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Formulation, In Vitro and In Vivo Evaluation of Gefitinib Solid Dispersions Prepared Using Different Techniques

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Abstract: Gefitinib (Gef) is a poorly water-soluble antitumor drug, which shows poor absorption/bioavailability after oral administration. Therefore, this study was carried out to develop Gef solid dispersions (SDs) using different carriers and different techniques in order to enhance its dissolution and oral absorption/bioavailability. Various SD formulations of Gef were established using fusion and microwave methods utilizing Soluplus, Kollidone VA64, and polyethylene glycol 4000 (PEG 4000) as the carriers. Developed SDs of Gef were characterized physicochemically and evaluated for in vitro dissolution and in vivo pharmacokinetic studies. The physicochemical evaluation revealed the formation of Gef SDs using fusion and microwave methods. In vitro dissolution studies indicated significant release of Gef from all SDs compared to the pure Gef. Optimized SD of Gef (S2-MW) presented significant release of Gef (82.10%) compared with pure Gef (21.23%). The optimized Gef SD (S2) was subjected to in vivo pharmacokinetic evaluation in comparison with pure Gef in rats. The results indicated significant enhancement in various pharmacokinetic parameters of Gef from an optimized SD S2 compared to the pure Gef. In addition, Gef-SD S2 resulted in remarkable improvement in bioavailability compared to the pure Gef. Overall, this study suggested that the prepared Gef-SD by microwave method showed marked enhancement in dissolution and bioavailability.

Keywords: gefitinib; fusion method; microwave method; polymeric carriers; pharmacokinetics; solid dispersion

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1. Introduction

Lung cancer is subdivided into small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC), while the latter accounts for 85% of lung cancer diagnosed patients [1]. Lung cancer has a high incidence rate and the highest mortality rate in males compared with other types of cancers [1,2]. At the beginning of the 21st century, a group of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) was introduced to the market for the treatment of NSCLC in advanced stages and resistant to chemotherapy [1]. TKIs are known as small molecules that are selective and used in targeted therapy for NSCLC, in which the adverse effects are less than chemotherapeutic drugs, and which are known for their cytotoxicity for normal cells [2]. Gefitinib (Gef) is a selective TKI that was approved by United States Food and Drug Administration (FDA) for the treatment of locally and metastatic NSCLC after chemotherapy treatment [3]. Gef binds with EGFR, which is over-expressed on cancer cells and inhibits the signal transduction process, which is responsible for cell proliferation and reduction tumor progression [2,3]. The affinity of Gef is higher in mutated EGFR cancer cells with exon 19 deletion or exon

21-point mutation L858R compared to wild type EGFR, and for that reason the selectivity increases with Gef [2].

As per biopharmaceutical classification system (BCS), Gef is classified as a BCS class II drug that has low solubility and high permeability. The dose of Gef is 250 mg once daily until tumor progression decreases. Gef is a water-insoluble dibasic compound with pKa values of 5.4 and 7.2, which shows a pH-dependent solubility in the gastrointestinal (GI) fluids. It has an extremely low aqueous solubility and its oral absorption is limited by its poor dissolution rate. Due to the poor solubility, around 60% of the drug is absorbed in GI tract (GIT). The bioavailability could be greatly increased by improving the solubility and dissolution rate of the drugs [2,4].

The solid dispersion (SD) technique is the most widely accepted and successful strategy to enhance the solubility and release of poorly water-soluble drugs [5,6]. The SDs can be prepared by using different techniques [7]. The SDs offer several advantages as drug delivery carriers, which includes reduction in drug particle size at molecular level, enhancement in porosity, improvement in wettability, and conversion of crystalline form of drugs into amorphous forms [7–12]. Different formulation strategies such as microparticles-based spray-dried SDs [8], polymeric nanoparticles [9–14], solid lipid nanoparticles [15,16], nanostructured lipid carriers [17], nanosuspension [18], PEGylated immunostimulatory nanocarrier [19], nanoemulsion [20], and nanoliposomes [21] of Gef have been studied in order to enhance its dissolution/bioavailability and anticancer therapy. The Gef is a poorly water-soluble compound with poor oral bioavailability [4]. SDs are known to enhance the solubility and bioavailability of poorly water-soluble drugs [7,8]. Hence, this technique was used for Gef. The studied carriers such as Soluplus®, Kollidone VA64®, and polyethylene glycol 4000 (PEG 4000) have been investigated extensively in literature for the preparation of amorphous SDs of poorly water-soluble drugs [7,22]. Therefore, these carriers were used for Gef in this study. Microwave assisted-SDs of Gef have not been studied for their dissolution, solubility, and bioavailability enhancement. Hence, the aim of the present research was to develop the SDs of Gef utilizing microwave technology in comparison with fusion method in order to obtain improvement in its in vitro dissolution rate and bioavailability upon oral administration. Various carriers such as Soluplus®, Kollidone VA64®, and PEG 4000 were utilized in the preparation of Gef SDs.

2. Materials and Methods

2.1. Materials

Gef (purity: 98.60%) was obtained from "Beijing Mesochem Technology (Beijing, China)". Soluplus® and Kollidone VA64® were obtained from "BASF (Ludwigshafen, Germany)". High-performance liquid chromatography (HPLC) grade solvents and PEG 4000 were obtained from "Sigma Aldrich (St. Louis, MO, USA)". Other materials were of analytical/pharmaceutical grades and used without any purification.

2.2. Preparation of SDs

2.2.1. Microwave Method

Gef-SD formulations were prepared using microwave irradiation technique with conventional microwave. Soluplus, Kollidon VA64, and PEG 4000 were used to prepare Gef-SD formulation in various ratios shown in Table 1. Drug to polymers ratio was chosen to be 1:4 *w*/*w*, therefore, Gef was blended with Soluplus + PEG 4000 and Kollidon VA64 + PEG 4000 to all formulations. After weighing of Gef and polymers, the powders were poured into a porcelain dish and blended using a glass stirring rod until all powders were homogenously blended. Each porcelain dish was placed in the center of the microwave and the energy of the microwave was selected as 900 watts. The microwave started to heat the porcelain dish; for every 30 s, the microwave heating was paused and stirring was continued in order to blend the mixture to ensure that all powders were uniformly exposed to the heating energy. The porcelain dish was then placed back and resumed the microwave for heating until all the blended powders were melted and resulted in

homogenous mixture. The mixture was then removed from the microwave and left to cool at room temperature. After that, the mixture was placed in a vacuum desiccator containing anhydrous potassium chloride for 48 h to remove any moister. After drying, the mixture was sieved using sieve mesh 325 and the sieved powder was saved in an amber colored airtight container for further analysis [22].

Table 1. Gefitinib (Gef)-carrier ratio used to prepare solid dispersion (SD) (*w*/*w*).

Formulation Code	Gef	Soluplus	Kollidon VA64	PEG 4000
S1	1	3	0	1
S2	1	2.5	0	1.5
A1	1	0	3	1
A2	1	0	2.5	1.5
P	1	0	0	4
D	1	0	0	0

2.2.2. Fusion (Traditional) Method

A traditional method was used for melting both Gef and polymers in a ratio similar to that used for microwave irradiation method. After geometrical blending, the mixture was heated using a hot plate following proper mixing until a homogenous mixture formed. Then, the melted mixture was left to cool at room temperature and sieved using sieve mesh 325. The prepared powder was placed in a desiccator for 48 h till further use [22].

2.3. Physiochemical Characterization

2.3.1. Differential Scanning Calorimetry (DSC)

Each Gef-SD formulation was subjected to DSC analysis using "DSC−8000 Perkins Elmer (Waltham, MA, USA)" apparatus in a temperature range of 25–250 °C at the heating rate of 10 °C/min. The samples were evaluated with the purge of nitrogen at around 20 mL/min, and an autosampler and chiller were installed to this apparatus. The weight of each sample was 3 mg and fixed inside sealed aluminum pan. For the characterization and evaluation of the samples, a "Pyris manager software (Pyris Elmer, Waltham, MA, USA)" was used for the solid state characterization [22].

