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Original article

Dissolution enhancement of Gefitinib by solid dispersion and complexation with β-cyclodextrins: In vitro testing, cytotoxic activity, and tablet formulation

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ABSTRACT

Cancer is the leading cause of mortality worldwide. In patients with metastatic non-small cell lung cancer, epidermal growth factor receptor (EGFR) is often overexpressed. Gefitinib (GEF), an inhibitor of EGFR, is approved for the treatment of patients with metastatic non-small cell lung cancer (NSCLC). However, the low solubility and dissolution of GEF limits its bioavailability. Numerous methods, including solid dispersion (SD) and complexation, have been reported to enhance the dissolution of poorly soluble drugs. In this study, GEF complexes were prepared using methyl-β-cyclodextrin (MβCD) and hydroxypropyl-β-cyclodextrin (HPβCD) in two molar ratios (1:1 and 1:2), furthermore, GEF SDs were prepared using polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), and poloxamer-188(PXM) in three different ratios (1:2, 1:4 and 1:6 w/w). Dissolution studies were conducted on the prepared formulations. Dissolution results showed a 1.22–2.17-fold enhancement in drug dissolution after one hour compared to untreated GEF. Two formulations that showed higher dissolution enhancement were subsequently evaluated for in-vitro cytotoxicity and were formulated into tablets. The selected PVP–GEF (1:4 w/w) and MβCD–GEF (1:1M) formulas displayed improved cytotoxicity compared to untreated GEF. The IC_{50} values of the PVP–GEF and MβCD–GEF were 4.33 ± 0.66 and 4.84 ± 0.38 μM, respectively which are significantly lower ($p < 0.05$) than free GEF. In addition, the formulated tablets exhibited enhanced dissolution compared to pure GEF tablets. PVP–GEF SD tablets released (35.1 %±*0.4*) of GEF after one hour, while GEF-MβCD tablets released (42.2 % ± *0.7)* after one hour. In the meantime, tablets containing pure GEF showed only 15 % ± 0.5 release at the same time. The findings of this study offer valuable insights for optimizing the dissolution and hence therapeutic capabilities of GEF while mitigating its limitations.

1. Introduction

Cancer is a leading cause of mortality in many countries and a main obstacle to increasing life expectancy (Kern et al., 2023). Non-small cell lung cancers (NSCLCs) comprise approximately 85 % of lung cancer cases (Molina et al., 2008). Gefitinib (GEF) is an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor that binds to the adenosine triphosphate (ATP)-binding site of EGFR (Kris et al., 2003). GEF has been approved for use in patients with metastatic NSCLC, a condition often associated with EGFR overexpression (Kazandjian et al., 2016). EGFR overexpression enhances the activation of anti-apoptotic Ras signal transduction cascades, causing an increase in proliferation and survival of cancer cells (Wee & Wang, 2017). According to the biopharmaceutical classification system, GEF is a class II molecule, as it exhibits low solubility and high permeability. GEF is practically insoluble in water, and its solubility in aqueous buffers increases with decreasing pH. The octanol–water partition coefficient (log *P*) of GEF is 3.75, and it has two pKa values: 5.4 and 7.2 (Borg et al., 2020). Although GEF can be used to treat patients with NSCLC, its oral absorption is limited by its dissolution. The oral bioavailability of GEF is about 60 % (Borg et al.,2020). Higher doses of GEF are required for effective treatment, but it is associated with adverse effects such as vomiting and

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diarrhea (Trummer et al., 2012). The poor water solubility and low bioavailability compromise the therapeutic efficacy (Garizo et al., 2021).

The bioavailability of class II drugs can be significantly increased by improving the solubility and dissolution of drugs (Andrey et al., 2015; Chougule et al., 2011; Patel et al., 2014; Patel et al., 2015). Various techniques, including solid dispersion (SD) and complexation, have been suggested to improve the aqueous solubility of poorly soluble drugs (Sinha et al., 2010). Generally, the SD method is preferred to improve these drugs' solubility and oral bioavailability because of its relative simplicity, low cost, and scalability. In a previous study, Munir et al. reported an improvement in the dissolution and solubility of dex-ibuprofen (DEX) by complexation with hydroxypropyl-β-cyclodextrin (HPβCD) and they evaluated the effect of different hydrophilic polymers on the solubilization efficiency of HPβCD. Three different methods (physical trituration, kneading, and solvent evaporation) were used to prepare binary inclusion complexes with various drug-to-HPβCD weight ratios. By preparing a formulation with a weight ratio of 1:4 (DEX: HPβCD) using the kneading method, researchers were able to increase the solubility and the release of DEX. The addition of hydrophilic polymers, poloxamer-188 (PXM-188) and poloxamer-407 (PXM-407), at weight ratios ranging from 2.5 to 20 % significantly enhanced the complexation efficiency and solubility of DEX/HPβCD (Munir et al., 2022). Other studies also showed improved dissolution of drugs using SD or complexation with HPβCD. The SD of pioglitazone was formulated via microwave-induced fusion using two carriers, PXM-188 and HPβCD, in various ratios. The formulations showed a noticeable increase in drug release as the concentration of the carriers increased (Mishra et al., 2011). Furthermore, an inclusion complex of saquinavir mesylate with HPβCD using a kneading method demonstrated an increase in solubility and dissolution rate compared with the pure drug (Mahajan et al., 2013). Another study aimed to enhance the physicochemical qualities of diacerein, a poorly soluble medication, using sorbitol as a water-soluble carrier. SDs were prepared through solvent evaporation and physical triturating, using different ratios of diacerein-to-sorbitol (1:0.5, 1:1.5, and 1:2.5; w/w). Sorbitol was demonstrated to be effective at increasing the solubility of poorly soluble medications (Fouad et al., 2021).

Several methods have been investigated to enhance the solubility and dissolution of GEF. The use of self-emulsifying drug delivery systems has been shown to increase the solubility/and dissolution of GEF by 2.14 times compared to pure drugs (Reddy & Vahini, 2020). Mustafa et al. prepared SDs of GEF using a spray-drying technique to combine the drug with different ratios of polyvinylpyrrolidone (PVP) and hydroxypropyl methylcellulose (HPMC). The authors reported that HPMC-based SDs increased GEF dissolution and release at pH 7.2 (Mustafa et al., 2022). Alshehri et al. prepared GEF SD with polyethylene glycol (PEG) 4000 by using fusion and microwave methods. The authors reported that GEF SD markedly enhanced dissolution and bioavailability(Alshehri et al., 2021).

This study aimed to enhance the dissolution and solubility of GEF via SD with hydrophilic polymers and via complexation with cyclodextrins, to evaluate the cytotoxic activity of the SDs and complexes in vitro, and to formulate them into tablets with enhanced dissolution characteristics.

2. Materials and methods

2.1. Materials

GEF (purity: 98.60 %) was obtained from Beijing Mesochem Technology (Beijing, China). Hydroxypropyl beta cyclodextrin (HPβCD) and Methyl beta cyclodextrin (MβCD) were purchased from Signet Chemical Corporation Pvt Ltd. (India). Polyeheleneglycol 4000 (PEG) and Poloxamer-188(PXM) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Polyvinyl pyrrolidone-K30(PVP) was purchased from Fluka Co. (Germany). Microcrystalline cellulose (Avecil PH102) was purchased from Riedel-de Haën (Seelze, Germany).

