Axial needle fracture force is known as the smallest force needed to deform or split microneedle when applied to the microneedle axis parallel (needle failure). A displacement force test station was used to calculate the axial needle fracture force (Testometric AX, UK). While the test station pressed a 100 microneedle array of patch contrary to a rigid surface at 1mm/min rate, the force abruptly fell upon needle failure; the force of microneedle failure was the maximum force applied immediately before needle fracture [14].

Ex-vivo skin permeation study

The abdominal skin of adult male Westar rats weighing 250 ± 10g obtained from animal house of college of pharmacy/ university of Kerbala, were used for *ex-vivo* permeation study of the microneedle patches. The protocol of this study was approved by the committee of ethics in college of pharmacy/university of Baghdad.

Animal skin (rat) was fixed in the Franz diffusion cell between the donor chamber and the receptor chamber in a way that the stratum corneum toward the donor chamber of the Franz diffusion cell. The available skin surface area for diffusion was 3.8 cm^2. To maintain sink conditions, 50 mL of phosphate buffer pH 7.4 (+ 10% ethanol) was added in receptor chamber. The system temperature was kept at 32 ± 1.0°C. The 10% ethanol was added to increase FBP solubility and reach the sinck condition.

Receptor media was continuously stirred using magnetic stirrer at 50 rpm, in a way that the rat skin surface just flushes the diffusion fluid. Applying the simple patches and microneedle patches containing 50 mg of drug over the used rat skin by gentle thumb pressure in a donor chamber was done. At time interval of 1, 2, 3, 4, 5, 6, 7, 8 and 12, 18 and 24hr, a sample of 2 mL were taken from the permeation media in the receptor compartment and substituted as soon as possible with the same volume of receptor fluid. The samples were investigated for drug content spectrophotometrically at λ_{max} 247 nm. Each trial was achieved in triplicate. The cumulative quantity of FBP permeated at each time intervals and different considerations like, steady-state flux (J_{ss}), and apparent permeation coefficient (P_{App}) were calculated [15].

Statistical analysis

The outcomes of the experimental work are demonstrated as a mean of triplicate models ± SD and were examined in relation to the one-way analysis of variance (ANOVA) to see if the changes in the applied variables are statistically significant at the ($P < 0.05$) level and non-significant at the ($p > 0.05$) level.

3. RESULT AND DISCUSSION

As described in formulations composition) Table 2) different MN formulations were prepared and the results of the current study displayed that all MN patches prepared with PVA and PVP-K30 showed homogenous polymer mixtures with the resulting MNs having sharp needle tips. (Figure 3) shows the shape and dimensions of MNs of the selected formula using SEM.In addition to microneedle patches, simple patches without microneedles (2:2 ratio of PVA: PVP-K30) containing FBP nanoparticles and pure FBP were also prepared, in order to allow their comparison with MN patches containing the FBP nanoparticles and pure FBP.

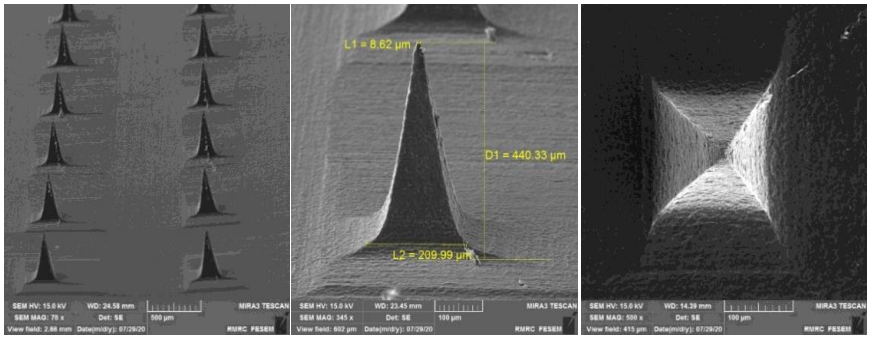


Figure 3: Microneedle's shape and dimensions of selected formula (M5) using SEM.