2.3.2. Scanning Electron Microscopy (SEM)

The SEM study was performed to evaluate the change in the crystallinity of the pure Gef after preparing the SDs. Each sample Gef (D), Gef + PEG 4000 (P), Gef + Kollidon + PEG 4000 (A2), (Gef + Kollidon + PEG 4000 (A1), and Gef + Soluplus + PEG 4000 (S2) were taken on the grid and evaluated for the change in the structure. The study was performed by using the "Electron Microscope (JSM 6360A, JOEL, Tokyo, Japan)". The samples were coated with gold and observed under the microscope at high resolution to check the change in the morphology [22].

2.3.3. Fourier Transforms Infrared (FTIR) Spectroscopy

FTIR analysis was carried out using "Perkins Elmer FTIR spectrum BX (Pekin Elmer, Marlborough, MA, USA)" in the range of 4400 to 350 cm^{-1}. The FTIR spectra of free Gef and different SDs were interpreted in order to evaluate any bonding interactions between Gef and polymers [22].

2.3.4. Powder X-ray Diffraction (PXRD)

The PXRD spectra of the pure Gef and prepared Gef. SDs were evaluated to check the changes in the molecular state of the crystalline Gef after preparing the SDs. The comparison was performed between the high intensity Gef and the prepared SD. The study was performed on the "X-Ray diffractometer (Ultima IV, Rigaku Inc. Tokyo, Japan)" with a scanning rate of 0.5/min in the scanning range of 3–60°. The characteristic peak

of each sample was assessed by collecting the data by monochromatic radiation (Cu Kά1, λ = 1.54 Å), operating at a voltage of 40 kV and current of 40 mA [22].

2.4. In Vitro Dissolution Studies

The in vitro dissolution test was performed according to FDA dissolution monograph using "USP 24 dissolution apparatus SOTAX AT MD Smart apparatus (SOTAX AG, Basel, Switzerland)" type II with a paddle rotating at 50 rpm equipped with an automated sampler. Each formulation (500 mg SD containing 100 mg of Gef) was filled in a hard gelatin capsule and placed in the dissolution apparatus. The temperature was set at 37 ± 0.5 °C. These studies were performed for 60 min with a dissolution medium of 0.1N HCl at pH 1.5. An aliquot of 2 mL was withdrawn at five different intervals (5, 10, 15, 30, 45, and 60 min) and replaced with the same amount of drug-free fresh medium for each sample [8]. The samples were filtered and centrifuged for 5 min with 10,000 rpm and analyzed for drug content using HPLC method, as reported previously [23].

2.5. In Vivo Pharmacokinetic Study

2.5.1. Bioanalytical Method and Chromatographic Conditions

In this study, a validated ultra-performance liquid chromatography-mass spectrometry/mass spectrometry (UPLC-MS/MS) method was employed to determine the concentration of Gef in plasma. The analysis of Gef was performed using "UPLC system (UPLC: Waters Acquity, Milford, MA, USA)". The chromatographic conditions involved the use of a ACQUITY UPLC BEH HILIC column (50 mm × 2.1 mm, 1.7 µm) with a mobile phase of acetonitrile and 0.1% formic acid (70:30, v/v) at a flow rate of 0.25 mL/min using imatinib as the internal standard [24]. The eluted compounds were detected by tandem mass spectrometry using "TQ detector (Waters Corp., Milford, MA, USA)" equipped with an electrospray ionization source operating in positive ionization mode. The quantification was performed with multiple reactions monitoring (MRM) mode. The selection of ionization pairs (m/z) was shown as follows: Gef: 447.23 → 128.15 (cone voltage 40 V, collision energy 26 V), imatinib: 494.3 → 394.2 (cone voltage 54 V, collision energy 28 V). The retention times for Gef and imatinib were 1.16 min and 1.06 min, respectively. The run time for this analysis was 2.0 min. The injection volume was 5 µL. The standard calibration range for Gef was 5–200 ng/mL. The intra-day and inter-day precisions were 9.54 and 11.24%, respectively. The LOD and LOQ for the present assay were 5 ng/mL and 15 ng/mL, respectively.

2.5.2. Study Design

Healthy Wister rats (weighing 180–250 g) were supplied from "Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia". All the animals were subjected to standard laboratory conditions of temperature and humidity in addition to strictly following institutional guidelines for animal studies. Ethical approval was taken from "Ethical Committee, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia (ethical approval number: KSU-SE−19–66)" in order to perform in vivo pharmacokinetic studies. Two groups consisted of six rats each that were fasted 24 h prior to administration of the respective formulation. The administration of a pure drug suspended in CMC 0.3% for group A and S2 formulation for group B was performed. The oral administration of 40 mg/kg of Gef was given to both groups followed by blood samples withdrawn from the vein of rat's tail at different time intervals (0.5, 1, 3, 6, 12, and 24 h) [25]. The samples were centrifufed at 2500 rpm for 5 min in order to separate the plasma [13].

2.5.3. Sample Preparation

The plasma samples were processed by protein precipitation method [13]. Simply, 0.1 mL of plasma sample was combined with 20 µL internal standard imatinib (25 µg/mL) and 0.38 mL methanol, then vortexed for 1 min. After centrifugation at 15,000 rpm for

10 min, 300 µL of the supernatant liquid was transferred to a sample vial and 5 µL was injected into the UPLC/MS/MS for quantitative analysis.

2.5.4. Pharmacokinetic Parameters

The different pharmacokinetic parameters, like peak plasma concentration (C_{max}), time to reach peak concentration (T_{max}), mean residence time (MRT), area under the curve (AUC), elimination rate constant (K_{el}), and plasma half-life ($t_{1/2}$) of Gef was calculated using the PK Solver 2.0 software by non-compartmental analysis.

2.6. Statistical Analysis

The experimental data are presented as mean ± SD. The data were statistically evaluated by student's t-test and ANOVA using "Graphpad prism (InStat 7; San Diego, CA, USA)" at a significance level of $p < 0.05$.

3. Results and Discussion

3.1. DSC Spectra

3.1.1. Fusion Method

The DSC spectra of pure Gef and various SDs prepared using fusion method are presented in Figure 1. The pure Gef showed a sharp endothermic melting peak at 198 °C, which corresponds to its melting point. The prepared SDs A1 and A2 (with Kollidon), S2 (Soluplus), and P (PEG 4000) showed a large shift of melting point from 198 °C to 52–55 °C. This shifting of the peak was possible due to the complete solubility of Gef in the used carrier system. The crystalline Gef converted to amorphous state after solubilization in the carrier. In the case of formulation P prepared with PEG 4000, a sharp large peak at 54 °C was recorded, which corresponds to its melting temperature. The formulations (A1 and A2) prepared using Kollidon and PEG 4000 showed an endothermic peak at 52 °C; there was slight difference in the peak height between both formulations. The change in carrier led to differences in the peak heights of the formulations.