2.2. Construction of a calibration curve

To determine the wavelength of maximum absorption (λ_{max}), a 100 μg/mL stock solution of GEF in methanol was prepared. Absorbance measurements of the solution were made over a range of 200–400 nm using a scanning spectrophotometer [UV-1700, Shimadzu, Japan] volumes of the stock solution were diluted with phosphate. ate buffer pH 6.8 to a final concentration of 5–25 mcg/mL. The absorbance of each dilution was measured at the λ_{max} of GEF(331 nm), and linear least-square regression analysis was performed. To detect any interference or overlap of the used polymers, 50 mcg/mL solution of each polymer free and mixed with 10 mcg/mL GEF solution was scanned in the same range.

2.3. Phase solubility studies

Excess GEF (10 mg) was mixed with 10 mL of distilled water containing different HP-β-CD or M-β-CD concentrations (125–100 mM), as described by Higuchi and Connors (1965). After shaking for 24 h at 25 °C ± 1 °C in a shaking water bath to reach equilibrium, samples were collected in triplicate and subsequently filtered through a 0.45 μm polyvinylidene difluoride (PVDF) membrane filter. Diluted samples were analyzed using a UV-1700 UV spectrophotometer (Shimadzu, Japan) at a wavelength of 331 nm against CD-in-water blanks with identical concentrations. The apparent stability constant (K_c) of the soluble complexes was calculated from the phase solubility diagrams using the equation:

$$K_c = Slope/S_o(1 - slope) \quad (1)$$

Where S_o is the intercept of the phase solubility diagram

2.4. Preparation of GEF solid dispersions (SD)

The SD of GEF was prepared by the solvent evaporation method. A ratio of (1:2,1:4 and 1:6 w/w) of GEF: PVP, GEF: PEG, and GEF: PXM was used. First, the polymer was dissolved in 10 mL of ethanol in a porcelain dish. The weight of GEF was added, and the solution was heated in a water bath at [70 ^{0}C] until complete evaporation, then dried and stored in a dry place until use (Alghaith et al., 2022).

2.5. Preparation of solid inclusion complexes

GEF and HPβCD (1:1 and 1:2 w/w molar ratios) or MβCD (1:1 w/w 1 and 1:2 M ratios) inclusion complexes were prepared by kneading Method. The calculated amounts of GEF and cyclodextrin were sieved through a No. 60 mesh sieve and then mixed in a mortar to form the physical mixture. For kneading, acetone/water mixture (1:1) was added dropwise to form a paste and then triturated in a mortar for 30 min. The obtained kneaded mass was dried at 40 ^{0}C for 24 h. The dried mass was pulverized and stored in a dry place for further investigation (Alghaith et al., 2022).

2.6. In-vitro dissolution testing

USP apparatus 2 (Caleva Ltd., Model 85 T) was used. In each of the six flasks, 500 mL of phosphate buffer (pH 6.8) was equilibrated to 37 ± 0.5 °C, and the paddle speed was set at 75 rpm. To each flask, a specific amount equivalent to 10 mg GEF from each formulation or one tablet was added. At intervals of 10, 20, 30, and 60 min, 5 mL samples were taken and replaced with an equal amount of fresh dissolution medium. For each formula, release runs were performed in triplicate and the absorbance was measured at 331 nm. The cumulative percentage of drugs released was determined as a function of time.

Table1
Composition of tablets formulated using PVP–GEF SD1:4, GEF–MβCD 1:1, or GEF powder. Weights are given in mgs.

Component	F1	F2	F3
GEF-PVP SD	250	–	–
GEF–MβCD	–	250	–
GEF powder	–	–	50
Crospovidone	25	25	25
Avicel pH 102	220	220	420
Magnesium stearate	5	5	5
Total	500	500	500

2.7. Fourier transform infrared spectroscopy (FTIR)

FTIR was performed on a Bruker ALPHA spectrometer (Bruker Optics, Ettlingen, Germany) to investigate the interaction of GEF with the carriers. The FTIR spectra of GEF, the polymers, their physical mixtures, and their SD or complexes were recorded in the wavelength region of 4000–400 cm^{-1}.

2.8. Powder X-ray (PXR) diffractometry

The crystallinities of the pure drug and its SD or solid inclusion complexes were investigated using PXR diffractometry. Powder sample X-ray diffraction patterns were obtained using a RIGAKU diffractometer

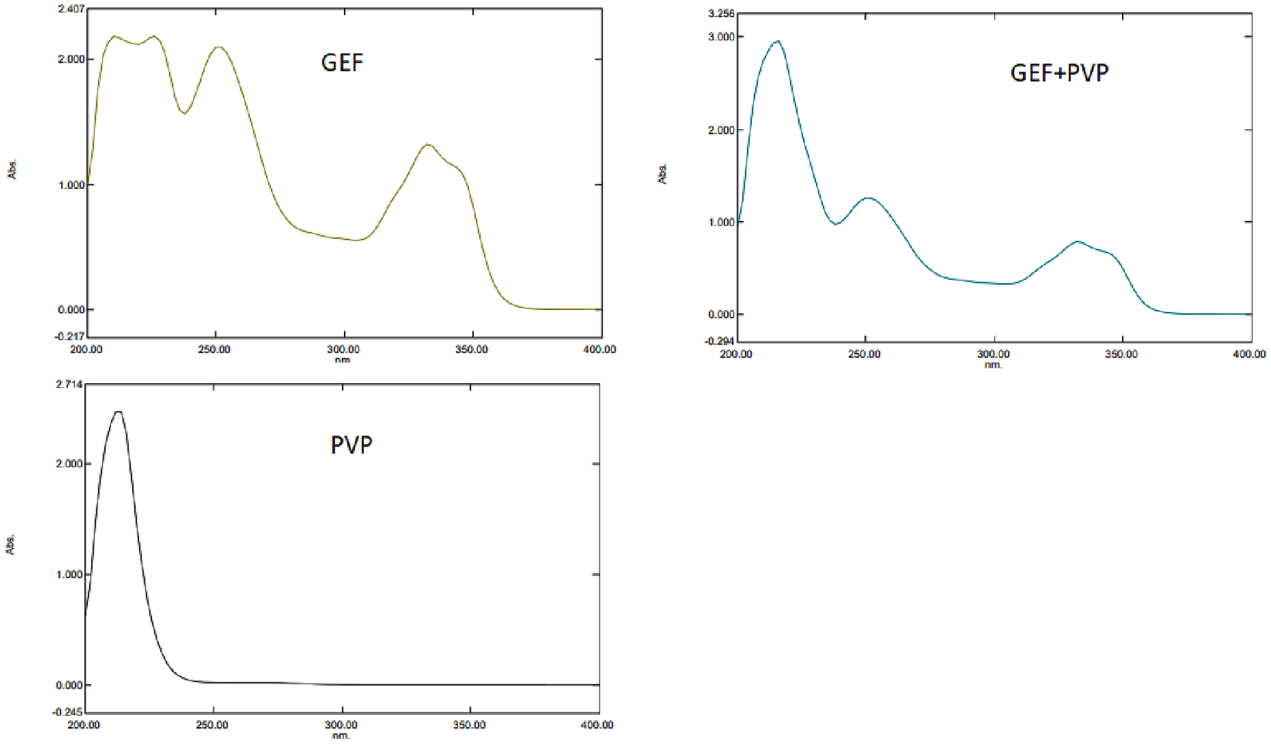

Fig. 1. UV spectra of GEF, PVP, and their mixed solution in methanol.