Mechanical properties of MN

The proficiency of a MNs array to be successfully introduced in the skin is critical to its use, as the stratum corneum must be pierced for the MNs array to give its effect. Incorporation of drug substances including nanoparticles into the polymeric solution in the production of a dissolving MN can produce a weakening effect on the MNs. Therefore, mechanical examinations must be done as an essential part of construction studies for dissolving MNs arrays. Mechanical studies were done for all the successful microneedle patches. (Figure 4) shows the axial needle fraction force of the selected MN6 patch using testometric apparatus. The results show that the axial needle fraction force ranged from 16.34±0.52 N to 28.43±0.76 N. The outcomes were shown that as the ratio of PVP-K30 increased in the polymeric solution blend the needle fraction force

decrease (Table 3), the same results were shown by P. D. Andi et al. [17] and Mofidfar M. et al. [18].

Table (3): Mechanical Analysis Data of the prepared MN Formulas

Formulas	Needle fraction force (N) per 100 microneedle*	* Folding Endurance
MN1	16.34±0.52	253±13
MN2	23.45±0.22	238±15
MN3	21.46±0.36	46±5
MN4	19.83±0.63	65±6
MN5	28.43±0.76	226±15
MN6	26.53±0.23	256±14
MN7	-	276±14

* mean ± standard deviation.

