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Research Article

Development and Evaluation of Ibuprofen-Loaded Glycerin Nanoparticles for Topical Application

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Abstract

The aim of this study was to prepare ibuprofen-loaded glycerin nanoparticle (IBU-GNP) based hydrogel for improved antiinflammatory effect. Ibuprofen (IBU) has low oral bioavailability and topical permeability because of poor solubility and transdermal permeability barriers. Glycerin nanoparticles (GNPs) are emerging nanotechnology for solubility improvement with the help of glycerin. Precipitation technique was opted to prepare IBU-GNPs, and the prepared nanoparticles (NPs) were subjected to appropriate in-vitro characterization techniques. IBU-GNPs revealed a uniform average diameter of 492.9 ± 44.2 nm. IBU-GNPs were formulated in hydrogel dosage form for topical application using carbopol 934 (2% w/v). Physical characterization, in-vitro release, and in-vivo antiinflammatory study in comparison to marketed IBU gel was performed for IBU-GNP hydrogel. The in-vitro drug release data revealed quick and sustained release behavior, which best fit the Higuchi model and followed Fickian diffusion as the release mechanism. IBU-GNP hydrogel rapidly lowered inflammation induced by carrageenan, followed by a sustained antiinflammatory activity, confirming an *in vitro* release pattern.

Keywords: Ibuprofen, nanoparticles, antiinflammatory, topical permeability, glycerin1.

1. Introduction

Ibuprofen (IBU) is one of the most common non-steroidal anti-inflammatory drugs (NSAIDs). It is prescribed as an analgesic, antipyretic, and anti-inflammatory drug. The chemical name of IBU is 2-(4-isobutylphenyl) propionic acid.(Potthast et al. 2005, Rainsford 2009, Kiran et al. 2015) IBU belongs to biopharmaceutical classification system class II, which means it has high permeability and low solubility that is 21 mg/L at 25°C (Sütő, Berkó, Kozma, Kukovecz, Budai, et al. 2016). Consequently, when administered orally, poor solubility results in low bioavailability, increased dosage, large inter-subject and intra-subject variation, and large variations in blood drug concentrations under fed and fasted conditions.(Potthast et al. 2005, Pintu et al. 2012, Faruki, Razzaque, and Bhuiyan 2013). Accordingly, IBU is used in high dose (2400 mg/day) for the management of arthritis. Unfortunately, high dose of IBU leads to cardiovascular and gastrointestinal adverse reactions. Therefore, cardiovascular and gastrointestinal safety can be ensured by topical application of IBU (Harirforoosh, Asghar, and Jamali 2014).

Moreover, poor permeability of IBU through human skin makes it difficult to attain optimum bioavailability. The stratum corneum and the external lipid rich layer of the epidermis are major permeability barriers for transdermal absorption of IBU. In previous studies, various methodologies were used to increase solubility of IBU, such as prodrugs, inclusion complexes, and solid dispersion method, and microcapsules formulation. The dissolution and bioavailability of IBU from these formulations differed widely. Additionally, these strategies were time consuming, costly, exhibited poor flow characteristics, and were found wanting in handling difficulties.(Lakshmi et al. 2011) Therefore, an attempt was made to prepare nanoparticles (NPs) for the enhancement of IBU solubility with the aid of simple and economical method.

Previously, NPs were prepared for solubility enhancement by various methods involving solvent evaporation (Niwa et al. 1993), complex coacervation (Ducel et al. 2004), salting out method (Allémann et al. 1993), microencapsulation by supercritical fluid technique (Yousif, AlMarzouqi, and Mohsin 2016) and monomer emulsion evaporation method.(Niewolik et al. 2021) The major drawbacks of earlier methods were complexity of procedure and low loading efficiency.(Jiang et al. 2005b) Thus, the simplest and cost effective way to improve the solubility of IBU is to prepare ibuprofen-loaded glycerin nanoparticles (IBU-GNPs). IBU-GNPs for skin application were prepared by precipitation method because of ease, effectiveness, simplicity, spontaneity and ability to reproducible NPs with high loading capacity. This method is used to incorporate hydrophobic drugs easily into NPs' core. In precipitation method, the solution of drug and polymer in a suitable solvent is mixed into an anti-solvent with continuous stirring to form precipitated NPs. Glycerin being a polyol compound increases solubility of IBU in IBU-GNPs (AbouTaleb, Hamd, and Abdellatif 2017, Govender et al. 1999). The small size of the IBU-GNPs will ensure close contact between the NPs and the lipid bilayer of the stratum corneum, resulting in increased penetration of the drug into the skin (Sütő, Berkó, Kozma, Kukovecz, Budai-Szűcs, et al. 2016).