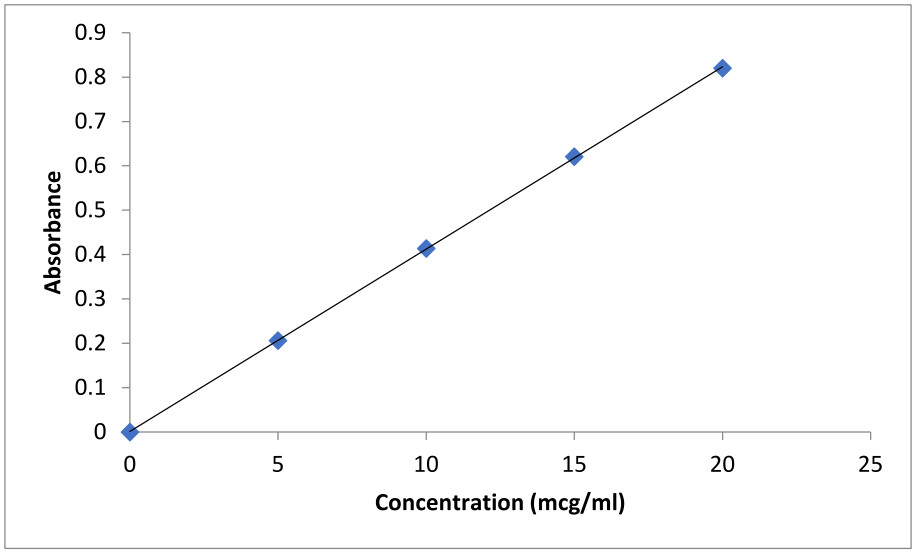

Fig. 2. The calibration curve of GEF ln phosphate buffer pH 6.8.

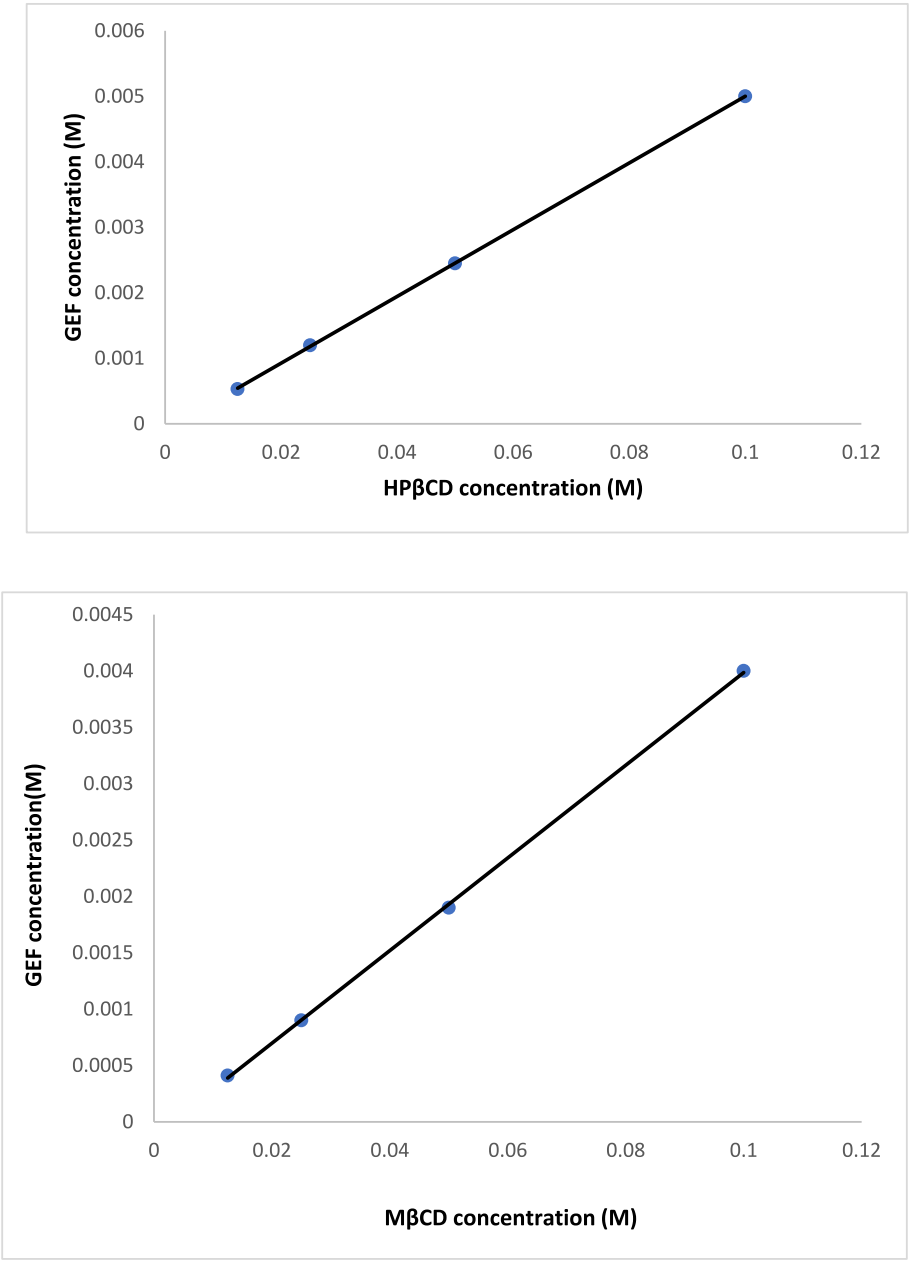

Fig. 3. Phase solubility curves of GEF with cyclodextrins solutions.

Table 2
GEF solubility curves parameters.

Cyclodextrin	Slope	Intercept	R^2	K_s M^{-1}
HPβCD	0.0509	0.0001	0.999	536
MβCD	0.0411	0.0001	0.999	428

(Japan) fitted with a curved monochromatic graphite crystal, an automatic divergence slit, and a PW/1710 automatic controller. Cu Kα radiation (λ ~ 1.5418 Å) served as the X-ray source, and measurement conditions were as follows: voltage, 40 KV; current, 40 mA. The patterns of diffraction were achieved using the continuous mode of scanning in step sizes of 2 °C from 4 °C to 140 °C.

2.9. Determination of the cytotoxicity of the formulae

A549 cells were obtained from the Leibniz Institute DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany). Cells were cultured in T75 cell culture flasks (supplier, country) in a humidified environment (5 % CO_2, 37 °C) using Dulbecco's modified Eagle's medium. The medium contained 10 % fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were maintained in a sub-confluent state and the medium was replaced every 48 h. A549 cells were then plated in 96-well cell culture plates to test the cytotoxic effect of compounds, at a seeding density of 6×10^3 per well and were allowed to adhere overnight. The cells were then treated with GEF, PVP, MβCD, GEF–PVP SD, and GEF–MβCD complex at various concentrations and incubated for 48 h. Then, 10 μl of MTT (5 mg/ml) was added to each well and the plates were incubated at 37 °C in 5 % CO_2 for 3 h. Dimethyl sulfoxide was added to solubilize the formazan products, and the plates were placed in a shaker for 10 min. The absorbance of each well was measured at 490 nm. IC_{50} was calculated using a dose-dependent curve, and cell viability was calculated using the following equation:

Table 3
Improvement in the solution of solid dispersions of GEF prepared using hydrophilic polymers or GEF subjected to complexation using hydroxypropyl-β-cyclodextrin (HPβCD) or methyl-β-cyclodextrin (MβCD). The percentage of dissolved GEF is the average of 3 replicates.