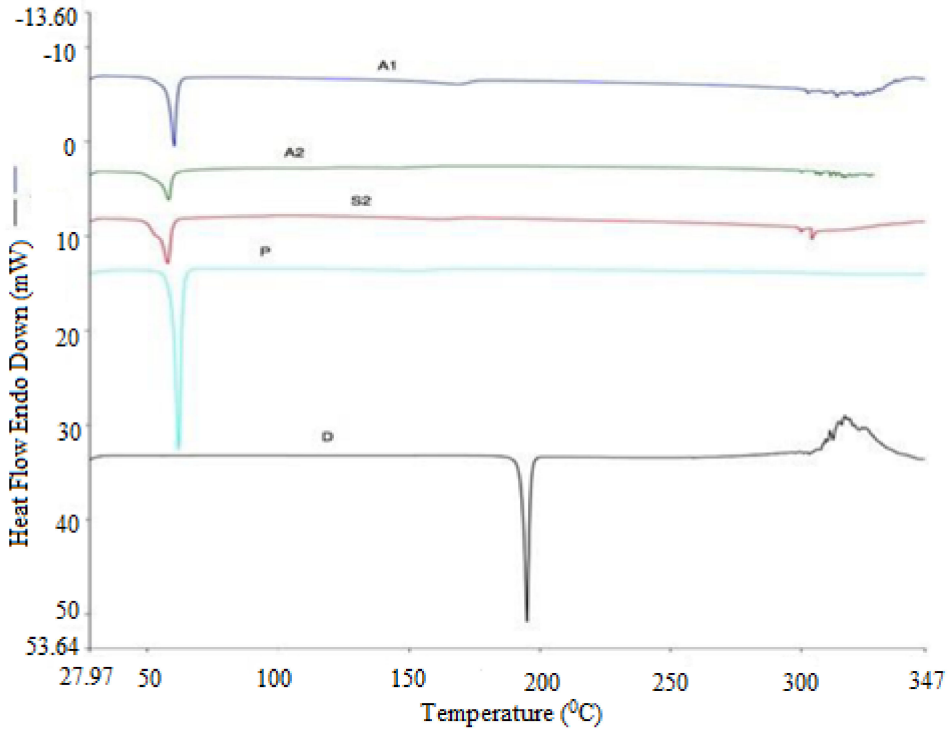

Figure 1. Differential scanning calorimetry (DSC) thermograms for pure drug (D) and various SDs prepared using fusion method.

3.1.2. Microwave Method

The DSC spectra of pure Gef and various SDs prepared using the microwave method are presented in Figure 2. The pure Gef showed a sharp endothermic melting peak at 198 °C, which corresponds to its melting point. The prepared SDs A1 and A2 (with Kollidon), S2 (Soluplus), and P (PEG 4000) did not show any peak near the melting peak of Gef. The formulations showed a melting peak between 52–55 °C, which corresponds to the carrier peak. The absence of Gef peak in the SDs may be due to the complete solubility of Gef in the used carrier system. The SDs prepared with the microwave irradiation method did not show any peak due to molecular dispersion of Gef in the carrier. There was a slight difference in the peak height between the samples prepared with the fusion method and microwave method. The SDs prepared by microwave method showed shorter peaks of SDs prepared with different carriers. These findings supported the results that SDs prepared by microwave method enhanced the solubility of the drug by converting crystalline drug to amorphous state. The microwave irradiated samples completely solubilized the poorly soluble drugs in the presence of microwave rays.

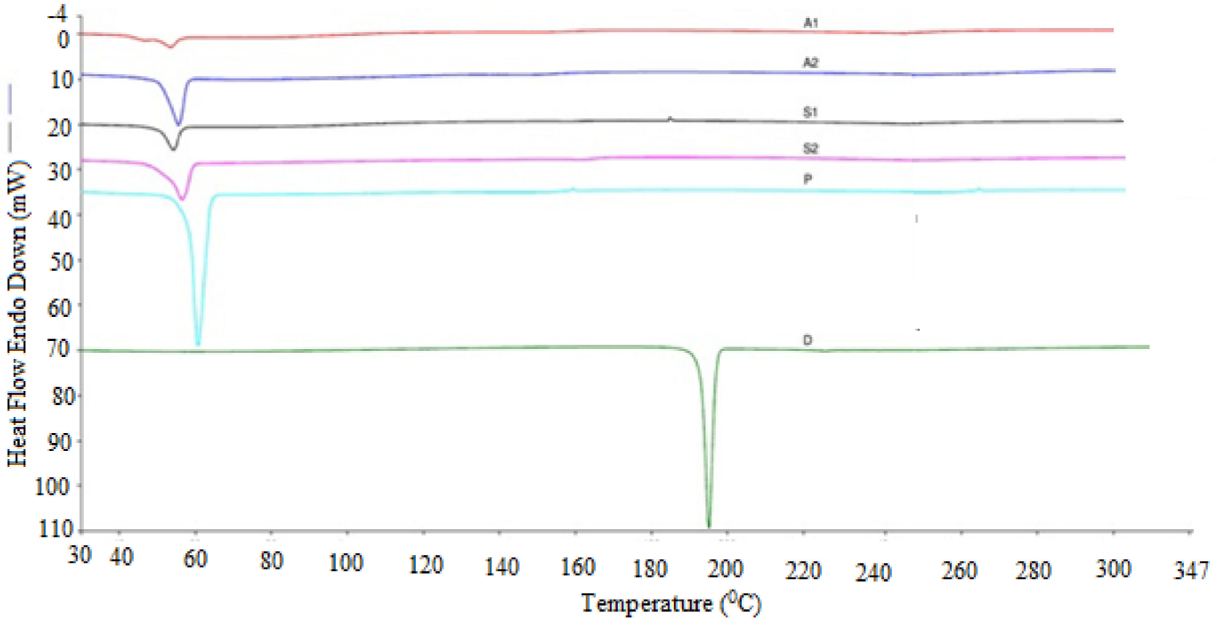

Figure 2. DSC thermograms for pure drug (D) and various SDs prepared using microwave method.

3.2. SEM Evaluation

3.2.1. Fusion Method

The SEM images for pure Gef and various SDs prepared using fusion method are presented in Figure 3. The surface morphology of the pure Gef and the prepared samples were evaluated to obtain the changes in the morphological structure after preparation of SD. The surface morphology of the pure Gef was found to be a well-defined crystal structure with sharp acicular structure. The prepared formulation (A1, A2, and S2) showed the change in the surface morphology. The particles were slightly converted to amorphous structure. The formulation (P) prepared with PEG 4000 showed marked changes in the structure. The particle showed amorphous and porous structure with irregular rough surface. The change in the particles shape confirms the formation of SD and the crystalline Gef completely solubilized in the used carrier PEG 4000.

3.2.2. Microwave Method

The SEM images for pure Gef and various SDs prepared using microwave method are presented in Figure 4. The surface morphology of the pure Gef was found to be a well-defined crystal structure with sharp acicular structure. The microwave irradiated

formulations (A1, A2, and S2) showed significant changes in the surface morphology. The particles were converted to amorphous structure, which was further confirmed with the PXRD and DSC study. The formulation (P) prepared with PEG 4000 showed greater changes in the structure. The particle showed an amorphous structure with irregular rough surface. The change in the particles shape confirms the formation of SD and the crystalline Gef completely solubilized in the used carrier PEG 4000 [10].

