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Formulation and In-Vitro Evaluation of Flurbiprofen Nanoparticles for Transdermal Delivery

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ABSTRACT

The skin has been a major route for delivery of a wide range of drugs both locally and systemically. Transdermal drug delivery system (TDD) has been widely applied for local and systemic delivery of drugs. Transdermal drug delivery system has been be applied to deliver drugs systemically as an alternative for oral route due to multiple reasons such as the elimination of gastric irritation through oral intake of drugs and avoidance of first pass metabolism which improve the release of active agent over time. The development of nanotechnology played a huge impact in the field of drug design especially in enhancing the solubility of poorly soluble drugs (class II), hence improving bioavailability of these drugs**.** Flurbiprofen is a non-steroidal anti-inflammatory drug (NSAID), it's categorized under class II of biopharmaceutics classification of drugs with poor solubility and good permeability. Flurbiprofen nanoparticles were prepared by using nanoprecipitation method. Different polymers (PVA, PVP and poloxamer 188) in different concentrations were used as stabilizers in this research. The results shows that all of the prepared FBP nanoparticles formulas showed a particle size result within nano range with variations from 9nm to 158nm. The release profile of the drug from nanoparticles were improved compares to the pure drug.

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KEYWORDS

Nanoparticles, Evaluation, Formulation, Delivery.

INTRODUCTION

The skin has been a major route for delivery of a wide range of drugs both locally and systemically. Transdermal drug delivery system (TDD) has been widely applied for local and systemic delivery of drugs.TDD has the advantages of being self-administered and the effect of first pass metabolism is avoided ,but the penetration of the drugs through the stratum corneum was always the main restriction[(1)(2)].

Many technologies have been investigated to improve the absorption of transdermal drug delivery included enhancement of physical-permeation through microneedles, electroporation, iontophoresis, electroporation and nanocarriers[(3)]. These multiple techniques have distinct mechanisms, but all of them shared the same goal through formation of holes by disruption of stratum corneum to permit the passages of molecules [(4)].

Microneedles made up of micron sized structures resemble needles, hence the name. The length of these needles in the range of 100 to 1000 mm which are able to perforate the stratum corneum without reaching the nerve endings, thus there is no generation of pain[(5)].In addition of being painless, safe and economic, microneedles technology has the benefit of possessing specialized characters such as biocompatibility, biodegradability, swellability and dissolving features[(6)].

Transdermal drug delivery system has been be applied to deliver drugs systemically as an alternative for oral route due to multiple reasons such as the elimination of gastric irritation through oral intake of drugs and avoidance of first pass metabolism which improve the release of active

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agent over time. This case is important for drugs under class II category in which the solubility is the rate limiting step [7].

The development of nanotechnology played a huge impact in the field of drug design especially in enhancing the solubility of poorly soluble drugs (class II), hence improving bioavailability of these drugs [8].

Flurbiprofen (FBP) is a non-steroidal anti-inflammatory drug (NSAID), it's categorized under class II of biopharmaceutics classification of drugs with poor solubility and good permeability. FBP is a potent inhibitor of platelet aggregation, also has an important role in the management of osteoarthritis and gout [9].

The physicochemical and pharmacokinetic properties of FBP made the drug suitable candidate for transdermal delivery [10].FBP has poor water solubility (10.45±3.2µg/ml) with low molecular weight 244.26 Daltons ,log p (octanol/water) is 4.2 and short half-life of 2-6 hours [11][12] . The aim of this study was to improve the solubility and bioavailability of FBP through nanoparticles mediated transdermal microneedles drug delivery system.

MATERIAL AND METHOD

Nine formulas (F1-F9) of FBP nanoparticles were prepared by using nanoprecipitation method. A certain quantity of FBP was totally dissolved in water miscible solvent (ethanol) and injected at speed of 0.5 mL/ min using syringe infusion pump into the water containing different stabilizers in different ratio with continuous stirring of 1000 rpm. Precipitation of FBP nanoparticles occurred immediately upon mixing. Then the obtained nanoparticles are sonicated at 37 °C for 5 min and the organic solvent was then evaporated at 40 °C temperature using hotplate [13].

The compositions of the prepared formulations of FBP nanoparticles are listed in table (1).