Carrier	GEF: carrier ratio	% GEF dissolved at 60 min	Enhancement compared to free GEF
PEG	1:2	34.42 ± 0.6	1.22
	1:4	38.84 ± 0.8	1.38
	1:6	39.97 ± 0.7	1.43
PXM	1:2	38.98 ± 1.4	1.4
	1:4	42.53 ± 1.5	1.52
	1:6	45.58 ± 1.7	1.62
PVP	1:2	51.8 ± 0.7	1.85
	1:4	58.0 ± 0.5	2.1
	1:6	60.4 ± 0.8	2.18
HPβCD	1:1	39.38 ± 3.1	1.44
	1:2	48.05 ± 3.2	1.67
MβCD	1:1	58.62 ± 2.7	2.11
GEG	1:2	60.1 ± 3.1	2.17
(untreated)		28.0 ± 1.5	1.0

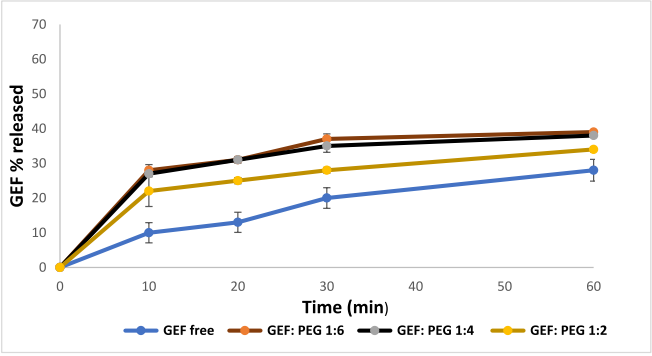

Fig. 4. The dissolution of gefitinib (GEF) in its free form, and as a solid dispersion with polyethylene glycol (PEG) in different ratios, in a pH 6.8 buffer. Values are presented as mean ± SE. N = [3].

$$\text{Cell viability}\ (\%) = [(\text{OD}_{490}\ \text{of the treated sample}) / (\text{OD}_{490}\ \text{of the untreated sample})] \times 100 \quad (2)$$

2.10. Tablet formulation

Tablets containing PVP–GEF SD (1:4w/w) or GEF–MβCD complex (1:1 M) (equivalent to 50 mg GEF) were manufactured using direct compression. The components of the formulations shown in Table 1 were mixed in a turbula mixer (type S27, Erweka, Apparatebau, Germany) for 10 min and then directly compressed into tablets using a single-punch tablet machine (type EKO, Erweka, Apparatebau, Germany) with 8 mm concave punches. The hardness of the tablets was maintained within the range of 6–8 kp, and the tablet weight was maintained at approximately 500 mg. Avecil pH 102 (obtained from Riedel-de Haën (Seelze, Germany) was used as the diluent, magnesium stearate as the lubricant, and crospovidone as the disintegrant.

2.11. Statistical analysis

GraphPad Prism software was used to analyze the data. Data are expressed as mean ± standard deviation (SD). The degree of significance was determined using a paired *t*-test. P-values < 0.05 were regarded as statistically significant.

3. Results

3.1. Calibration curve of GEF

The λ_{max} of GEF in methanol was determined as 331 nm. There is no interference or overlap of the used polymers with GEF absorbance. Repressive spectra of GEF, PVP, and their combined solution are shown in Fig. 1. Subsequently, a dilution series was made using phosphate buffer pH 6.8, and the absorbance of the dilutions was measured at 331 nm. The calibration curve was constructed in the concentration range of 5–20 mcg/mL (Fig. 2). The regression equation was:

$$A = 0.0394C (R^2 = 0.999) \quad (3)$$

Where A, is GEF absorbance and C, is its corresponding concentration.

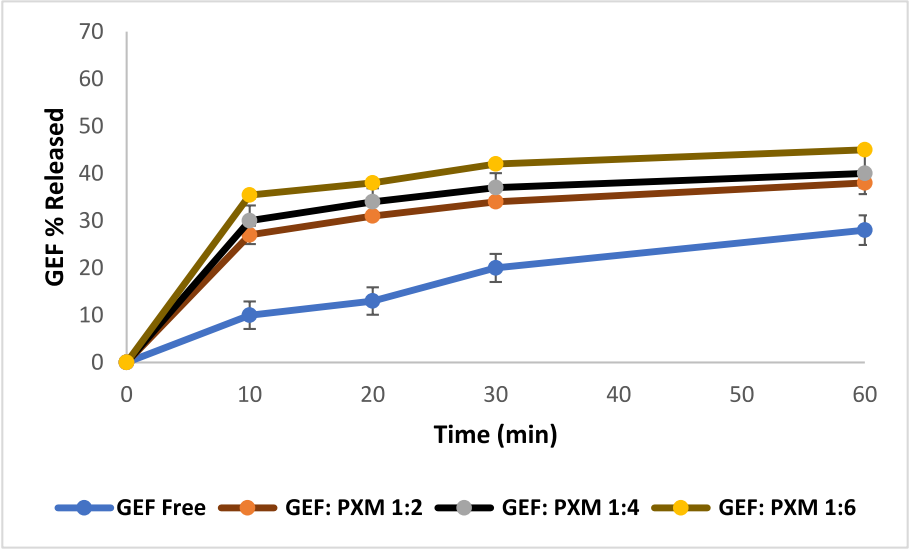

Fig. 5. The dissolution of gefitinib (GEF) in its free form, and as a solid dispersion with poloxamer-188 in different ratios, in a pH 6.8 buffer. Values are presented as mean ± SE. N = [3].

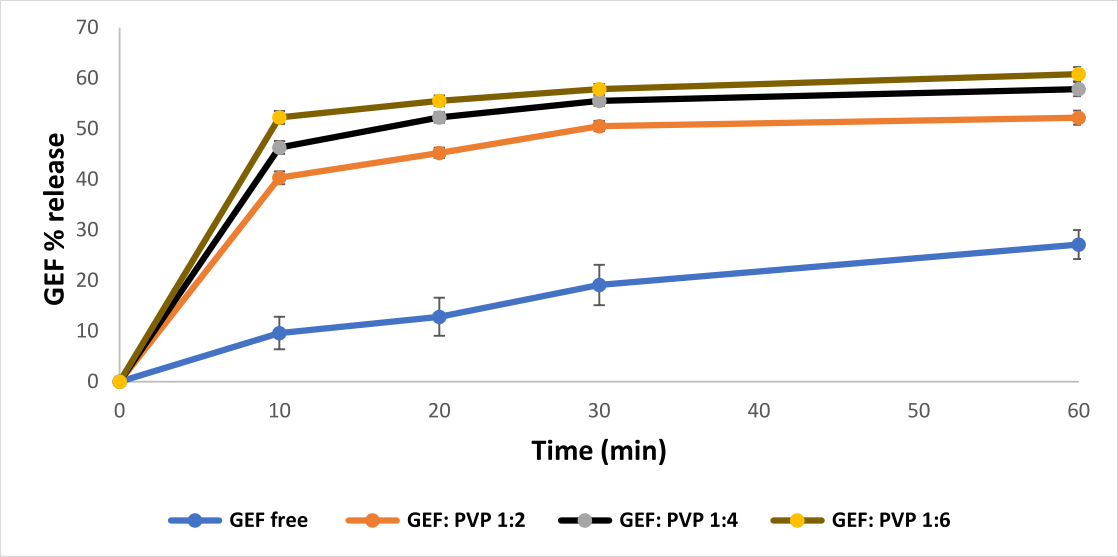

Fig. 6. The dissolution of gefitinib (GEF) in its free form, and as a solid dispersion with PVP-k30 in different ratios, in a pH 6.8 buffer. Values are presented as mean ± SE. N = [3].