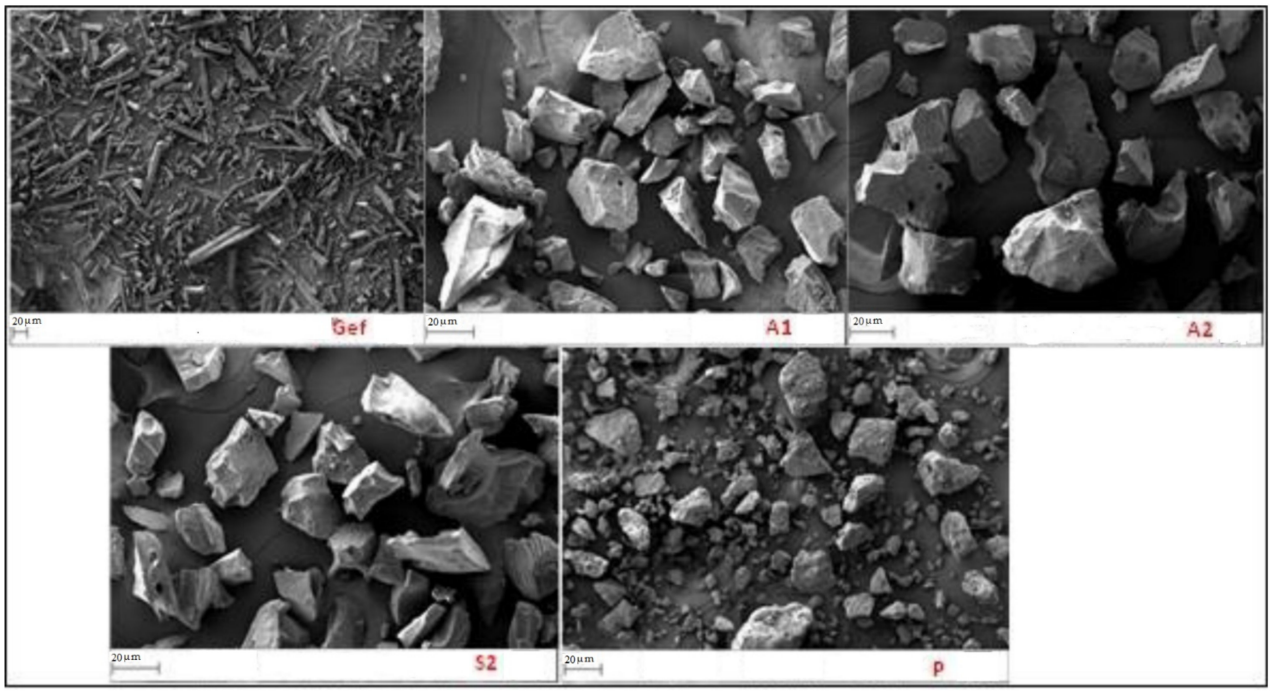

Figure 3. Scanning electeron microscope (SEM) images of pure Gefitinib (Gef) and various SDs (A1-F, A2-F, S2-F, and P-F) prepared using fusion method.

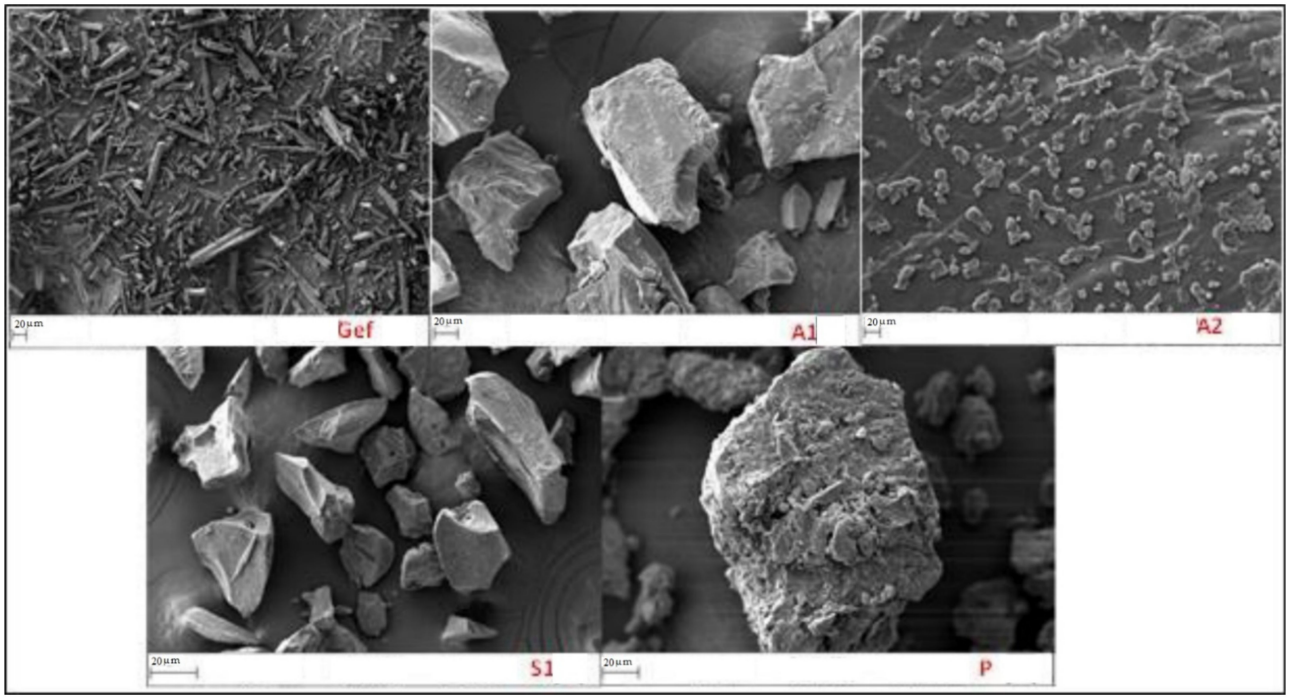

Figure 4. SEM images of pure Gef and various SDs (A1-MW, A2-MW, S2-MW, and P-MW) prepared using microwave method.

3.3. FTIR Spectroscopy

3.3.1. Fusion Method

The FTIR spectra for pure Gef and various SDs prepared using fusion method are presented in Figure 5. The FTIR spectra of pure Gef exhibited various spectral peaks for the existing functional groups. The functional group NH showed a sharp peak at 3399 cm^{-1} for the pure Gef. The other peaks were observed at 2956 cm^{-1} for CH_2, 1624 cm^{-1} for C=N, and 1578 cm^{-1} for C=C of aryl group. The functional group present in the pure Gef also exhibited peaks for the C-O group at 1110 cm^{-1}. The presence of a peak at 929 cm^{-1} further confirms the presence of a C-F group in the pure Gef, which is in alignment with the structure of pure Gef. The prepared SD (P) showed similar peaks as that of pure Gef with slight variations. The peaks for NH observed a slight variation in peak at 3400 cm^{-1} for NH groups. The peaks for alkyl group also exhibited a slight variation at 2808 cm^{-1} compared to pure Gef. The peak for the C=N group was not observed in the carrier P. The peaks for C=C aryl group, C-O group, and C-F group were also observed at 1431, 1102, and 956 cm^{-1} as compared to the pure Gef. The other formulation (S2) exhibited slight variation in the FTIR spectra; the peak for CH_2 group was recorded at 2890 cm^{-1} for formulation S2. The peaks for C=N group exhibited a drastic change in the peaks for both the formulation at 1673 cm^{-1} in compliance with the pure Gef. The spectra exhibited an absence of peaks for aryl and C-O group for both the formulations. The formulation S2 exhibited a peak for the C-F group at 953 cm^{-1}. The other SD (A2) showed peaks in compliance with the pure Gef. The marked characteristic peaks of Gef were present in the prepared SDs. The above FTIR spectral peaks conclude that the main characteristics peaks were present in the formulation as compared to the pure Gef with slight deviation. The change in the peaks was due to the presence of carrier peaks and formation of SDs. The spectra also indicated a minimal interaction between the functional groups of the pure Gef and the PEG 4000, Soluplus, and Kollidon [10].