The hydrogel can be obtained by using various agents such as Carbopol, Pluronic F127, Natrasol 250 and Satiaxane CK 91. Carbopol is a polymer of polyalkenyl ethers or divinyl glycol, cross-linked with acrylic acid. Carbopol is an ideal candidate for topical hydrogels because of its hydrophilic nature that helps in formulation and their non-irritant and non-toxic behavior with no hypersensitivity when applied to human skin (AbouTaleb, Hamd, and Abdellatif 2017, Guterres, Alves, and Pohlmann 2007, Sahoo, Pani, and Sahoo 2014).

The aim of present study was to prepare IBU-GNP based hydrogel in two steps; firstly, the IBU was incorporated into glycerin to make IBU-GNPs and then the hydrogel loaded IBU-NPs were formulated by using Carbopol 934. The prepared IBU-GNPs and the hydrogel formulations were subject to appropriate physical characterization techniques. Finally, the in- vitro release, and in-vivo anti-inflammatory responses were determined for IBU-GNP hydrogel and compared to the marketed IBU gel.

2. Material and Methods

2.1. Materials

IBU was purchased from AstraZeneca Pharma (India Ltd.). Glycerin was acquired from AnalaR Chemicals Ltd. (Poole, England). Carbopol 934 and carrageenan were procured from Sigma

Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade and were used without any further purification.

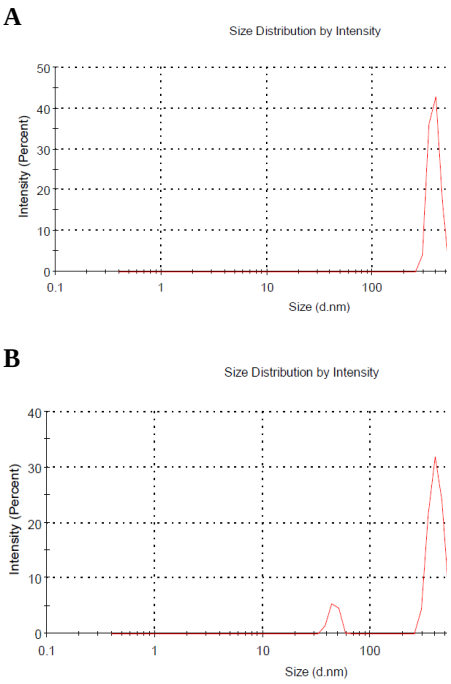

Figure 1. Particle size distribution of IBU-GNPs (A) and blank GNPs (B).

2.2. Preparation of IBU-GNPs

Precipitation method was utilized to prepare IBU-GNPs with glycerin, methanol, and distilled water. We have developed IBU-GNPs by dissolving 400 mg IBU and 100 mL glycerin in a solvent that is miscible in water. For this purpose, 100 mL methanol was used. The mixture was sonicated (Elma E60H Elmasonic, Germany) for 20 minutes to achieve homogenous solution and poured into a beaker, containing 400 mL distilled water, with continuous stirring on magnetic stirrer at 500 rpm. The precipitates of IBU-GNPs were formed, which were kept on stirring overnight until methanol phase evaporated. The obtained IBU-GNPs were purified from large aggregates by filtration through syringe filters with pore size of 0.45 µm. This process was repeated for blank GNPs without addition of IBU. The obtained formulations were subjected to high speed homogenization (Heidolph Instruments, Germany) to prevent crystallization (Li et al. 2017, AbouTaleb, Hamd, and Abdellatif 2017).

2.3. Characterization of IBU-GNPs

2.3.1. Particle Size, Zeta Potential and Polydispersity Index (PDI)

Particle size, PDI and zeta potential of blank GNPs and IBU-GNPs was measured by zetasizer (ZS 90 Malvern, UK). The samples were appropriately diluted and subjected to homogenization before transferring them into standard zeta cuvette, and analyzed in zetasizer at 25^{o}C temperature (AbouTaleb, Hamd, and Abdellatif 2017, Gul et al. 2022).

2.3.2. Scanning Electron Microscopy (SEM)

Surface morphology of blank GNPs and IBU-GNPs was observed by SEM (VEGA3 TESCAN LMU). The blank GNPs and IBU-GNPs were dried before analysis. The samples were coated with gold to avoid charge-up of samples.(Yousif, AlMarzouqi, and Mohsin 2016) The sample stub prepared was positioned in SEM, and photomicrographs were achieved at different scanning voltages.

2.3.3. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was performed for raw IBU, glycerin and IBU-GNPs by using FTIR spectrometer (L160000A, PerkinElmer, USA). The wavelength range of 4000 – 600cm^{-1} were utilized to achieve IR spectra of all samples. The sample of raw IBU was in dried powder form, while the glycerin and IBU-GNP were in liquid forms (Sütő, Berkó, Kozma, Kukovecz, Budai-Szűcs, et al. 2016, Pintu et al. 2012).