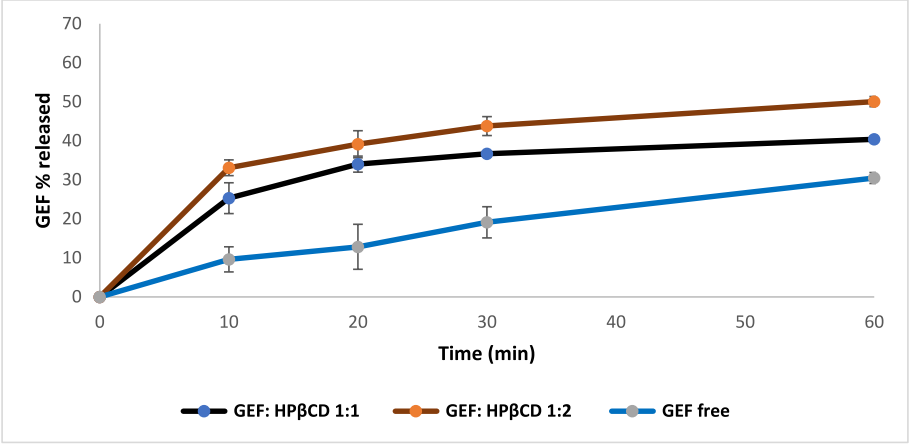

Fig. 7. The dissolution of gefitinib (GEF) in its free form, and as a complex with hydroxypropyl-β-cyclodextrin (HPβCD) in different ratios, in a pH 6.8 buffer. Values are presented as mean ± SE. N = [3].

3.2. Phase solubility studies

The phase solubility profiles obtained for the HPβCD–GEF and MβCD-GEF inclusion complexes are shown in Fig. 3. The aqueous solubility of GEF linearly increased with the cyclodextrin concentrations. Obtained from the slope of the linear phase solubility diagram, Kc (the apparent stability constant) was found to be 536.50 M^{-1} for HPβCD and 428.60 M^{-1} for MβCD (Table 2).

3.3. Dissolution studies

The dissolution of GEF from the prepared SDs is presented in Table 3 **and** Figs. 4–6**.** The percentage of dissolved GEF was enhanced by approximately 1.12–2.18 fold (Table 3**).** The percentage of GEF dissolved from PEG SDs increased from 34.42 to 39.97 by increasing the GEF: polymer ratio from 1:2 to 1:6. In addition, the percentage of GEF dissolved from poloxamer SDs increased from 39.97 to 45.58 by increasing the GEF: polymer ratio from 1:2 to 1:6. Furthermore, the % GEF dissolved from PVP SDs increased from 51.80 to 60.4 by increasing the GEF: polymer ratio from 1:2 to 1:6.

Fig. 7 and Table 3 show that the HPβCD–GEF inclusion complexes, prepared using kneading, at GEF-to-HPβCD ratios of 1:1 and 1:2 released 39 % ± 3.1 % and 48.0 % ± 3.2 %, respectively, of GEF after 60 min. In contrast, 28 % ± 1.5 % of pure GEF was released in the same period. Furthermore, MβCD–GEF inclusion complexes prepared using kneading (1:1 and 1:2) released 58.6 ± 2.7 & and 60 % ± 3.1 % of GEF (Fig. 8 **&** Table 3).

PVP-GEF SD (1:4) and Mβ-CD-GEF (1:1) showed high dissolution enhancement were selected for further investigations and tablet formulation.

3.4. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of pure GEF and the components of PVP–GEF SD and MβCD–GEF complex are presented in Figs. 9 **& and** 10, respectively. The FTIR spectrum of pure GEF exhibited various spectral peaks corresponding to its known functional groups. The sharp peak at 1500 cm^{-1} in pure GEF represents the N—H bending vibration. The C—O functional group of GEF also exhibited a peak at 1112 cm^{-1}. The presence of peaks at 1250 cm^{-1} and 770 cm^{-1} further confirmed the presence of a C—F and C—Cl functional group in pure GEF (Figs. 9a & 10a). All of these peaks appeared with less intensity due to the dilution effect of the

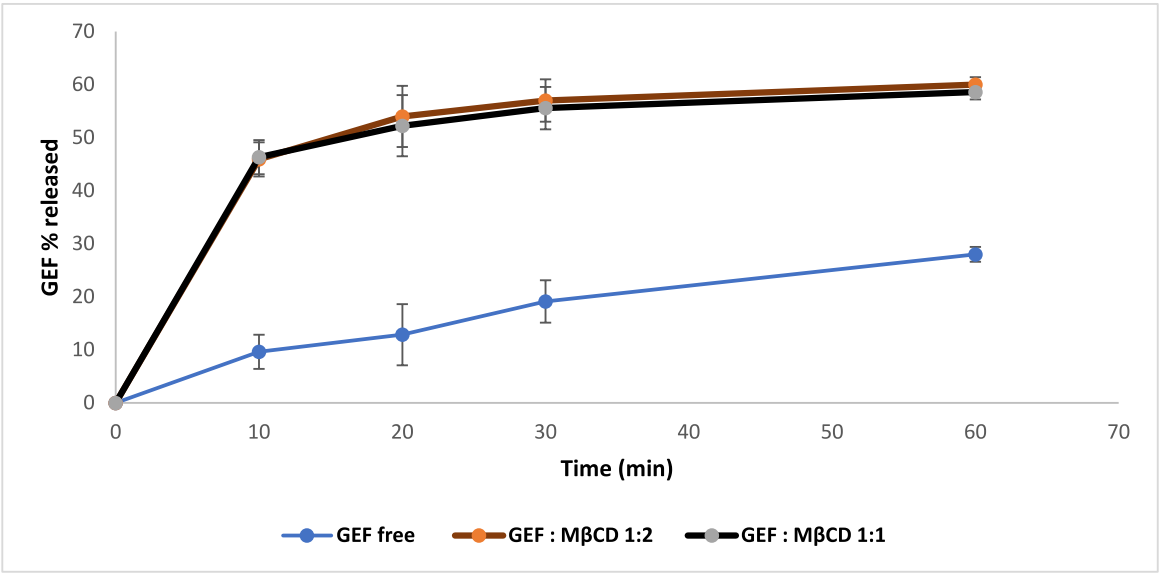

Fig. 8. The dissolution of gefitinib (GEF) in its free form, and as a complex with methyl-β-cyclodextrin (MβCD) in different ratios, in a pH 6.8 buffer. Values are presented as mean ± SE. N = [3].