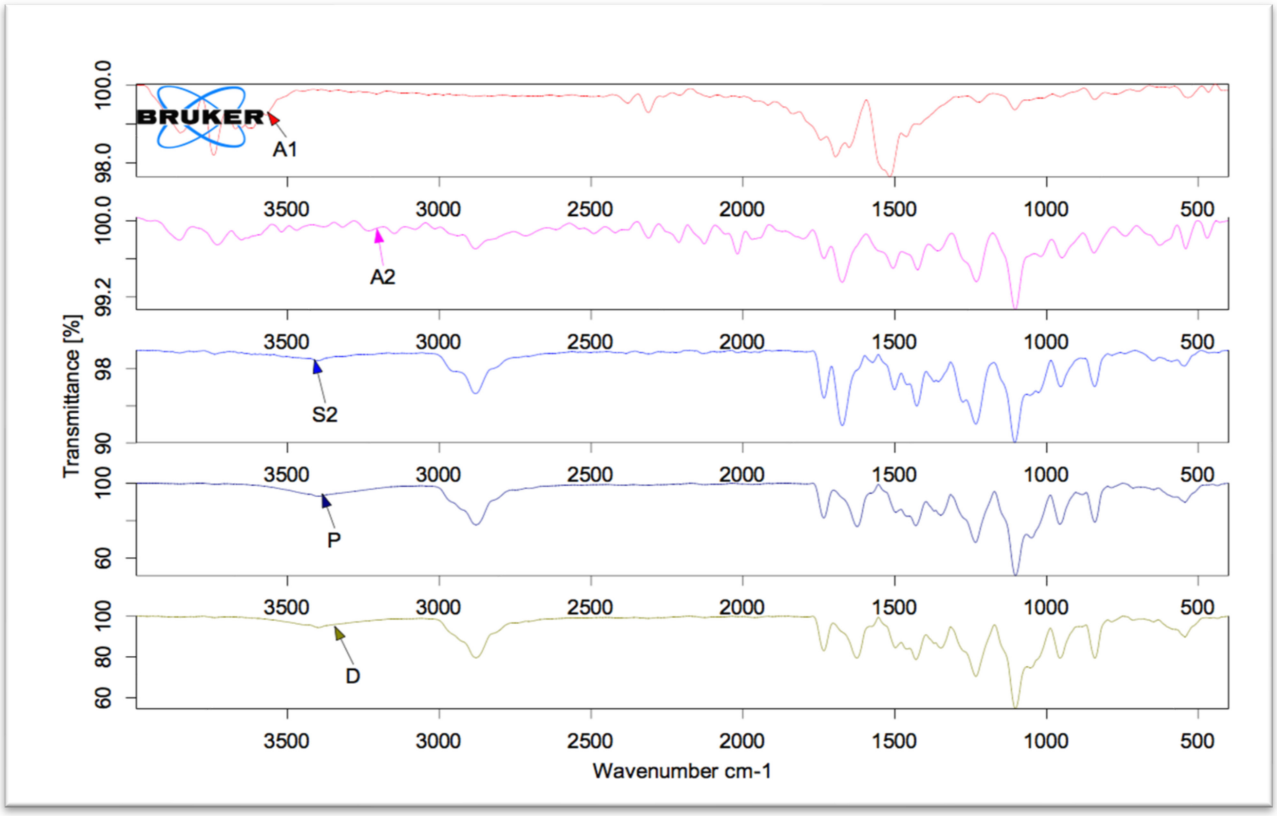

Figure 5. Fourier transforms intra-red (FTIR) spectra for pure Gef and different Gef-SDs formulations prepared using fusion method.

3.3.2. Microwave Method

The FTIR spectra for pure Gef and various SDs prepared using microwave method are presented in Figure 6. The FTIR spectra of pure Gef exhibited various spectral peaks for the existing functional groups. The prepared SD (P) showed similar peaks as that of pure Gef with slight variation. The peaks for NH were observed with a slight variation in the peak at 3400 cm^{-1} for NH groups. The peaks for the alkyl group also exhibited a slight variation at 2808 cm^{-1} compared to the pure Gef. The peak for C=N group was not observed in the carrier P. The peaks for C=C aryl group, C-O group, and C-F group were also observed at 1500, 1110, and 962 cm^{-1} as compared to the pure Gef. The other formulation (S2) exhibited slight variation for the NH group at 3401 cm^{-1}, whereas formulation S1 exhibited a sharp change in the NH peak at 3448 cm^{-1} as compared to the pure Gef. The formulation (S2) also exhibited a peak for CH_2 group at 2890 cm^{-1} but S1 exhibited the absence of peaks for NH group as compared to the pure Gef. The peaks for C=N group exhibited a drastic change for the formulation S2 at 1675 and S1 at 1637 cm^{-1} in compliance with the pure Gef. The spectra exhibited an absence of peaks for aryl and C-O group for both the formulations. The formulation S2 exhibited a peak for C-F group at 961 cm^{-1} but the spectra of S1 exhibited no traces for the peaks of C-F group as compared with the pure Gef. The other SDs (A1 and A2) showed peaks in compliance with the pure Gef with the absence of peaks for C=C group at 1500 cm^{-1}. The above FTIR spectral peaks conclude that the main characteristics peaks were present in the formulation as compared to the pure Gef with slight deviation. The change in the peaks was due to the presence of carrier peaks and formation of SD. The spectra also indicated a minimal interaction between the functional groups of the pure Gef and the PEG 4000, Soluplus, and Kollidon [10].

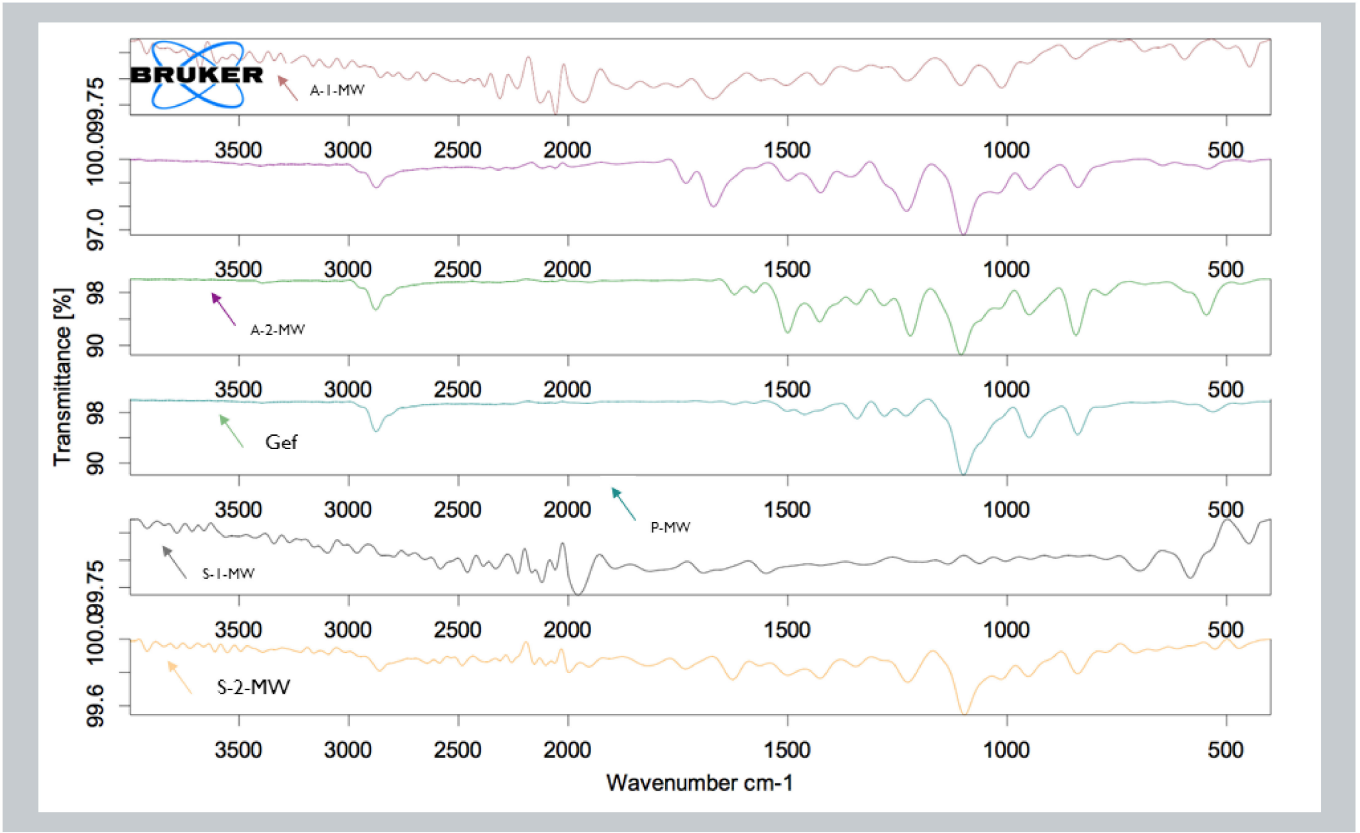

Figure 6. FTIR spectra for pure Gef and different Gef-SDs formulations prepared using microwave method.