Table 1: composition of formulas

Formula Code	FBP Conc. mg/ml	VOLUME INJECTED ml	PVP CONC %	Poloxamer 188 CONC %	PVA CONC %	SOLUTION VOL ml
F1	10	5	0.05	-	-	50
F2	10	5	0.1	-	-	50
F3	10	5	0.2	-	-	50
F4	10	5	-	0.05	-	50
F5	10	5	-	0.1	-	50
F6	10	5	-	0.2	-	50
F7	10	5	-	-	0.05	50
F8	10	5	-	-	0.1	50
F9	10	5	-	-	0.2	50

Characterization of FBP Nanoparticles

Particle size analysis

Dynamic light scattering (DLS) techniques were used to evaluate the particle size distribution, mean diameters, and polydispersity index of anoparticles at room temperature using a particle size analyzer (Marlven, UK). Measurements were taken three times for each study [14].

Zeta potential

Zeta potential considered as property of suspensions. The difference between the bulk solution (dispersing medium) and the hydrodynamic shear surface is described (slipping plane). It may be used to improve nanoparticle formulation stability over time. The zeta-plus analyzer was used to calculate it (Marlven, UK) [15]. Measurements were performed in triplicate.

Entrapment Efficiency (EE)

By calculating the concentration of free FBP in the dispersion medium, the entrapment efficiency (EE percent) was calculated indirectly. The amount of free drug was determined using an ultrafiltration technique. Briefly, 5.0 ml of FBP nanoparticles solution was placed in the upper chamber of Amicon® Ultra Centrifugal tube, a molecular cut off size (MWCO) 10 kDa and centrifuged for 30 min at 3,000 rpm. The ultrafiltration containing the free drug and the concentration of unentrapped FBP was determined spectrophotometrically at λmax 247 nm. The (EE%) was calculated using the following equation:

EE %=wT−wFwT×100

Where;

WT = weight total $drug$ is the weight of initial drug used,

Wf = weight $free\ drug$ is the weight of free drug detected in the supernatant after ultrafiltration of the aqueous dispersion[16].The measurements were performed in triplicate and values were the mean ± SD.

Surface Morphology

The morphological examination of the nanoparticles was performed using scanning electron microscopy (SEM; TESCAN, UK)[17, 18] .

In vitro drug release studies

Ten milliliter of nanodispersion (50mg drug) were placed in dialysis bags (8000-14000 D), which were sealed and placed in 900 mL dissolution medium (phosphate buffer pH 7.4 containing). Drug release study was carried out employing the USP type II dissolution apparatus (Pharma test, Germany) at 37 °C± 0.5 and 50 rpm for 2 hr. At each time interval of 5, 10, 15, 20, 30, 45, 60, 75, 90and 120 min. aliquots 5 mL of sample was collected and replaced with fresh buffers. The collected samples were analyzed spectrophotometrically at λmax 247 nm [17]. The measurements were performed in triplicate and values were the mean ± SD.

Compatibility Study

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of FBP with the prepared FBP nanoparticles were performed using FTIR spectroscopy (Shimadzu, Japan) and compared with the standard FTIR spectrum of the pure drug. Spectra were recorded between 400 and 4000 cm−1 range[19].

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 determine if the changes in the applied factors are statistically significant at level of ($P < 0.05$) and non-significant at level of ($p > 0.05$).

RESULT AND DISCUSSION

Solvent / antisolvent precipitation technique used to formulate FBP nanoparticles. This method has advantage of single step, no need of high shear/ stirring rate or high temperature. This technique is suitable for compounds that are soluble in ethanol or acetone.

The particle size of all the prepared formulas were characterized by Malvern DLS zetasizer. The particle size distribution of nanoparticles collected from a particle analyzer is described by the polydispersity index, which is a parameter. The particle size distribution index (PDI) indicates the long-term stability of nanoparticles by measuring the diameter, distance, or variance within the particle size distribution. Higher PDI values suggest a larger particle size range and the polydisperse quality of the sample, while lower PDI values indicate monodisperse formulation [20].

All of the prepared FBP nanosuspension formulas showed a particle size result within nano range with variations from 9nm to 158nm as shown in table 2.