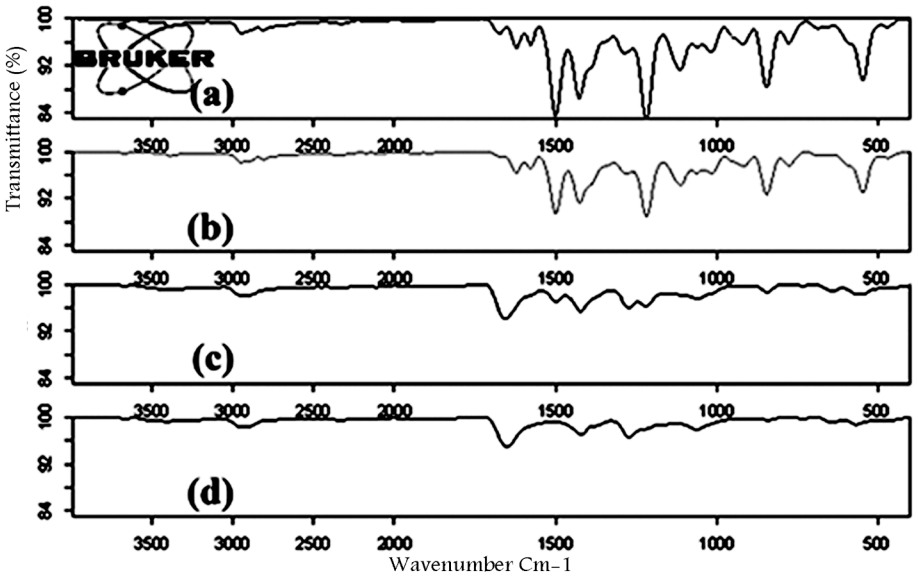

Fig. 9. Fourier transform infrared spectra of (a) pure gefitinib (GEF), (b) a physical mixture of GEF and polyvinylpyrrolidone (PVP), (c) a PVP–GEF solid dispersion, and (d) PVP.

polymer or disappeared (Figs. 9b & 10b) and some disappeared due to the interaction with the polymer (Figs. 9c & 10c).

3.5. Powder X-ray diffraction (PXRD

The changes in the PXRD spectra of GEF, when subjected to SD preparation with *PVP* carriers *or inclusion comply with* MβCD, were investigated. The PXRD spectra of pure GEF, the components of PVP-GEF SD, *a*nd the MβCD –GEF *complex* are presented in Figs. 11 and 12, respectively. Pure GEF showed multiple sharp, high-intensity diffraction peaks at 19.5°, 24.2°, 26.5°, 38.1°, and 44.3°, demonstrating that it was present in its natural crystalline form (Fig. 11a & 12a). The physical mixtures have the same characteristic peaks with less intensity due to the polymer dilution effect (Fig. 11**b&** 12**b**). On the other hand, the PVP–GEF SD spectrum showed the disappearance of the characteristic GEF peaks at 19.5–26.5 degrees, indicating the presence of GEF in its amorphous state (Fig. 11**c**). A similar pattern was observed for the MβCD–GEF complex (Fig. 12**c**) which could be attributed to the entrapment of GEF in the MβCD cavity and inclusion complex formation.

3.6. Cytotoxicity assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the cytotoxicity of free GEF, PVP–GEF SD 1:4 w/w, and MβCD–GEF 1:1M on A549 cells following 48 h of treatment. IC_{50} is expressed as half maximal inhibitory concentration. PVP and MβCD controls showed no cytotoxic effect up to 1000 and 500 μg/ml, respectively (Fig. 13). These concentrations were higher than the concentrations of the respective carriers used in the GEF formulations. Free GEF showed an IC_{50} value of 9.97 ± 1.8 μM, PVP–GEF and MβCD –GEF exhibited higher toxicity compared to free GEF. The IC_{50} values of the PVP–GEF and M-β-CD–GEF were 4.33 ± 0.66 and 4.84

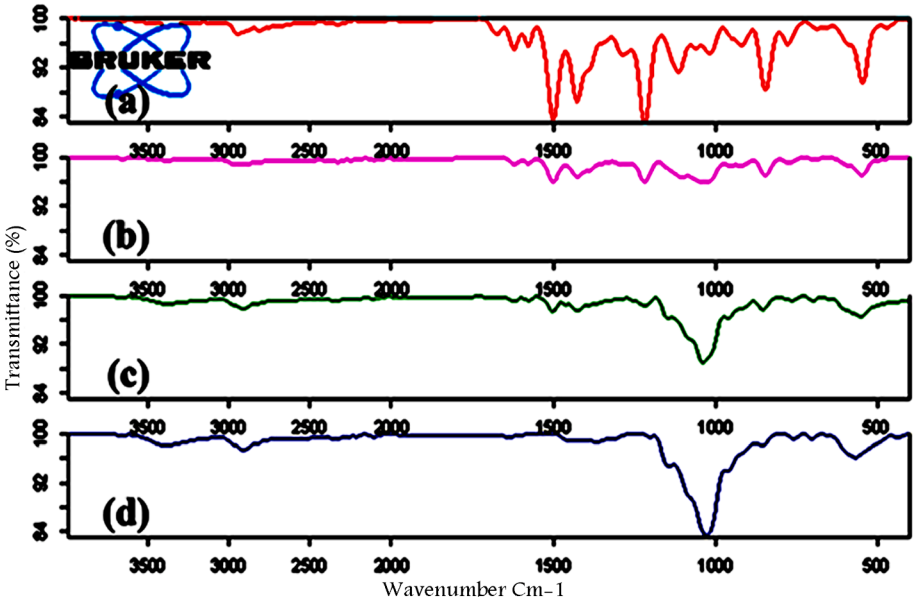

Fig. 10. Fourier transform infrared spectra of (a) pure gefitinib (GEF), (b) a physical mixture of GEF and (MβCD), (c) MβCD –GEF complex, and (d) M-βCD.

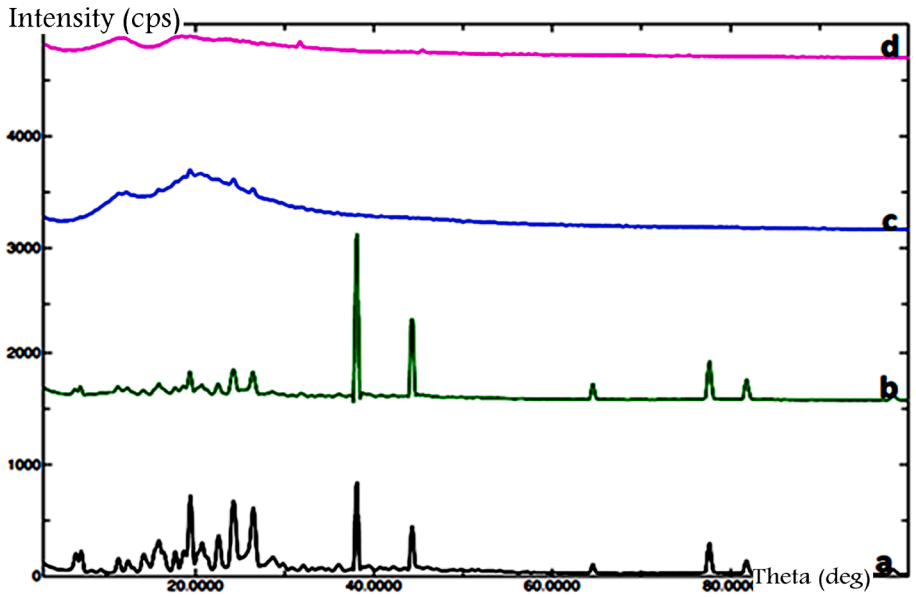

Fig. 11. The powder X-ray diffraction spectra of (a) pure gefitinib (GEF), (b) a physical mixture of GEF and polyvinylpyrrolidone (PVP), (c) a PVP–GEF solid dispersion, and (d) PVP powder.