2.3.4. Entrapment Efficiency

Entrapment efficiency of IBU-GNPs suspension was determined by sonicating NPs suspension for 5 minutes. 0.2 mL of IBU-GNPs was collected using syringe

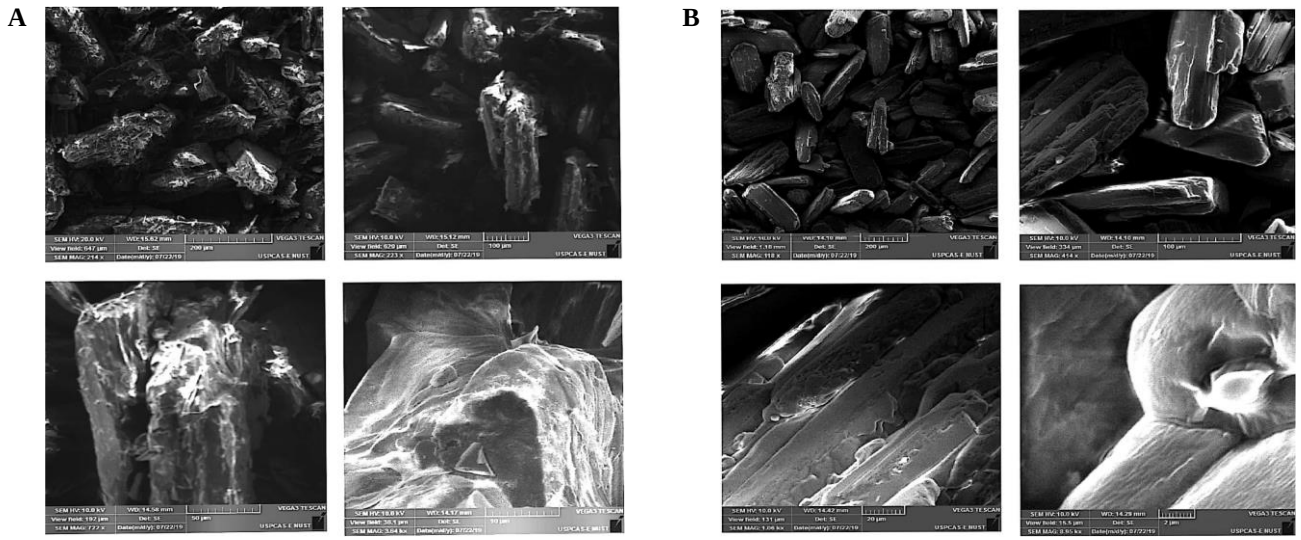

Figure 2. SEM images of blank GNPs (A) and IBU-GNPs (B). Scale bar represents 200, 100, 50 and 10 µm and 200, 100, 20, 2 µm for blank GNPs and IBU-GNPs, respectively.

.filter of 0.45µm membrane and appropriately diluted with methanol. The prepared sample was transferred to cuvette and analyzed to determine the absorbance by UV spectrophotometer (Jasco V530) at a maximum wavelength of 221 nm(Vidal and Alegre 2013, Jiang et al. 2005a). Finally, the entrapment efficiency was calculated by the following formula:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Actual amount of IBU in IBU-GNPs}}{\text{Theoretical amount of IBU in IBU-GNPs}} \times 100$$

2.4. Preparation of IBU-GNPs Loaded Hydrogel

IBU-GNP hydrogel was prepared by adding appropriate volume of Carbopol 934 (2%, w/v) into the pre-filtered aqueous solution of IBU-GNPs. This step was performed in a water-bath (HH S-8, China) to provide 50°C temperature, with continuous manual stirring, until the gel became homogenous. The Carbopol in hydrogel produces free acids, so, sodium hydroxide solution (0.4%, w/v) was poured into the gel to achieve the desired pH. Later, the IBU-GNP hydrogel was sonicated for 10 minutes to achieve bubble free hydrogel. Blank-GNP loaded hydrogel was prepared, following the same procedure, by using blank-GNPs in place of IBU-GNPs (Abdellatif and Tawfeek 2016a, AbouTaleb, Hamd, and Abdellatif 2017).

2.5. Characterization of IBU-GNPs Loaded Hydrogel

2.5.1. pH

Digital pH meter (PHS-25CW) was used for evaluation of pH of blank GNPs hydrogel and IBU-GNP hydrogel. For this measurement, 1 g of hydrogel was dissolved in 100 mL methanol and sonicated for 5 minutes. Then pH meter electrodes were dipped directly into the sample at 25°C and pH was noted. The pH measurement was done in triplicate samples (Rasool et al. 2010, Kaur 2013).

2.5.2. Viscosity

Viscosity of IBU-GNP hydrogel and blank GNPs hydrogel was determined by spindle No. 4 equipped to Brookfield digital viscometer. The analysis was performed for triplicate samples at 25°C temperature, and at different rotation speeds (1 and 10 rpm) to determine the effect of shear stress on formulations (Jug et al. 2005).