Formulas 1, 8, and 9 have been shown to have the smallest total particle size, so they have been chosen for further investigation. When PVA was used as a stabilizer, the particle size was found to be the smallest. This may be attributed to PVA's strong affinity for both hydrophilic and hydrophobic surfaces and the fact that PVA adsorbs FBP more than PVP and poloxamer 188 [21].

Table 2: Particle size, PDI, Zeta potential and EE% of the prepared formulas.

Formula	Particle size	PDI	Zeta potantial	EE%
F1	17.8 ± 1.2	0.271 ± 0.02	4.2 ± 0.3	66 ± 4.5
F2	32.7 ± 2.4	0.233 ± 0.03	-20.1 ± 2.4	57 ± 3.5
F3	32.2 ± 2.9	0.267 ± 0.02	-16.6 ± 1.4	53 ± 4.2
F4	158 ± 18.5	0.414 ± 0.04	-4.7 ± 4.5	85 ± 4.9
F5	149 ± 13.6	0.252 ± 0.02	-18 ± 2.1	79 ± 5.7
F6	165 ± 19.6	0.374 ± 0.03	-5.25 ± 0.75	74 ± 7.4
F7	32.4 ± 4.2	0.785 ± 0.08	-15.7 ± 1.3	79 ± 7.5
F8	9.9 ± 0.98	0.148 ± 0.01	-1.41 ± 0.02	72 ± 9.4
F9	10.5 ± 1.3	0.161 ±0.02	-6.09 ± 0.8	64 ± 5.8

*Average ± Standard Deviation (n=3)

The entrapment efficiency decreased significantly ($p<0.5$) with increasing polymer ratio for all the prepared formulations. A good relationship between particle size and %EE of the drug was recorded as small size particles possessed low entrapment efficiency. Furthermore, as the

polymer ratio increased, the attachment of polymer into the nanoparticles shell might increase leading to increase the hydrophilic properties of nanoparticles. The increased hydrophilic properties of the nanoparticles might decrease the % EE leading to further drug loss in solution.

Formulas (F2, F6 and F8) were chosen to study the in-vitro drug release profiles of the prepared FBP- nanoparticles depending on particles size, PDI, zeta potential and % EE.

In vitro drug dissolution studies

In vitro drug release studies of the pure FBP and selected formulas (F2, F5 and F8) were carried out in phosphate buffer saline PH (7.4) containing 10% ethanol to simulate in vivo release in the skin. To maximize FBP solubility and thus meet the sink state, ethanol was applied to the phosphate buffer solution. The results reveal that all of the chosen formulas have a significantly higher dissolution rate ($P<0.05$) than the raw drug (figure 1). According to the Noyes– Whitney equation, the rate of solid dissolution is directly proportional to the surface area of the solid exposed to the dissolution medium. These findings matched those of Mansouri et al, who prepared ibuprofen nanoparticles using a solvent/antisolvent precipitation technique and discovered that the prepared nanoparticles dissolved 2.33 times faster than the raw medication. [13].