3.4. Powder X-ray Diffraction

3.4.1. Fusion Method

The PXRD spectra for pure Gef and various SDs prepared using fusion method are presented in Figure 7. The changes in the PXRD spectra of the pure Gef were evaluated after preparing the SD with different carriers. The pure Gef showed multiple, sharp high-intensity diffraction peaks at 19.3°, 24.1°, and 26.4°, which revealed the existence of its natural crystalline form. The different carriers like Kollidon (38.1° and 43.2°), PEG 4000 (18.7° and 21.5°), and Soluplus (38.1° and 42.1°) showed their characteristic diffraction peaks at respective 2 theta values. The prepared formulations (P, S1, S2, and A2) showed significant changes in the high intensity peaks of Gef. The peak height and intensity were reduced due to the conversion of crystalline Gef into amorphous state after being completely dispersed into the used hydrophilic carrier. The other observed peaks in the formulations were of the used carriers [10].

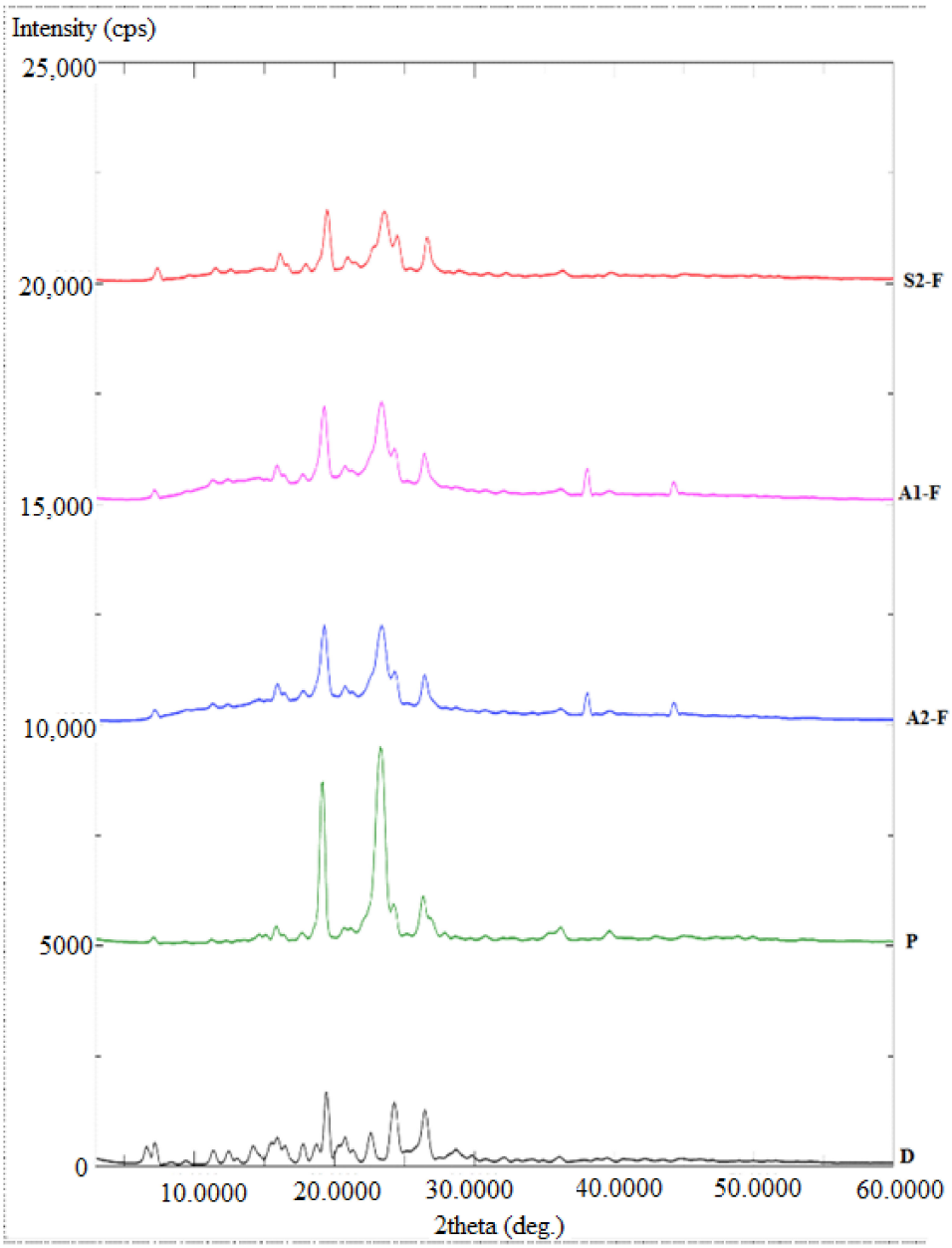

Figure 7. Powder X-ray diffraction (PXRD) spectra for pure Gef and different Gef-SDs formulations prepared using fusion method.

3.4.2. Microwave Method

The PXRD spectra for pure Gef and various SDs prepared using microwave method are presented in Figure 8. The pure Gef showed multiple, sharp, high-intensity diffraction peaks at various diffraction angles. The different carriers like Kollidon (38.1° and 43.2°), PEG 4000 (18.7° and 21.5°), and Soluplus (38.1° and 42.1°) showed their characteristic diffraction peaks at respective 2 theta values. The prepared formulations (P, S1, S2, and A2) showed marked changes in the high intensity peaks of Gef. The peak height and intensity were reduced due to the conversion of crystalline Gef into amorphous state after being completely dispersed into the used hydrophilic carrier. The other observed peaks in the formulations were of the used carriers [10].