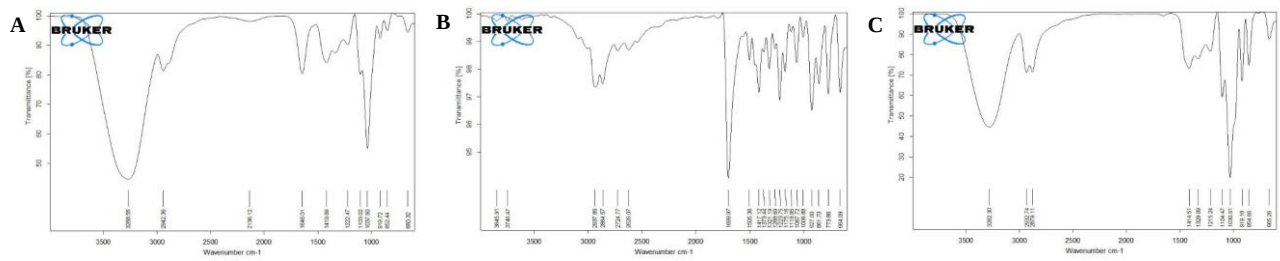

Figure 3. FTIR spectra of IBU-GNPs (A), ibuprofen (B) and glycerin (C).

2.5.3. Homogeneity

The blank GNP hydrogel and IBU-GNP hydrogel were visually analyzed for homogeneity in triplicate samples after placing each sample in separate container. Homogeneity of each sample was analyzed by pressing a small amount of hydrogel between the index finger and the thumb (AbouTaleb, Hamd, and Abdellatif 2017).

2.5.4. Spreadability

Spreadability of blank GNPs hydrogel and IBU-GNP hydrogel was measured for triplicate samples of each hydrogel by simply placing 1 g of each sample on a horizontal plate and measuring their diameter after 1 minute. 125 g was the standard weight fixed on upper plate (AbouTaleb, Hamd, and Abdellatif 2017).

2.5.5. Entrapment Efficiency

IBU-GNP hydrogel was evaluated for entrapment efficiency by ultraviolet (UV) spectrophotometer. For entrapment efficiency 100 mg of each hydrogel was extracted in beaker using 100 mL methanol as solvent.(Abdellatif and Tawfeek 2016a) The above mixture was sonicated for 5 minutes. The supernatant was filtered *via* membrane syringe filter of 0.45µm pore size. Then, serial dilutions were made to achieve the desired hypothetical concentration. The absorbance of sample was measured at 221 nm wavelength in UV-visible spectrophotometer (Vidal and Alegre 2013). The entrapment efficiency was calculated by using the following formula:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Actual amount of IBU in IBU-GNP hydrogel}}{\text{Theoretical amount of IBU in IBU-GNP hydrogel}} \times 100$$

2.5.6. Stability Studies

The stability study was performed on IBU-GNP hydrogel formulation by storing it at 4°C and 25°C for 30 days. The stability study included determination of chemical and physical stability parameters. The chemical stability was assessed by determining the free IBU released from IBU-GNP hydrogel through UV spectrophotometer as discussed. The physical stability was evaluated by optically observing IBU-GNP hydrogel for homogeneity and appearance (AbouTaleb, Hamd, and Abdellatif 2017).

2.6. In-vitro Drug Release

In-vitro drug release of IBU from IBU-GNP hydrogel and marketed IBU gel was assessed by 'dialysis bag diffusion technique' and samples were collected for 24 hours. Dialysis method was utilized because it reveals the release kinetics of hydrophobic drugs more effectively in nano-carrier systems.(Hua 2014) To conduct in-vitro release study, we placed IBU-GNP hydrogel equivalent to 800 µg IBU in dialysis bag (Spectrum Laboratories, Inc., Rancho Dominguez, USA) having molecular weight cut off 10 kDa with the addition of 9.95 mL of phosphate buffered saline (PBS) (pH 7.4) in it. The bag was tied from both ends and suspended in a vessel of USP dissolution

Table 1 Appearance, homogeneity, spreadability, pH and viscosity.

Parameter	Blank GNPs	IBU-GNPs	IBU-GNP hydrogel	Blank hydrogel
Appearance	Colorless and clear	Colorless and clear	Colorless and clear	Colorless and clear
Homogeneity	Good	Good	Good	Good
Spreadability (g.cm/second)	-	-	6.10 ± 0.10	5.63 ± 0.06
pH	7.6 ± 0.1	7.4 ± 0.2	6.7 ± 0.2	6.4 ± 0.1
Viscosity (cP)	-	-	4688.0 ± 85.1	4184.0 ± 22.8

apparatus II (BETA-8L, Galvano Scientific), containing release medium 500 mL PBS (pH 7.4). To mimic in-vivo conditions, the dialysis system was held at 37°C temperature and rotated at 100 rpm. At scheduled intervals, 10 mL sample was withdrawn from each vessel followed by the replacement with equal volume of fresh PBS to preserve sink conditions. Then UV spectrophotometer, at a wavelength of 221 nm, was used to analyze these samples for IBU contents. Similarly, the above procedure was repeated for marketed IBU gel for comparative study (Jiang et al. 2005a, Aldawsari et al. 2015, Gönüllü et al. 2015, Sütő, Berkó, Kozma, Kukovecz, Budai-Szűcs, et al. 2016).