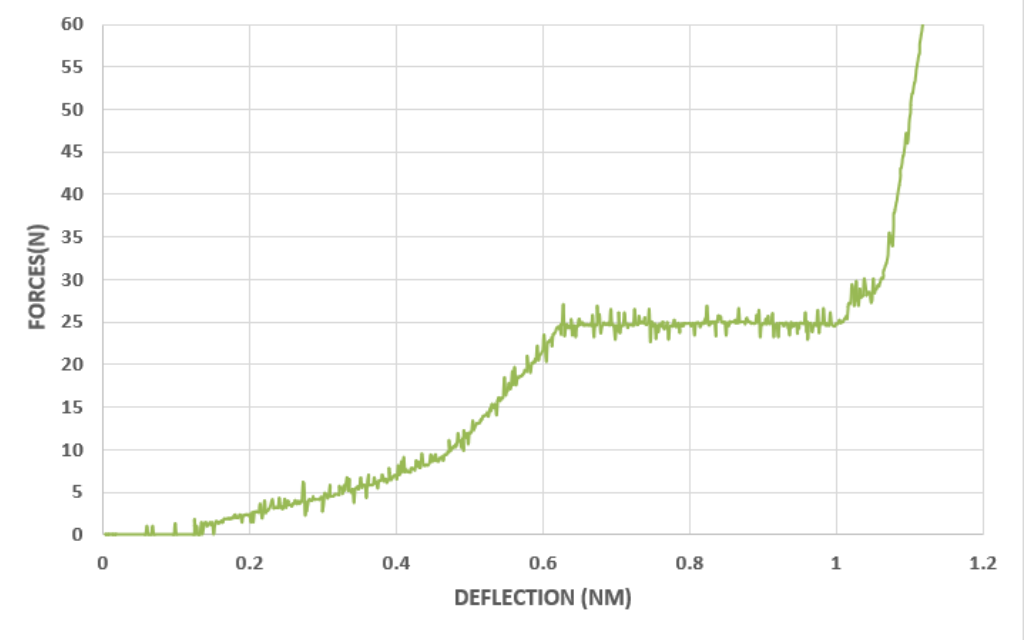


Figure 4: Axial needle fraction force of MN6 using testometric apparatus

The formulated microneedle patches showed an acceptable quantity of medicament ranged from 92.3 to 112.7 %. The accepted range of content uniformity labeled in BP is ranged from 85 % to 115%, so the results of content uniformity is obeying this range which indicate that the technique used in preparing microneedle patches is very efficient. In this basis, it was discovered that the medication was distributed evenly within the prepared patches.To obtain transdermal drug delivery, patients must apply a patch for a long period of time. The PVA/ PVP-K30 associate MNs array assists complete inclusion and the needles were quickly dissolved by the skin interstitial fluid to reduce patch- induced adverse effects [19].From (figure 5) it was observed that after 24 hr. the FBP permeated from the selected FBP-NP patch MN6 which contain pure FBP microneedle patch was 68.1%. While for simple ordinary patch, MN7 containing raw FBP was 6.6%. These results attributed to microneedle penetration of stratum corneum which improves the