± 0.38 μM, respectively, which are significantly lower ($p < 0.05$) than free GEF.

3.7. GEF release from formulated tablets

PVP–GEF SD1:4 and GEF-MβCD1:1M were formulated into tablets containing amounts equivalent to 50 mg of GEF (F1 and F2 in Table 1). In addition, tablets containing 50 mg of GEF powder (**F3,** Table 1) were prepared for comparison. The release profiles of the tables are shown in Fig. 14. F1, which contained PVP–GEF SD released (35.1 % ± *0.4*) GEF after one hour. F2 contains GEF- MβCD showed a higher release (42.2 % ± *0.7)* after one hour. In the meantime, F3 which contains 50 mg of pure GEF released only 15 % ± 0.5 of GEF at the same time (Fig. 14).

4. Discussion

4.1. Assay suitability and phase solubility study

The λmax of GEF in methanol was determined as 331 nm. There is no interference or overlap of the used polymers with GEF absorbance. The correlation coefficient of the calibration curve ($R^2 = 0.999$)

The λ_{max} of GEF in methanol was determined as 331 nm. There is no interference or overlap of the used polymers with GEF absorbance (Fig. 1**)** linearity of The calibration curve ($R^2 = 0.999$) Indicates the suitability of the assay (Fig. 2**).**

The aqueous solubility of GDF linearly increased with the increase in HP-β-CD and M-β-CD concentrations. The solubility diagram indicated the formation of a 1:1 HP-β-CD–GEF, and 1:1 M-β-CD–GEF inclusion complexes, as its shape showed a linear host–guest correlation (AL type) and its slope was less than 1 (Chowdary and Srinivas, 2006)

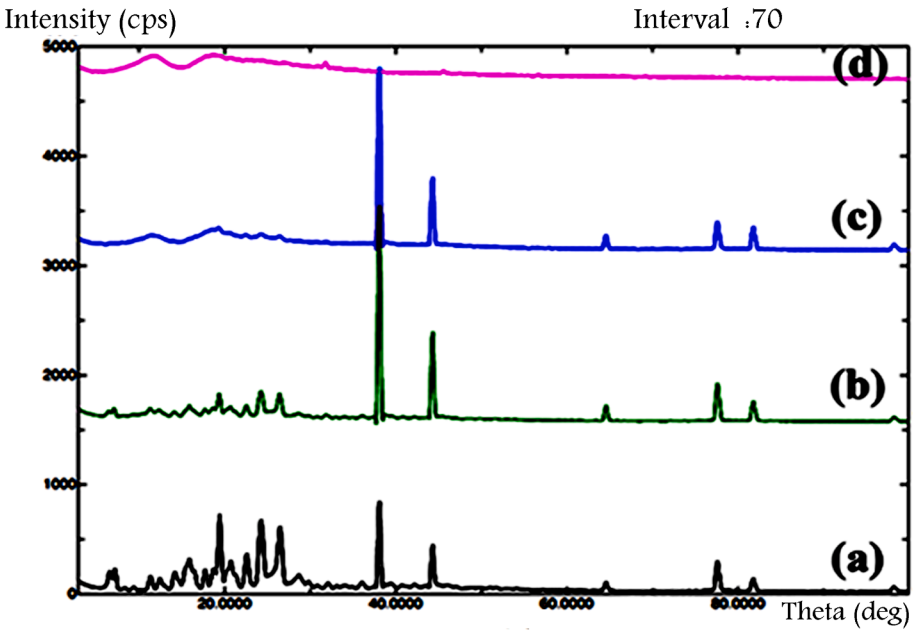

Fig. 12. The powder X-ray diffraction spectra of (a) pure gefitinib (GEF), (b) a physical mixture of GEF and methyl-β-cyclodextrin (MβCD), (c)MβCD –GEF complex, and (d) MβCD powder.

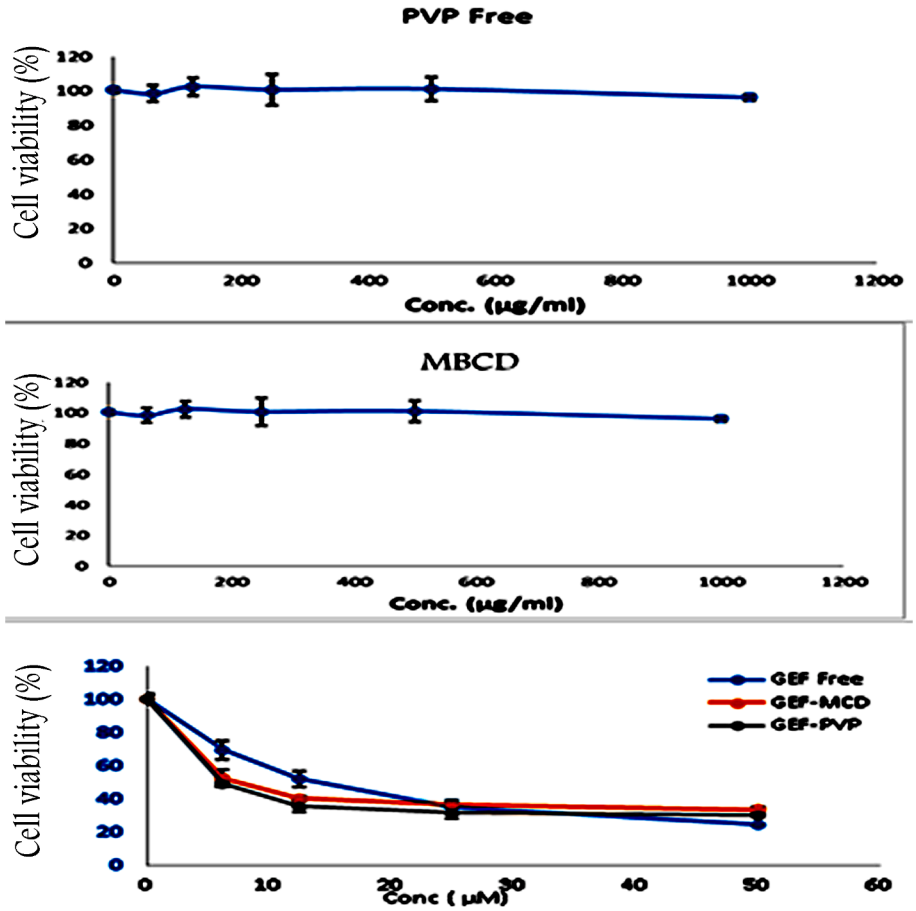

Fig. 13. Cytotoxicity of free polyvinylpyrrolidone (PVP), free methyl-β-cyclodextrin (M-β-CD), pure gefitinib (GEF), a PVP–GEF solid dispersion, and a MβCD –GEF complex on A549 cells following 48 h of treatment. Values are presented as mean ± standard deviation. N = [3].

