Research Article

Solubility Enhancement of Synthesized Quinazolinone Derivative by Solid Dispersion Technique

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ABSTRACT

The current study pacts with the development of solid dispersions of quinazolinone derivative with polaxamer407 endeavorto improve the solubility profile. Quinazolinone derivative solid dispersions were prepared by melt-fusion technique using polaxamer407 as carrier in different ratios. Acute oral toxicity (OECD 423) study was done to check the toxicity of the compound in Swiss Albino Mice and cell line study on HT-29 &L929 normal fibroblast cells were conducted by MTT assay. To attain an optimized formulation, solid dispersions were characterized and evaluated through phase solubility, drug content, wettability in-*vitro* release studies etc. A moderate toxicity on normal fibroblasts L929 cell line with the highest cytotoxic activity on HT-29 human colon carcinoma cell line was observed with an effective dose of 200mg. The phase diagram showed a linear increase due to the weight fraction of polaxamer in pH 6.8. The *in-vitro* dissolution rate of quinazolinone derivative was improved in the solid dispersion attributed to improve wettability, decrease in crystallinity and the increase in the amorphous fraction of the drug. The drug release from solid dispersion followed zero order fickian diffusion kinetics. The results suggested that the prepared solid dispersions are a promising approach to enhance the solubility of quinazolinone derivative.

Keywords: Quinazolinone derivative, *In-vitro* cytotoxicity, OECD 423, Polaxamer 407, Solid dispersion, Solubility, Wettability.

INTRODUCTION

7-chloro-3-(4-chlorophenyl)-2-phenyl quinazolin-4(3H)-one, a quinazolinone derivative exhibits pharmacological and biological activities such as anti-inflammatory[1], anticancer[2], anticoccidial[3], analgesic[4], anticonvulsant[5], antimicrobial[6], antimalarial[7]. 7-chloro-3-(4-chlorophenyl) -2-phenyl quinazolin-4 (3H) -one has an empirical formula $C_{20}H_{12}Cl_2N_2$ with molecular weight 368 g/mol and structural formula (Figure 1). The mechanism of action of drug mainly involves the inhibition of thymidylate synthase receptor that prevents the growth of cancerous cells.[8]The evaluation of toxic action of the synthesized compound is very important prior to formulation and toxicity test on animal models is a key stage to ensure the safer dose of the compound. Acute oral toxicity study under OECD 423 guideline on Female Swiss Albino mice was conducted which aimed to establish the therapeutic dose of the compound from which 1/10th of maximum dose was selected. Another challenge in drug development and formulation of new molecules is its poor aqueous solubility.[9]Many newly synthesized compounds show lipophilicity with slow drug release and hence expected to have dissolution limited absorption.[10] Solubility can be enhanced by various formulation and chemical approaches like polymer complexation, crystal habit modification, micronization, nanosuspension, use of surfactant, cosolvent, pH alteration, solvent evaporation method, lyophilisation , electrospining, supercritical fluid technology ,eutectic mixtures, solid solutions and solid dispersions.[10, 11] Recent research works shows solid dispersion as the best technique for enhancing the solubility of poorly soluble compounds. Solid dispersions can be first, second and third generations. In this study second generation solid dispersion was used in which thermodynamically stable crystalline drug was dispersed in polymeric amorphous carrier. This improves bioavailability and dissolution of the drug at the absorption site.

Here solid dispersion is prepared by melt-fusion method[12, 13], in which the active ingredients get molecularly dispersed into the hydrophilic carrier. The dispersion process improves solubility by inhibiting the crystal nature of drug and converts into an amorphous form .by exceeding free energy, entropy and enthalpy, increased wettability, drug-carrier solubilisation by diffusion layer, to reduce particle size with a decrease in interfacial tension with hydrophilic carrier and ensures sink condition for dissolution to increase bioavailability.[14]