permeation through the skin. These results indicate that formulation of FBP as nanoparticles improve permeation through skin by 4.1 folds as nanoparticles improve the solubility of FBP. The chosen formula (MN6) delivers FBP more effectively via the stratum corneum than a simple patch by 10.3 times. The *in-vitro* permeation study of the microneedle patch in comparing with the normal simple strip using rat skin showed significant improvement ($P<0.05$) in the penetration of FBP (Figure 5).Transdermal flux (J) represented the slope of the permeation profile produced the steady-state flux of the individual formulation. The slope of FBP release for MN6 was found to be $525.72\ \mu g/cm2.hr$. While the transdermal flux for ordinary patches were found to be $1076.6\ \mu g/cm2.hr$. and $42.403\ \mu g/cm2.hr$, respectively.

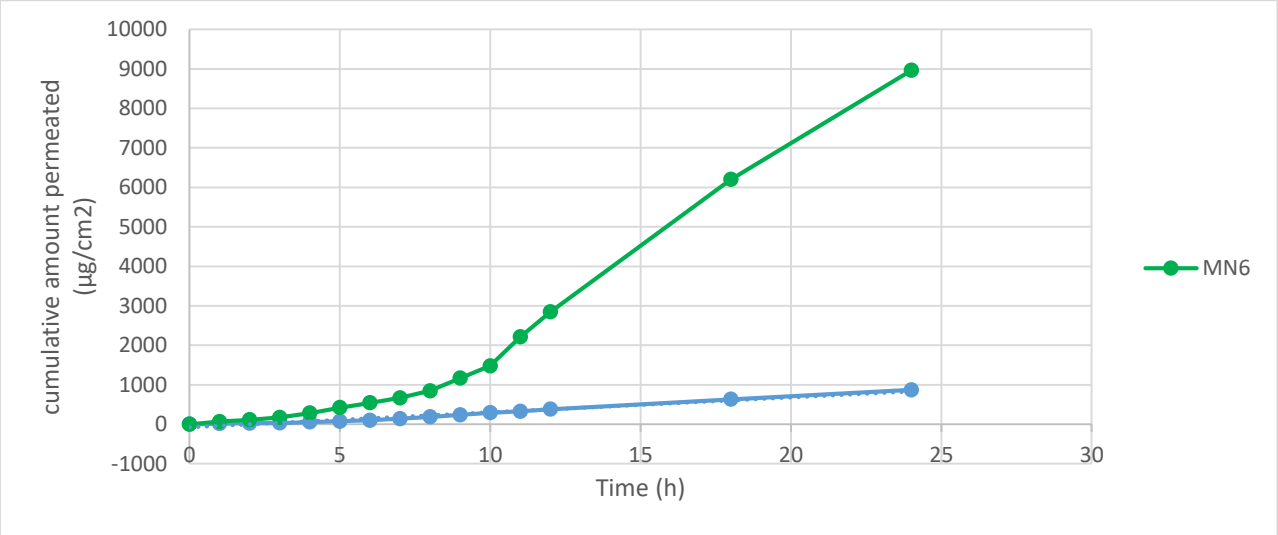


Figure (5): cumulative amount permeated from MN6 compared to ordinary patch

Table (4): Permeation Parameters of FBP

Formulas	Flux (J_{ss}) (µg/ cm2. hr) *	Permeability coefficient (P) (cm/ hr)*
MN6	525.72±12.5	$10.5*10^{-3} \pm 0.0016$
MN16	42.403±3.7	$0.84*10^{-3} \pm 0.00003$