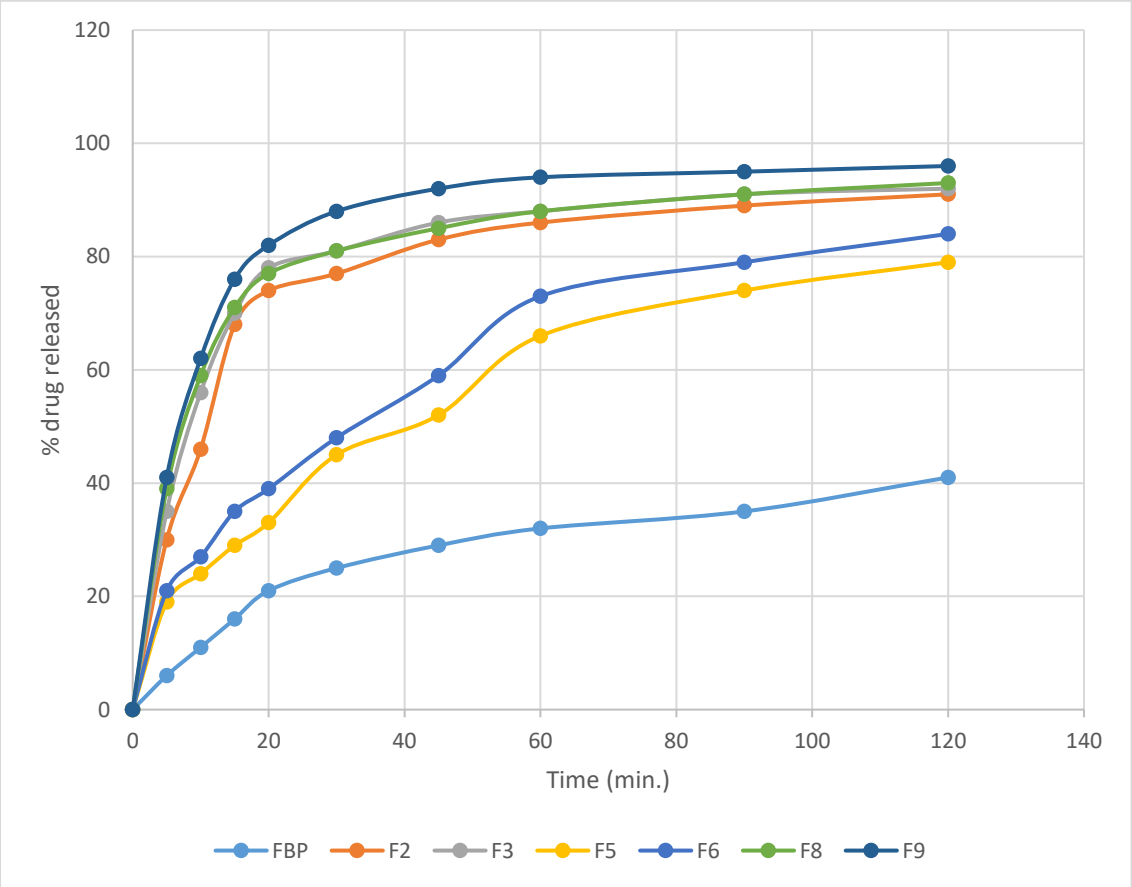

Fig.1: Dissolution profile of the prepared FBP nanoparticles (F2,F3,F5, F6, F8 and F9) in PBS (pH 7.4).

Furthermore, since these formulas involve the largest concentration of stabilizer, the dissolution of formulas (F3, F6, and F9) was also investigated (figure 1). The dissolution behaviour of formulas (F3, F6, and F9) was slightly faster than formulas (F2, F5, and F8), according to the results. Although these formulas have greater particle sizes, this may be attributed to increased wettability as a consequence of increasing polymer concentration in these formulas. These findings are consistent with Roni et al., who investigated the effects of HPMC and poloxamer on clonazepam dissolution and discovered that both HPMC and poloxamer increased the dissolution rate of clonazepam by many folds [22]. Based on these results, formula (F8) was selected for further investigations.

Scanning electron microscope (SEM)

The SEM (figure 2) photograph of the chosen formula (F8) revealed that FBP nanoparticles have a uniform shape and evenly spaced particle size, which is consistent with the zeta plus analyzer data

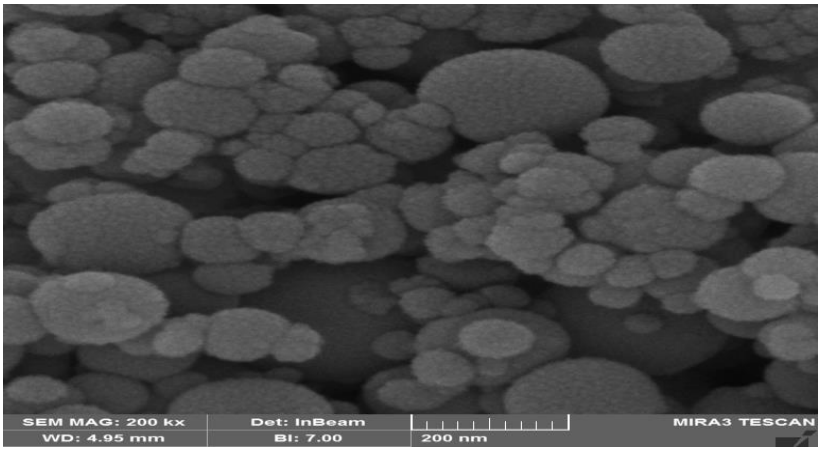

Fig.2: SEM of FBP nanoparticles (F8)