2.7. In-vivo Anti-inflammatory Study

2.7.1. Experimental Animals

Male Wistar rats (weighing 200-250 g) were received for anti-inflammatory study from Riphah Institute of Pharmaceutical Sciences, Islamabad, Pakistan. The animals were brought 1 week prior to experimentation date for acclimatization to the laboratory environment. The animals were kept in cages with sawdust on the floor throughout the study. They were retained in well maintained environment of 25 ± 1°C temperature, alternative light-dark cycles and relative humidity of 40-60%. The animals were fed with standard quantity of rat pellet diet with free access to water. This animal study was performed according to National institute of health policies, and rules of Institutional Research and Ethics Committee of Riphah Institute of Pharmaceutical Sciences, Islamabad (Approval # REC/RIPS/2017/00).

Table 2. Release kinetics of IBU from IBU-GNP hydrogel.

Kinetic model	R^2	Slope
Zero order kinetics	0.7928	K0 = 5.921
First order kinetics	0.8836	K1 = 0.058
Hixon-Crowell kinetics	0.8617	KHC = 0.178
Higuchi model	0.9116	KH = 5.160
Korsmeyer-Peppas kinetics	0.8999	n = 0.368

2.7.2. Study Design

Carrageenan-induced rat paw edema model was utilized to investigate the anti-inflammatory response of IBU-GNP hydrogel (Bhaskar et al. 2009, Rasool et al. 2010, Abdullah et al. 2011, Lakshmi et al. 2011, Abdellatif, El Hamd, and Saleh 2016). The animals were randomly divided into four groups (n=5) as below;

Group I: only saline injected, normal group (neither carrageenan injected nor formulation applied).

Group II: only carrageenan injected, inflamed control group.

Group III: inflamed by carrageenan injection and treated with IBU-GNPs hydrogel formulation

Group IV: inflamed by carrageenan injection and treated with marketed IBU gel formulation.

On the experimentation day, either 1 g of IBU-GNP hydrogel or marketed IBU gel

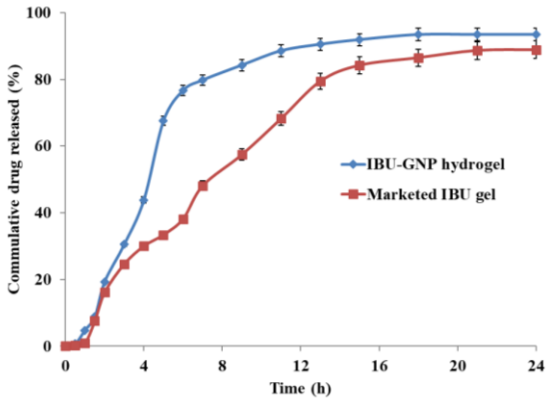

Figure 4. ***In vitro*** **release pattern of IBU from IBU-GNP hydrogel. The study was performed by using dialysis bag diffusion technique for 24 h in comparison to marketed IBU.**

was applied on the left hind paw of each rat in respective groups with 50 times gentle rubbing by index finger. After 30 minutes, 0.1 mL of carrageenan (1 % w/v, made in normal saline) was subcutaneously injected into sub plantar tissues to induce edema on left hind paw. Similarly, 0.5 mL of normal saline and carrageenan 1 % was injected to the un-inflamed and inflamed control group, respectively.

2.7.3. Measurement of Edema Volume and Percent Inhibition of Paw Edema

Plethysmometer (UGO Basile 7140, Italy) was used to measure edema volume of all groups for 8 hours with interval of 1 hour (0, 1, 2, 3, 4, 5, 6, 7 and 8 hours). Percent inhibition of paw edema indicates anti-inflammatory activity of experimental groups, as compared to the control group.(Biswal et al. 2014) This percent inhibition was calculated by the following equation:

$$\text{Percent inhibition (\%)} = \frac{Vc - Vt}{Vc} \times 100$$

Where,
Vc = paw volume of control group
Vt = paw volume of experimental group

2.8. Statistical Analysis

All the studies were performed in triplicate and the results were expressed as mean ± standard deviation. Regression analysis and ANOVA were also applied to the data to analyze statistical significance of data obtained in the calibration curve formation and drug release study.

3. Results and Discussion

The precipitation method was employed in the present study to prepare the IBU-GNPs. This method had been successfully used in past for the preparation of GNPs of loratadine, where the solubility, permeability and transdermal bioavailability increased (AbouTaleb, Hamd, and Abdellatif 2017). IBU has low solubility and high permeability, which puts a limitation to its bioavailability (Yasuhiro et al. 2012, Vidal and Alegre 2013). IBU-GNPs prepared by precipitation method not only enhanced bioavailability of IBU but also displayed sustained release behavior when formulated in hydrogel. Moreover, it minimizes the vascular events that are associated with repeated dosing of IBU, as it has shorter biological half-life i.e. 2 hours (Sütő, Berkó, Kozma, Kukovecz, Budai-Szűcs, et al. 2016). The IBU-GNPs were incorporated into hydrogel dosage form using Carbopol 934 2% w/v (Abdellatif and Tawfeek 2016a). The IBU-GNPs hydrogel prepared in the current study had a significantly improved in -vitro release profile, and anti-inflammatory activity when compared with marketed IBU gel.