X-ray diffraction verified the transition in the crystalline condition of FBP nanoparticles figure (6). The X-ray peaks of FBP showed several sharp and symmetrical diffraction peaks of high intensity, indicating that FBP in crystalline structure, while XRD of prepared formulas 2, 5, and 8 showed disappear of these peaks which mean the conversion of FBP to the amorphous form. This finding is consistent with previous study, in which Junghanns et al. reported that, depending on the manufacturing method, the conversion of crystal active pharmaceutical ingredient to nanoparticles may result in either crystalline or amorphous products, especially when precipitation is used [19].

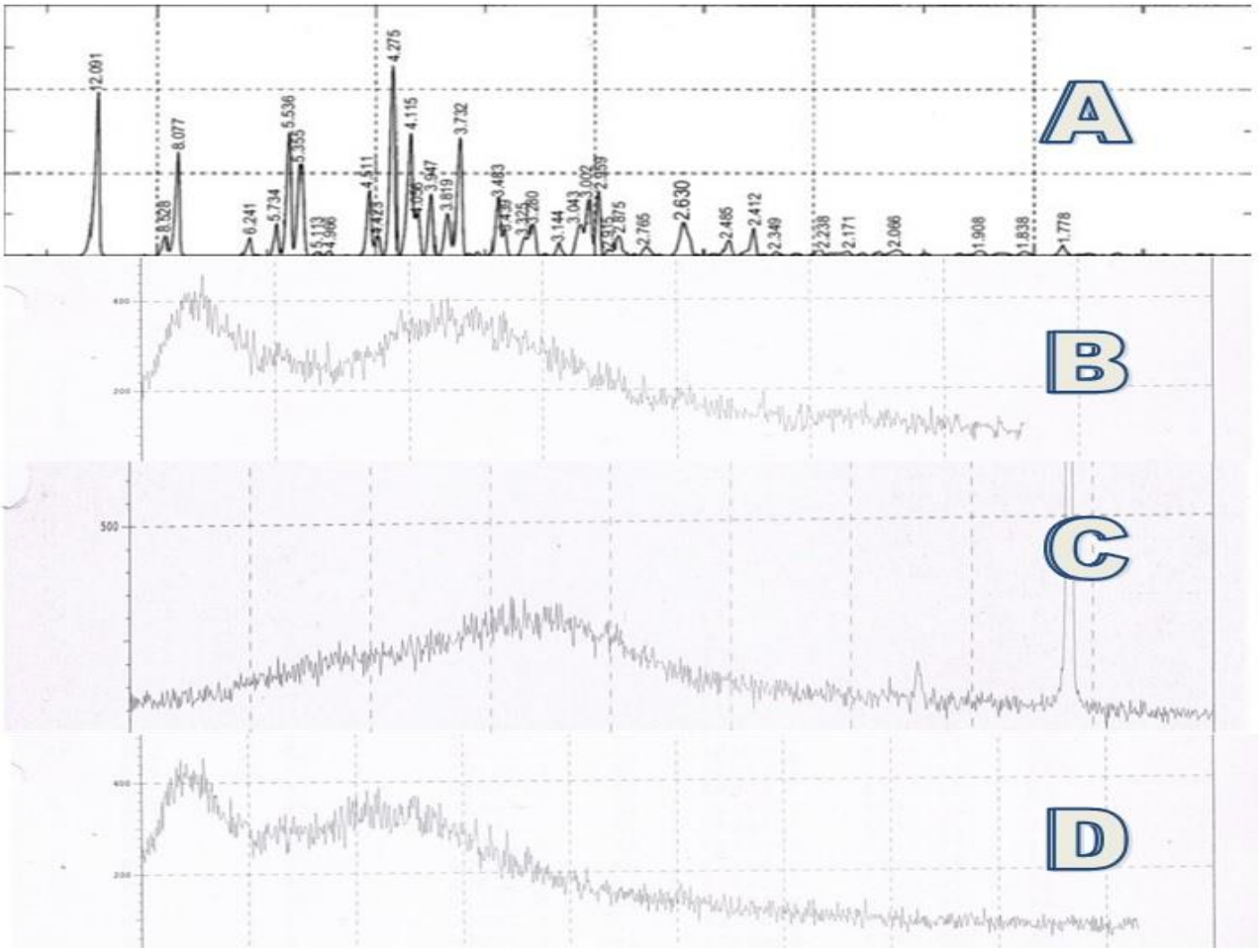
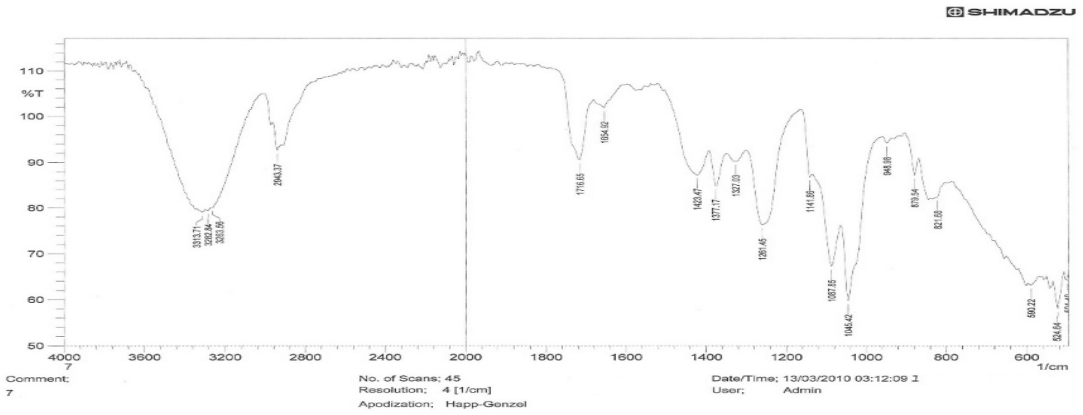