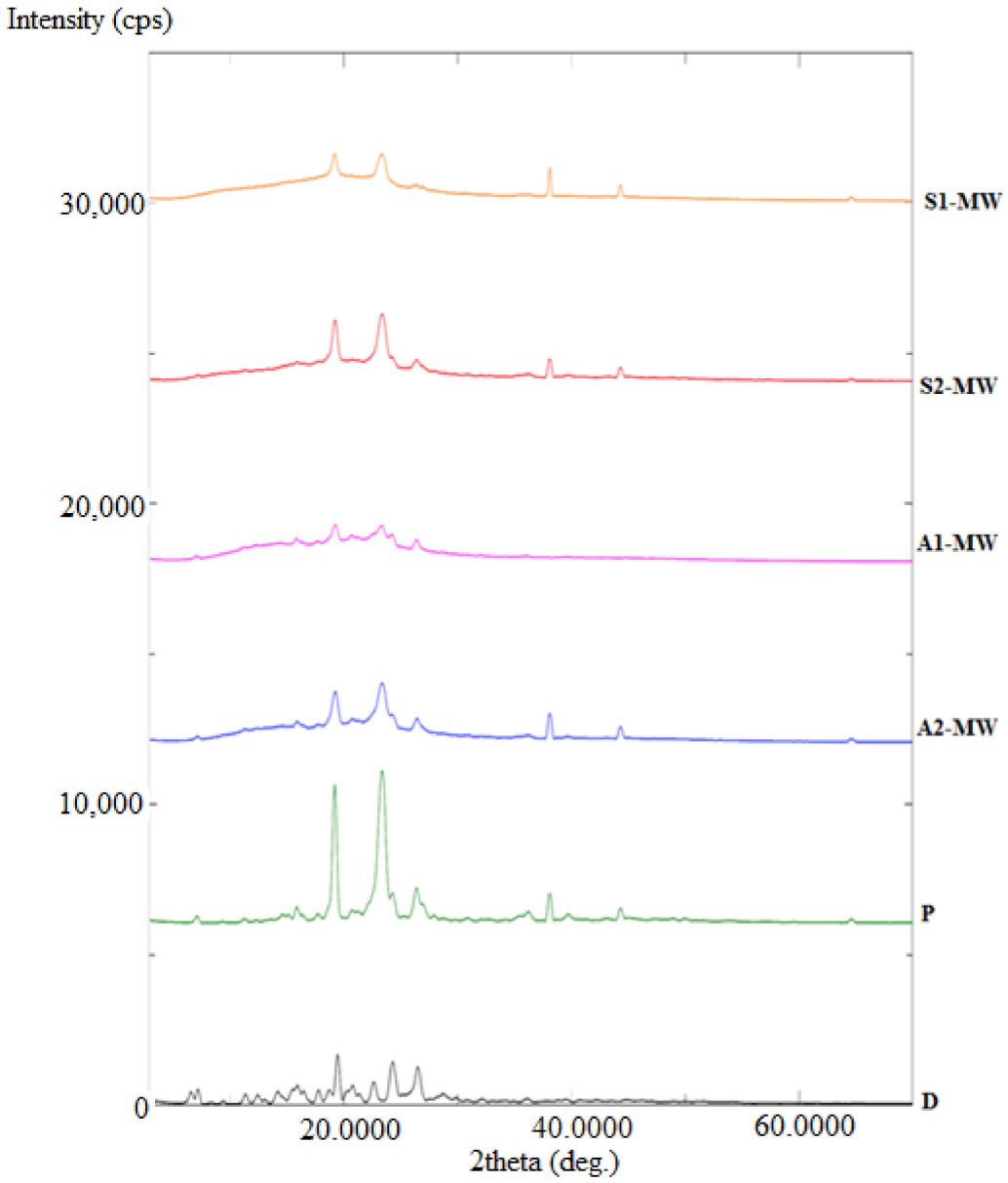

Figure 8. PXRD spectra for pure Gef and different Gef-SDs formulations prepared using microwave method.

3.5. In Vitro Dissolution

3.5.1. Fusion Method

The in vitro drug release profile of Gef from different SDs prepared using fusion method are presented in Figure 9. The significant enhancement in Gef release was observed

from different SDs compared to pure Gef. However, the release was found to be lesser in comparison to the SDs prepared using microwave method. The formulation composition Kollidon VA 64 + PEG 4000 (A2-F) depicted the maximum drug release of 45.2 ± 1.11%. The pure Gef showed poor drug release of 25.4 ± 0.8% in the given 60 min study time. The release order was found to be S2-F > A2-F > P-F > A1-F > pure Gef. The SDs prepared with fusion method do not show significant difference in the release pattern using carriers like Soluplus, Kollidon, and PEG 4000.

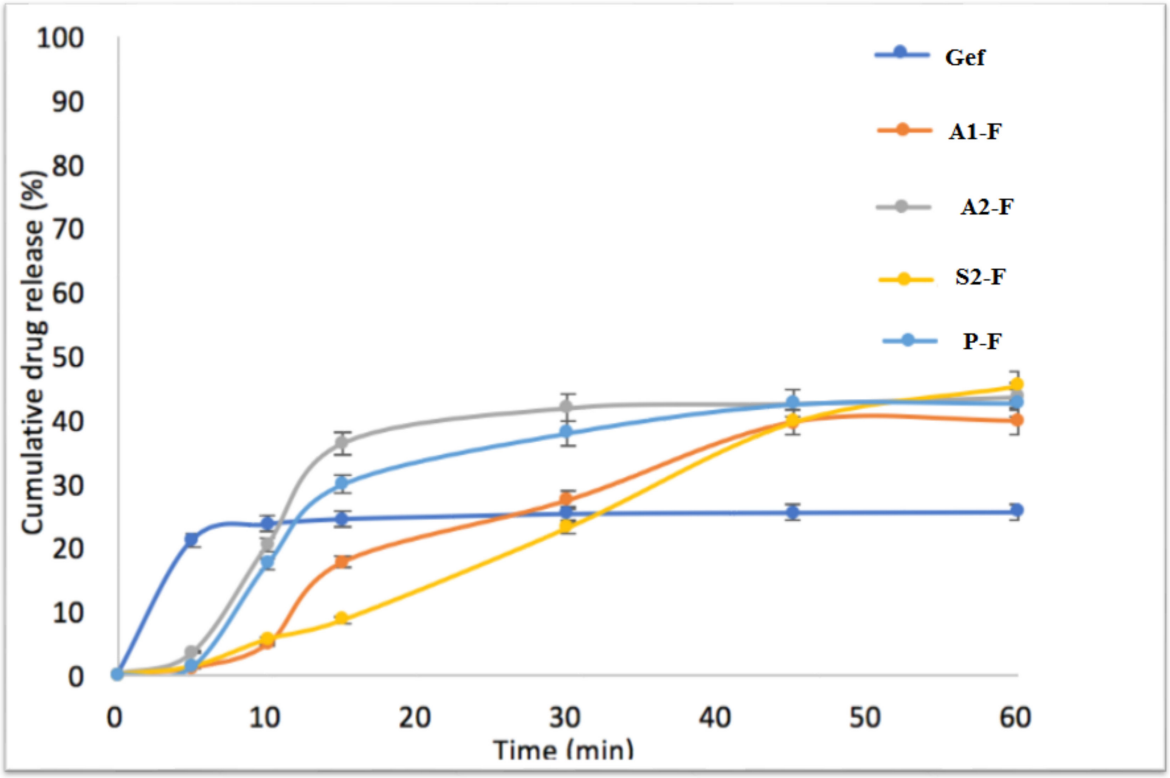

Figure 9. In vitro dissolution profile of Gef from pure Gef and various SDs prepared using fusion method (mean ± SD, n = 3.0).

3.5.2. Microwave Method

The in vitro drug release profile of Gef from different SDs prepared using microwave method are presented in Figure 10. The prepared SDs showed significant enhancement in drug release compared to the pure Gef. The pure Gef showed poor solubility with drug release of 21.23% in 60 min study time. The prepared Gef SDs showed the release pattern of S2-MW > S1-MW > A2-MW > A1-MW > P-MW. The used hydrophilic carriers showed marked enhancement in the Gef release. The difference in the release pattern among all SDs may be due to the variation in carrier property and the used ratio. The SDs prepared with Soluplus + PEG 4000 (S2-MW) showed maximum drug release (82.1 ± 5.1%) at the given time point. Gef is a poorly water-soluble compound in its pure form. However, it is water soluble in SDs due to change in its physicochemical properties. When SDs will enter into systemic circulation, Gef will be water soluble due to the amorphous nature of the drug in SDs. Therefore, the maximum drug release from S2-MW may be due to the maximum amorphization of Gef with the used carrier in the used microwave condition. The application of the microwave gives the production of uniform heat to the sample with the same rate, which helps to give better contact between the drug and carrier. This might be the main reason for the maximum drug release from SD (S2) prepared using microwave technology.