3.1. Preparartion of IBU-GNPs

The IBU-GNPs during the preparation process were initially clear and colorless, and with continuous stirring, it became cloudy which indicated the formation of IBU-GNPs in the solution. The solution was kept overnight on stirring to evaporate methanol and then filtered

through syringe filters of 0.45 µm. The prepared IBU-GNPs were later subject to further characterization.

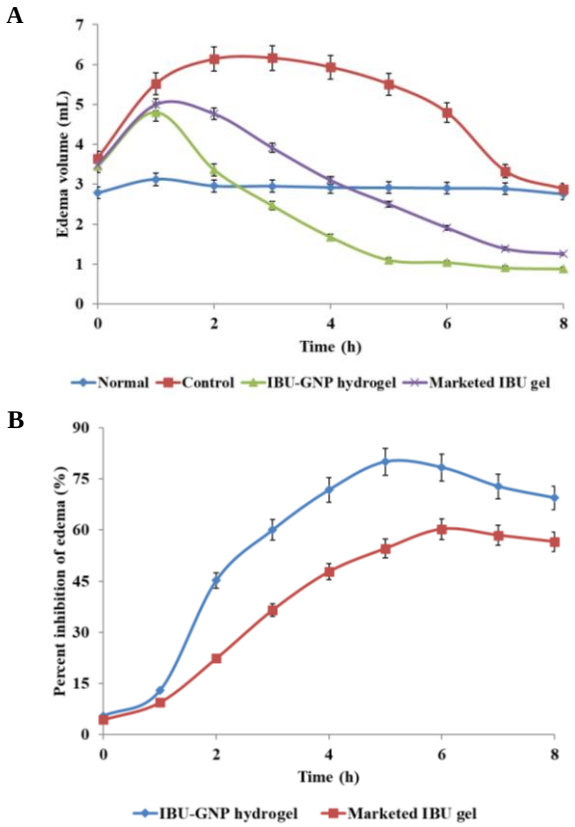

Figure 5. Edema volume (mL) (A) and percentage inhibition of edema (B) for IBU-GNP hydrogel and marketed IBU gel.

3.2. Particle Size, Zeta Potential and PDI

The particle size, zeta potential and PDI of IBU-GNP and blank GNP were determined by zetasizer. Figure 1a indicates that the IBU-GNPs had one peak with uniform Z-average diameter of 492.9 ± 44.2 nm at 100% intensity. The size of GNPs before the incorporation of IBU was larger and non-uniform than IBU-GNPs. The Z-average diameter of blank GNPs was 903.1 ± 59.8 nm, where two peaks were recorded; first peak at 405.3 nm with 88.4% intensity, and second peak at 46.0 nm with 11.6% intensity (Figure 1b). The zeta potential is the surface charge that depicts the stability of formed NPs. Generally, zeta potential value around -25 mV shows sufficient electrostatic repulsion between the NPs that prevents agglomeration and ensure long-term stability regarding the physical parameters. IBU-GNPs and blank GNPs revealed zeta potential of -12.9 ± 6.4 and -8.2 ± 3.7, respectively. A negligible change in zeta potential values after loading of IBU in GNPs depicts that no significant change in surface charge occurred during the preparation process; moreover, no drug was accumulated on the surface of NPs leading to the hypothesis that all IBU was loaded inside the GNPs. It represents the distribution of drug in polymer indicating the stability of IBU-GNPs. PDI depicts the overall particle size distribution in the solution. Ideally, the PDI value < 0.7 indicates the homogenous distribution with narrow range of particles in solution while the PDI value > 0.7 shows heterogeneity of particles.(Pereira et al. 2006, Rosenfeld et al. 2008, Lewis and Li 2010, Viéville, Tanty, and Delsuc 2011) In this research study, the PDI of IBU-GNPs and blank GNPs measured by zetasizer were 0.392 and 1, respectively, indicating the homogeneity and uniform distribution of particles in IBU-GNPs and homogeneity in blank GNPs. These results confirmed the coating of IBU with glycerin with high water solubility of IBU-glycerin complex in IBU-GNPs.

3.3. SEM

Morphology of IBU-GNPs and blank GNPs were determined by SEM analysis. Both the formulations were evaporated, on hot plate, until dried. The powder samples were coated with gold to enhance imaging. According to the Figure 2a (blank GNPs), and Figure 2b (IBU-GNPs), the surface texture IBU-GNPs was smoother and uniform in comparison to blank GNPs because after loading of drug, the IBU has occupied the pore volume.