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of FBP powder show carbonyl stretching band at 1696cm^{-1}, Flurbiprofen 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} can be assigned as the stretching modes of biphenyl rings. the bands observed in the 3120–3030 cm^{-1} region are assigned to the C-H stretching vibration. (figures 3, 4). There was no changes in the FTIR bands of FBP in the prepared FBP nanoparticle formula (F8) and pure FBP, which indicate no chemical interaction between the drug and excipients used.

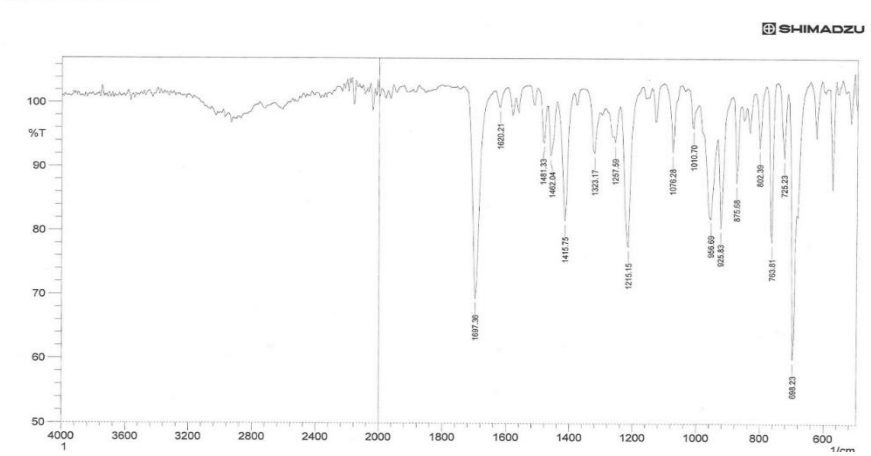

Fig.3: FTIR spectra of FBP pure drug

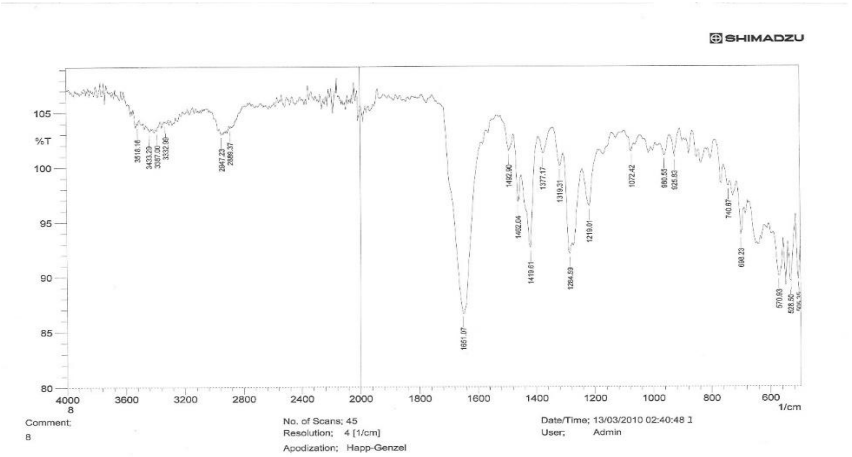

Fig.4: FTIR spectra of FBP nanoparticles (F8).

CONCLUSION

Flurbiprofen nanoparticles were prepared successfully using nanoprecipitation method. Drug: Polymer ratio of the system were important to obtain nanoparticles with desired size. PVP succeed to stabilize FBP nanoparticles and achieve uniform distribution. The encapsulation efficiencies were acceptable for all nanoparticles obtained. The release profile of the drug from nanoparticles were dependent on the type and concentration of the used polymers. The prepared FBP nanoparticles will introduced in transdermal microneedle patches in part two of this research.

CONFLICT OF INTERESTS

None.

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