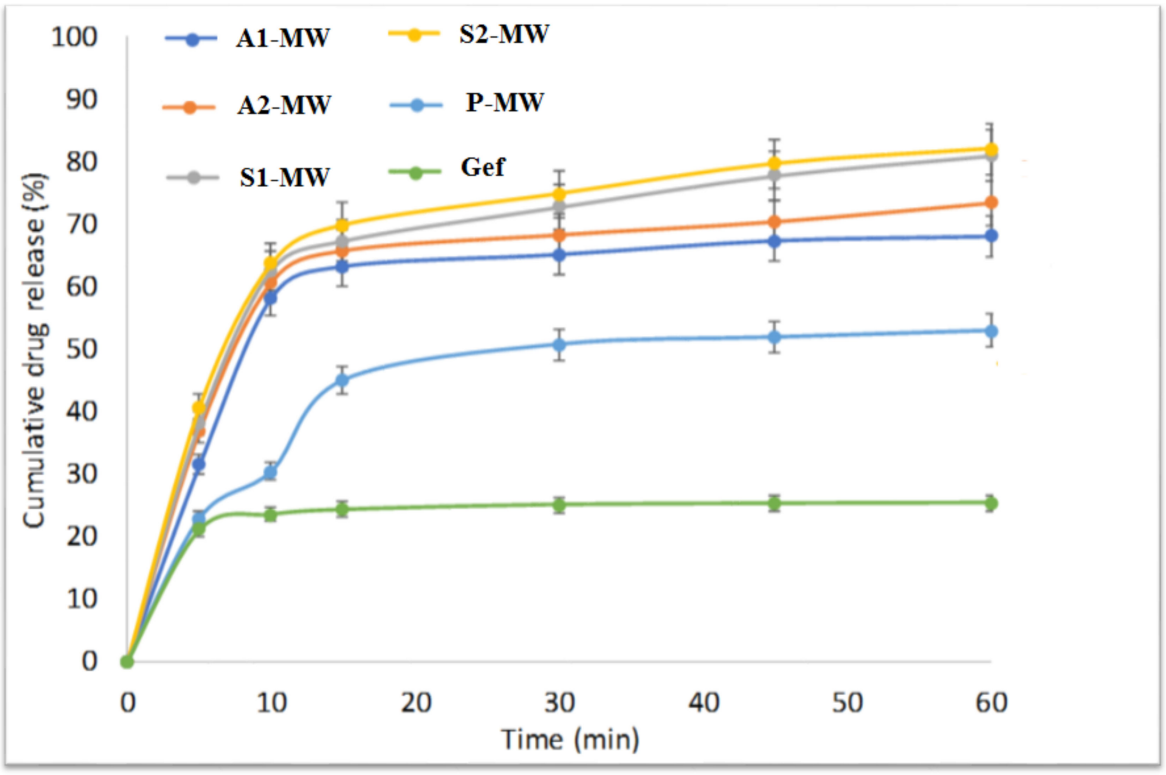

Figure 10. In vitro dissolution profile of Gef from pure Gef and various SDs prepared using microwave method (mean ± SD, n = 3.0).

3.6. In Vivo Pharmacokinetic Study

The prepared SDs are immediate release type formulations and hence release studies were carried out for 60 min. The in vivo absorption process is different. In vivo absorption studies are performed up to the complete elimination phase of the drug. Therefore, these studies were performed for up to 24 h in order to get the elimination points of the drug. The plasma-concentration-time profile curves for pure Gef and an optimized SD S2 are presented in Figure 11. The pharmacokinetic profiles of Gef following oral administration of pure Gef and Gef-SD (S2) are depicted in Table 2. A significant difference between the pharmacokinetic parameters was observed. After a single-dose oral administration, Gef SD (S2) exhibited ~ 1.6-fold higher C_{max} value for Gef-SD (23.56 ± 4.11 µg/mL) than the pure Gef (16.1 ± 2.45 µg/mL). The reason for higher C_{max} value is the greater solubility of Gef in the hydrophilic carrier. The $AUC_{0–24}$ value was found to be 154.55 µg·h/mL for pure Gef as compare to Gef-SD 158.38 µg·h/mL. The pure Gef showed the $AUMC_{0–24}$, $t_{1/2,}$ and K_{el} values of 1132.36, 6.98 h, and 0.099213 h^{-1}, respectively, whereas Gef-SD depicted 1162.11, 7.43 h, and 0.093174 h^{-1}, respectively. The above results suggested that the oral administration Gef-SD resulted in a remarkable improvement in bioavailability of Gef when compared to pure Gef. The enhanced Gef bioavailability after oral administration of Gef-SD was due to the enhanced solubility of Gef into the hydrophilic carrier that increases the solubility as well as the surface area, which results in significantly higher absorption of Gef from GIT upon oral administration.

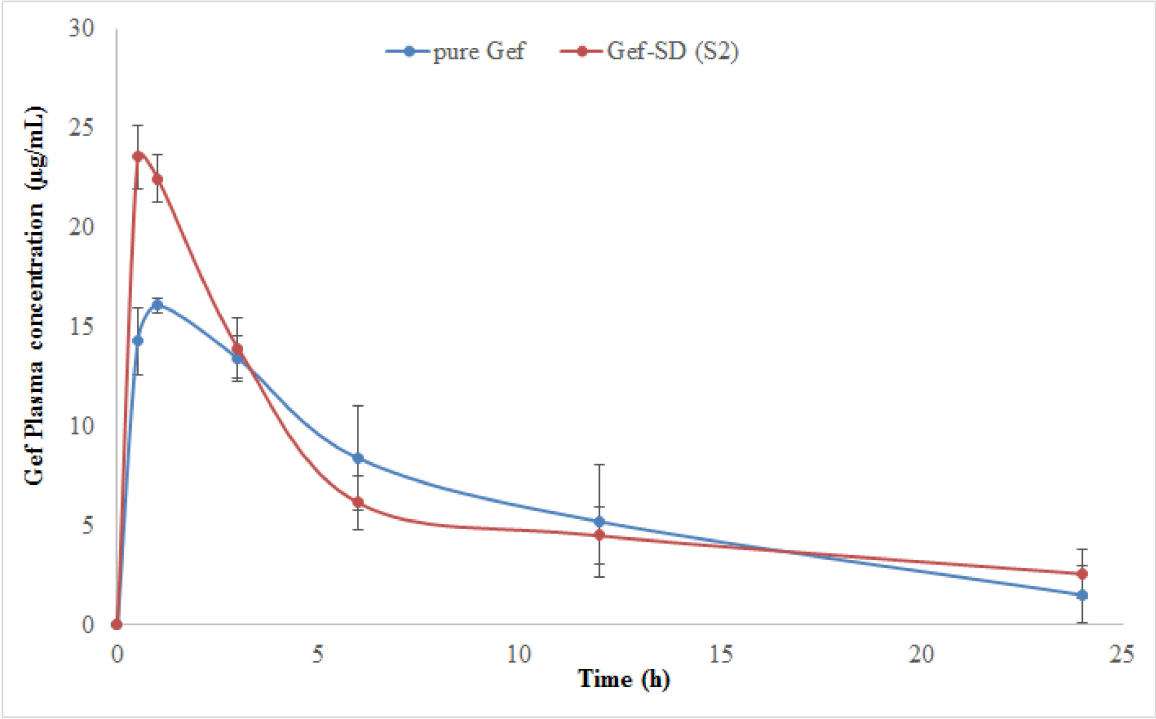

Figure 11. In vivo pharmacokinetic profile of pure Gef and Gef-SD (S2) after oral administration in rats (mean ± SD, n = 6.0).

Table 2. Pharmacokinetic parameters after oral administration of Gef-SD (S2) and pure Gef suspension (mean ± SD, n = 6.0).

Parameters	Pure Gef	Gef-SDs (S2)
C_{max} (µg/mL)	16.1 ± 2.45	23.56 ± 3.11 *
T_{max} (h)	1 ± 0.46	0.5 ± 0.13
$AUC_{0\rightarrow24}$ (µg·h/mL)	154.55 ± 14.33	158.38 ± 12.21
$AUC_{0\text{-inf}}$	169.87 ± 10.78	186.07 ± 16.65
$AUMC_{0\rightarrow24}$ (µg·h^2/mL)	1132.36 ± 59.43	1162.11 ± 67.54
$AUMC_{0\text{-inf}}$	1654.47 ± 84.87	2123.86 ± 7.65 *
$t_{1/2}$ (h)	6.98 ± 0.41	7.43 ± 0.33
K_{el} (h^{-1})	0.099213	0.093174

* $p < 0.05$, significant difference compared to pure Gef.

4. Conclusions

The present study was designed to prepare SDs of Gef using fusion and microwave methods. The physicochemical characterization results revealed the conversion of crystalline Gef into the amorphous state. The surface morphology of the pure Gef was found to be a well-defined crystal structure with sharp acicular structure. The prepared SDs showed changes in the surface morphology. The pure Gef showed poor solubility with drug release of 21.23% in 60 min study time. The prepared Gef SDs showed a significant enhancement in drug release pattern. The SDs prepared using microwave method showed greater dissolution rate compared to the fusion method. The pharmacokinetic study results suggested that the oral administration Gef-SD resulted in remarkable improvement in bioavailability when compared to pure Gef. The enhanced Gef bioavailability after oral administration of Gef-SD was due to the enhanced solubility of Gef into the hydrophilic carrier. The overall conclusion of this study is that the prepared Gef-SD by microwave method showed marked enhancement in dissolution and bioavailability.

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Informed Consent Statement: Not applicable.

Data Availability Statement: This study did not report any data.

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Conflicts of Interest: The authors declare no conflict of interest.

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