Figure (6): XRD diffractogram of (A): Pure FBP, (B): F2, (C): F5 and (D): F8.

FTIR analysis was performed to determine the chemical interaction or stability of FBP with the other components of the formula. The spectra of raw FBP and physical mixture (1:1) of FBP with polymers (PVA and PVP-K30) are shown in figures (7-8). FTIR spectra of pure drug FBP(Figure 9) show characteristic absorption peaks of carbonyl stretching band at 1696cm^{-1}, FBP pure exist as a dimer because of broadness of carboxylic acid stretching near 3000 cm^{-1} and C-F stretching peak at 1217 cm^{-1}. The medium bands at 1621, 1581, 1563, 1513 and1482 cm^{-1} were due to the stretching modes of biphenyl rings. The bands observed in the 3120–3030 cm^{-1} region



are due to the C-H stretching vibration. The appearance of these drug and polymer characteristic peaks in the FTIR spectrum of drug with polymer (physical mixture) means that the FBP is compliant with polymer and that there is no chemical reaction between the FBP and stabilizers used [20].

Figure (7): FTIR spectrum of pure FBP.

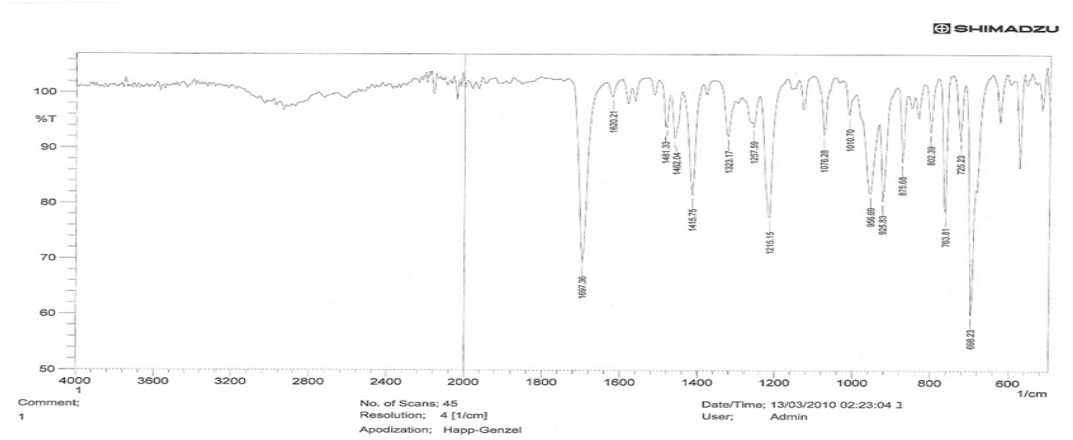


Figure (8): FTIR spectrum of FBP with PVA physical mixture

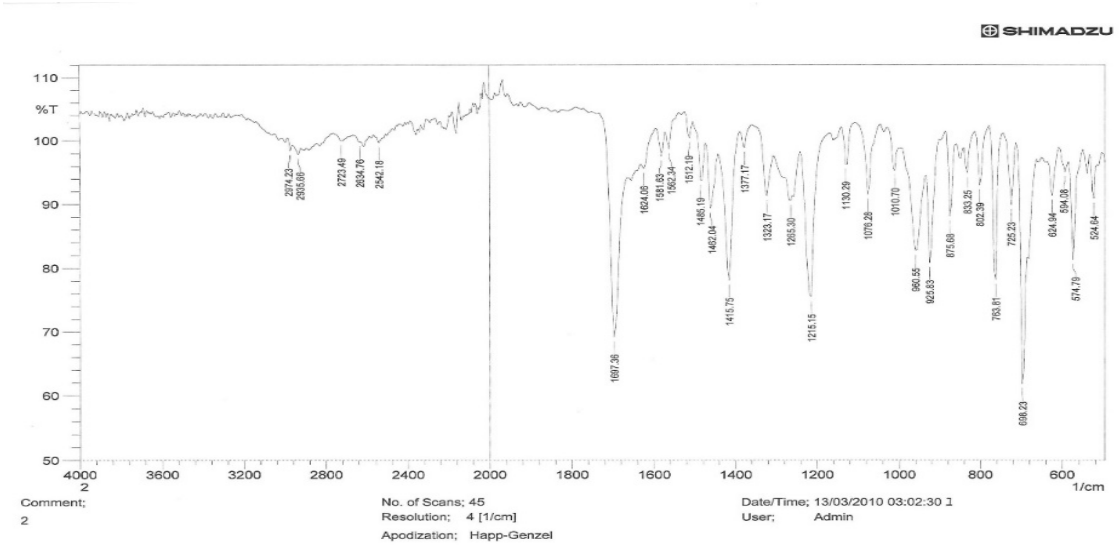


Figure (9): FTIR spectrum of FBP with PVP K30 physical mixture

Conclusion

In this study, the potential of microneedle loaded with FBP NPs for transdermal delivery were introduced. FBP permeation from patches containing FBP-NP and raw FBP were 27.6% and 6.6%, respectively. These results indicate that formulation of FBP as nanoparticles improve permeation through skin by 4.1 folds as nanoparticles improve the solubility of FBP. As opposed to MN7, which contains raw FBP as an ordinary patch, the chosen formula (MN6) delivers FBP more effectively via the stratum corneum than a simple patch by 10.3 times.

FBP is a great candidate for transdermal drug delivery. Poor bioavailability of FBP results in frequent dosing and poor patient compliance. Hence FBP nanoparticles loaded microneedle patch for transdermal delivery showed reduction in dosing frequency with better release and patient compliance.

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Conflict of interests

Declared None.

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