3.4. FTIR

FTIR analysis was performed on raw IBU powder, liquid glycerin, and IBU-GNPs. The FTIR spectrum of IBU (Figure 3b) indicated broad band with different transmittance at 2626cm^{-1}-2739cm^{-1}, which was due to the O-H stretching of carboxylic acid. The functional group of IBU i.e. carbonyl group of propionic acid showed stretching band at 1699cm^{-1}. The aromatic C=C bending bond was reflected by a small band at 1417cm^{-1}. On the other hand, the glycerin FTIR spectrum (Figure 3c) exhibited broad peak at 3282cm^{-1} due to stretching motion of alcoholic O-H group. The C-O bonding in glycerin showed a bending at 1030cm^{-1}. Coming towards the FTIR spectrum of IBU-GNPs (Figure 3a), the combined bands from IBU and glycerin are visualize as expected. The IBU-GNPs FTIR spectrum showed a broader band for O-H stretching motion at 3400-2800cm^{-1} resembling to that of the glycerin FTIR spectrum, which indicates the coating of IBU by glycerin. Secondly, in comparison to IBU FTIR spectrum, stretching band of carboxyl group, C=O of glycerin coated IBU, exposed at 1646cm^{-1}, was smaller than that of IBU. This indicates the loss of IBU-IBU hydrogen bonding which is the key feature of IBU at solid state that leads to encapsulation of IBU by glycerin (Yousif, AlMarzouqi, and Mohsin 2016). The spectrum of IBU-GNPs resembles mostly to the FTIR spectrum of glycerin, which supports the hypothesis of encapsulation of IBU by glycerin.

3.5. Entrapment Efficiency

The standard calibration curve was developed for the measurement of entrapment efficiency, and in-vitro study, by analyzing the serial dilutions of raw IBU at a wavelength of 221 nm in UV-visible spectrophotometer because IBU shows highest calibration sensitivity at 221 nm.(Vidal and Alegre 2013) The IBU-GNPs were analyzed in triplicate samples and the mean entrapment efficiency was found to be 96.36 ± 1.37%. The standard deviation is < 2% which depicts the reproducible concentration of IBU in GNPs (AbouTaleb, Hamd, and Abdellatif 2017). Thus, the IBU-GNPs hydrogel was formed.

3.6. Preparation of Hydrogel Loaded IBU-GNPs

The hydrogel dosage form is easy to apply with good aesthetic properties than simple gels, creams, and ointments (Raphael et al. 2015). Therefore, the IBU-GNPs and blank GNPs were incorporated into hydrogel with the help of Carbopol 934 (2% w/v).

3.7. Physical Characterization of IBU-GNP Hydrogel

The prepared hydrogel was colorless and clear. The hydrogel when pressed between the index finger and thumb was free from aggregates, with good homogeneity. The spreadability is an important parameter for hydrogel, as it reflects to the area which the gel will cover when applied to the affected skin. The higher spreadability of hydrogel indicates that small amount of sheer is required to spread it on the affected area (Abdellatif and Tawfeek 2016a). **Table 1** shows higher spreadability value of IBU-GNP hydrogel than blank GNP hydrogel. The pH mentioned in **Table 1** was determined in triplicate samples for IBU-GNPs, blank GNPs, and their hydrogel formulations. The mean pH of hydrogel formulation was similar to that of rat's skin pH, which reveals the compatibility of hydrogel with the rat skin (Abdellatif and Tawfeek 2016b, Abdellatif and AbouTaleb 2015). Higher viscosity of IBU-GNP hydrogel compared to blank GNP hydrogel supports that IBU-GNP hydrogel is appropriate for transdermal delivery of IBU.

3.8. Entrapment Efficiency of IBU-GNP Hydrogel

The entrapment efficiency of IBU-GNP hydrogel was determined by UV spectrophotometric analysis at wavelength of 221 nm. The mean entrapment efficiency of IBU-GNP hydrogel formulation was found to be 93.98 ± 0.76%, the standard deviation < 2% indicates the reproducibility. This result revealed that the incorporation of IBU-GNP in hydrogel dosage form does not affect its entrapment efficiency (AbouTaleb, Hamd, and Abdellatif 2017).

3.9. In-vitro Release Study

The in-vitro release study of IBU from IBU-GNP hydrogel and marketed IBU gel was conducted by 'dialysis bag diffusion technique', in which the specified amount of formulation and marketed gel was placed in the dialysis membrane immersed in vessel containing PBS (pH = 7.4) as release medium. The cumulative percent drug release was calculated by analyzing the acquired samples *via* UV spectrophotometric analysis (Figure 4). Marked differences were observed in the release pattern of IBU-GNP hydrogel, and marketed IBU gel. The IBU released in first 6 h from IBU-GNP hydrogel and marketed gel was 76.67% and 38.18% respectively. This pattern formed due to glycerin, as glycerin has greater solubility in PBS (AbouTaleb, Hamd, and Abdellatif 2017). The sustained release behavior for short half-life drug, like IBU, has great impact on the dosing frequency and patient compliance.(Yousif, AlMarzouqi, and Mohsin 2016) The IBU-GNP hydrogel exhibited sustained release behavior after 6 h that is much earlier than marketed IBU gel, which exhibited this behavior at 15 h. These results confirm that the initial burst due to higher solubility and sustained release behavior of IBU-GNP hydrogel will improve the patient compliance.