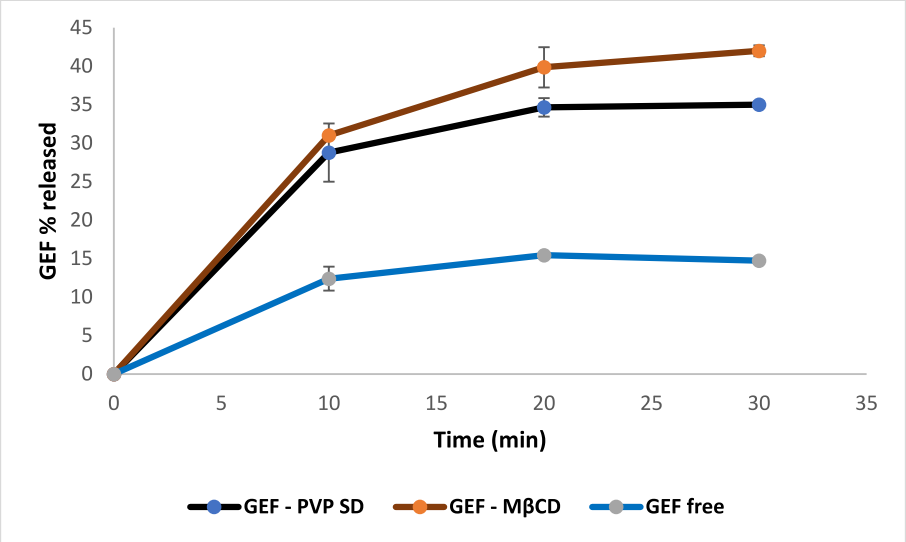

Fig. 14. The dissolution profile of tablets contains GEF-PVP SD; F1, GEF-MβCD; F2 and GEF powder; F3.

4.2. Dissolution studies

The effectiveness of several drugs that are poorly soluble in water is limited by their poor dissolution and bioavailability. GEF is a water-insoluble dibasic compound used for the treatment of NSCLCs. It has pKa values of 5.28 and 7.17, and its solubility is pH-dependent, which affects its solubility in gastrointestinal (GI) fluids (Godugu et al., 2016). SD preparation has been successfully used to enhance the solubility and dissolution of poorly water-soluble drugs, thereby improving their bioavailability(Chougule et al., 2011; Gohel & Patel, 2003; Gurunath et al., 2014). In addition, the solubility and dissolution of GEF have been shown to improve by complexation with CDs (Trummer et al., 2012). In this study, SDs of GEF were prepared using hydrophilic polymers (PEG 4000, a PXM-188, PVP K30) in three different ratios. In addition, GEF was subjected to complexation using HPβCD and methyl-β-cyclodextrin (MβCD) in two molar ratios to improve its dissolution (Table 3).

Because of the basic nature of GEF, its dissolution is pH-dependent. However, as the drug absorption occurs mainly in the intestine, all dissolution studies were conducted in a phosphate buffer medium (pH 6.8) to mimic the conditions inside the GI tract (Godugu et al., 2016). The prepared GEF- SDs enhanced the dissolution of GEF by approximately 1.124–2.18 fold (Table 3, Figs. 4–6). This enhancement could be attributed to the conversion of a portion of the GEF to an amorphous state, increasing its surface area and leading to a higher dissolution rate (Friesen et al., 2008; Mishra et al., 2011; Sinha et al., 2010).

Figs. 7 & 8 and Table 3 show that the HPβCD–GEF and MβCD –GEF inclusion complexes, prepared using kneading, at ratios of 1:1 and 1:2 M ratio. The HPβCD–GEF and M-βCD–GEF inclusion complexes showed a significantly higher dissolution than GEF alone ($P < 0.05$). In addition, an increased CD molar ratio led to an insignificant increase in drug release ($P > 0.05$).

The increased dissolution rate of GEF from HPβCD–GEF and MβCD –GEF inclusion complexes compared to that of free GEF could be attributed to the amorphization of GEF and the formation of soluble inclusion complexes, leading to an increased dissolution rate (Carrier et al., 2007; Lee et al., 2009; Vikas et al., 2018). PVP-GEF SD 1:4 and MβCD-GEF 1:1M showed high dissolution enhancement and therefore, were selected for further investigations.

4.3. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra in Figs. 9 and 10 showed spectral peaks corresponding to its known functional groups. All of the peaks in the physical mixtures appeared with less intensity due to the dilution effect of the polymer (Figs. 9b & 10b). The prepared PVP–GEF SD spectrum exhibited a marked decrease in the intensity or disappearance of peaks. The change in the peaks was due to the presence of carrier and formation of SD. The spectra also indicated a minimal interaction between the functional groups of the pure GEf and the PVP (Fig. 9c). A similar observation was recorded for the MβCD–GEF complex (Fig. 10c) which could be attributed to the entrapment of GEF in the MβCD cavity (Alshehri et al., 2021; Li et al., 2019).

4.4. Powder X-ray diffraction (PXRD)

PXRD is a technique used in materials science to analyze the structure of crystalline substances. It works by shining X-rays into a powdered sample and measuring the produced diffraction pattern. This pattern provides information about the arrangement of atoms in the crystal lattice of the material, allowing us to determine its properties and degree of crystallinity. As shown in Figs. 11 and 12. The prepared formulations (PVP–GEF SD and MβCD–GEF complex) exhibited significant changes in the high-intensity peaks of GEF. The peak heights and intensities at 19.5°, 24.2°, and 26.5° were reduced, possibly due to the conversion of crystalline GEF into a partially amorphous state. Other peaks observed in the formulations were a result of the carriers used (Alshehri et al., 2021; Li et al., 2019).

4.5. Cytotoxicity assay

A cytotoxicity assay is a method used to determine the degree to which a substance is toxic to cells. The results indicated that PVP–GEF and MβCD–GEF exhibited higher toxicity than free GEF. The IC_{50} values of the PVP–GEF and MβCD–GEF were 4.33 ± 0.66 and 4.84 ± 0.38 μM, respectively, which are significantly lower ($p < 0.05$) than free GEF (Fig. 13). This could be attributed to enhanced drug solubility and dissolution (Alghaith et al., 2022).

4.6. GEF release from formulated tablets

Attempts were made to formulate tablets containing the most promising prepared PVP–GEF SD and MβCD–GEF were formulated into tablets with strengths equivalent to 50 mg of GEF. The drug released from the tablets was significantly higher ($p < 0.05$) than that released from the tablet formulations prepared using the pure untreated drug as shown in Fig. 14. These findings open a window for further research to

improve dissolution properties and hence the bioavailability of GEF tablets.

5. Conclusions

In this study, GEF SDs were prepared using the solvent evaporation method, and GEF complexes with cyclodextrins were prepared by the kneading method. Both SDs and the complexes showed marked improvement in the dissolution of GEF, and a decrease in the IC_{50} compared to pure GEF. The PVP–GEF SD1:4w/w and MβCD –GEF1:1M were formulated into tablets. The prepared tablets showed a significant enhancement in dissolution ($p < 0.05$) compared to the tablets prepared with pure GEF, representing a practical approach for the effective delivery of the drug. Moreover, the potential to reduce the required dose of GEF could lead to fewer adverse effects, improving patient tolerance and adherence to treatment.

CRediT authorship contribution statement

Adel F. Alghaith: Data curation, Methodology, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition. **Gamal M. Mahrous:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – review & editing. **Ahmed S. Alenazi:** Methodology, Supervision, Validation, Visualization. **Suliaman M. ALMufarrij:** Formal analysis, Methodology, Writing – original draft. **Mohammed S. Alhazzaa:** Formal analysis, Methodology, Writing – original draft. **Awwad A. Radwan:** Data curation, Project administration, Software. **Abdullah S. Alhamed:** Methodology, Visualization, Writing – review & editing. **Mohamed S. Bin Salamah:** Data curation, Methodology. **Sultan Alshehri:** Conceptualization, Investigation, Software.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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