The drug release data of IBU-GNP hydrogel was fitted into different kinetic models. The best-fitted model was selected based on highest R^2 value. According to **Table 2**, the in-vitro release data of IBU-GNP hydrogel best fitted Higuchi model with a predominant R^2 value than other kinetic models. Thus, it has been proposed that IBU-GNP hydrogel follow diffusion mechanism to release IBU, this also supports the sustained release behavior pattern of IBU-GNP hydrogel. The "n" value was measured by fitting the data to Korsmeyer-Peppas model to find out which diffusion mechanism is followed for drug release. The value of n = 0.368 and less than 0.45, indicates that Fickian diffusion mechanism was followed by the hydrogel formulation (Gouda, Baishya, and Qing 2017).

3.10. In-vivo Anti-inflammatory Study

In-vivo anti-inflammatory activity was assessed by carrageenan-induced rat paw edema model. Carrageenan was injected to all groups except normal group to induce inflammation leading to significant increase in paw volume in comparison to normal group. Paw volume after 8 h was higher in control group than in the normal group. The reduction in paw volume in IBU-GNP hydrogel and marketed IBU gel treated groups begin at 1 h after the carrageenan injection, but these results were not significant enough to predict the anti-inflammatory activity of both formulations. IBU-GNP hydrogel was substantially more effective than marketed IBU gel in terms of reduction in edema volume after 8 h treatment. The edema volume of IBU-GNP hydrogel and marketed IBU gel groups after 8 h of carrageenan injection were reduced to greater extent than control group, which were 0.88 mL, 1.25 mL, and 2.88 mL, respectively. Percent inhibition of edema

was calculated for IBU-GNP hydrogel and marketed IBU gel in comparison to control group, which indicated the higher anti-inflammatory activity of IBU-GNP hydrogel than marketed IBU gel (Figure 5b). The increasing order of anti-inflammatory activity of IBU-GNP hydrogel from 1 h followed by maintained level of anti-inflammatory activity after 5 h is demonstrated in Figure 5a. This pattern of anti-inflammatory activity of IBU-GNP hydrogel resembles to its release pattern revealed in the in-vitro experiment.

3.11. Stability Determination

The stability study was performed on IBU-GNP hydrogel formulation after storing at 4°C, and 25°C for 30 days. The entrapment efficiency was measured in triplicate at 221 nm *via* UV-visible spectrophotometer. The results exposed that IBU-GNP hydrogel stored at 4°C had better entrapment efficacy than that stored at 25°C. While on optical observation both samples were clear and free from any kind of aggregates.

4. Conclusions

The IBU-GNP hydrogel was successfully developed by utilizing precipitation technique, for the transdermal delivery of IBU, with better performance in terms of enhanced drug solubility, augmented anti-inflammatory activity, and improved patient compliance with minimal cost. After promising results from zetasizer, SEM, and FTIR analysis of IBU-GNPs, it was incorporated into hydrogel dosage form using Carbopol 934 (2% w/v). The hydrogel was subjected to physical characterization, in-vitro release study, and in-vivo anti-inflammatory study. The in-vitro data revealed quick release followed by controlled release for prolonged period of time. The in-vitro data was best fitted to Higuchi model and followed Fickian diffusion as a release mechanism. The release pattern obtained by in-vitro experiment was confirmed by in-vivo study, as the IBU-GNP hydrogel rapidly lowered the inflammation induced by carrageenan injection followed by sustained level of anti-inflammatory activity. This sustained release pattern will help to reduce the dose and frequency of application of IBU-GNP hydrogel, which provides benefit to the patients with chronic arthritic disorders i.e. osteoarthritis and rheumatoid arthritis by reducing GIT side effects. From the current research, it can be concluded that IBU-GNP hydrogel is a competent NP based drug delivery system to effectively deliver IBU topically. Additional studies on safety, solid-state characterization, stability, and preparation of different dosage forms from IBU-GNPs are highly encouraged. The in-vivo bioavailability studies are required to validate the enhancement of solubility of IBU.

Conflict of interest

There are no conflicts to declare.

Funding

NA.

Study Approval

This study was approved by the Riphah Institute of Pharmaceutical sciences, Riphah International University, Islamabad, Pakistan.

Consent Forms

NA.

Data Availability

All the data related to this study is available with the authors.

Authors Contribution

GRE and SK conceptualized the study and wrote the final manuscript, MG, IR, FA & HA

helped in experimentation and writing the first draft, MG, and IR did the literature search and analysis, and SK supervised the whole project.

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