The drug release from solid dispersion involves two mechanisms: Drug and Carrier controlled release. In carrier controlled release mechanism, the hydrophilic carriers either absorb water by forming a concentrated layer of gel which act as a diffusion barrier. The drug release in drug controlled release mechanism takes place when intact to contact with water due to its insolubility in the concentrated layer.[15]The dissolution profile gets enhanced when the carrier ratio in solid dispersion increases because the drug disperses better, thereby decreases the crystallinity.[16] Carrier controlled release involves two mechanisms: diffusion carrier controlled release and erosion carrier controlled release. Carrier controlled follows a diffusion process when the drug and polymer are completely dispersed, and when the carrier

gets separated from the drug particles, then carrier controlled follows an erosion process.[17]

The present research work mainly focussed on the solubility enhancement of 7-chloro-3-(4-chlorophenyl) -2-phenyl quinazolin-4 (3H) -one by melt-fusion technique using hydrophilic carrier in 1:1, 1:3 & 1:5. In order to evaluate the solid dispersion, dissolution and solubility studies were performed.

MATERIAL AND METHODS

Materials

Polaxamer 407, Eudragit L-100, guar gum, pectin, microcrystalline cellulose, crosscarmellose cellulose, sodium starch glycollate were purchased from Nice Chemicals Pvt. Ltd. Cochin.

Synthesis

Transfer the solution of anthranilic acid (0.05 mol) and pyridine (25 mL) into a beaker, along with the addition of benzoyl chloride (0.05 mol) dropwise, which is maintained at 0-5 ˚C for 4 h. A solid product formed when the mixture was allowed to stir for 3hr at room temperature. Then the mixture was neutralized with 60% saturated sodium bicarbonate solution to produce a pale yellow solid product after filtered with water and recrystallized from ethanol. An equimolar mixture of intermediate was added in 4-chloro aniline and the mixture was refluxed for 10 hrs with 10 ml glacial acetic acid. The mixture was then poured into crushed ice and stirred vigorously to produce the solid product which was filtered and recrystallized with ethanol.[18]

Method Development

Preparation of Stock Solution

100 mg of the synthesized compound was made up to 100 ml into a 100ml standard flask with ethanol to give a solution of 1000 µg/ml.

Determination of λ max

1 ml of stock solution was pipetted out was made up to 10 ml into a 10ml standard flask with ethanol to obtain strength 100 µg/ml and scanned at 200-400nm using a UV spectrophotometer.[19]

Preparation of Standard Calibration Curve

Aliquots of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 ml were pipetted out into 10 ml volumetric flask and made up with ethanol. The linear calibration curve was plotted within the range from 1-10 µg/ml concentration analyzed by UV spectrophotometer by plotting absorbance against concentration.

Structural Elucidation

The synthesized compound was structurally characterized by NMR and mass spectrophotometer. An NMR spectral study was done with Bruker Fourier- NMR spectrometer. Mass spectra of the samples were recorded on a Waters e 2695-Waters 3100 instruments with ESI-PMT arrangement as the mode of ionization and type of detector respectively.[18]

In-Vitro Cytotoxicity of Quinazolinone Derivative

The MTT assay was performed to assess cytotoxicity in adenocarcinoma colorectal cancer (HT-29) cells and normal fibroblast cell (L929) exposed to different concentrations of synthesized compounds. Both the cells were seeded in 96-well plates maintained at 37ºC in a 5% CO_2 humidified incubator. Untreated cells were served as control for cell viability. In the prepared growth medium, the cells were treated with 6.25, 12.5, 25, 50, and 100 µg/ml concentrations of synthesized compound for 24, 48, and 72 hrs. 100µL of 5 mg/ml in sterile H_2O containing MTT solution was poured into each well and incubated for 4 hrs at 37°C under 95% atmosphere air and 5% CO_2. Prior to the addition of 200µL aliquots of DMSO, the MTT solution was removed followed by incubation for 10 min at 37°C. Optical density was read at 540 nm using DMSO as blank.

The percentage growth inhibition and percentage viability were calculated using equation.[20, 21]

$$\text{Percentage Inhibition (\%)} = \left(100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control}}\right)$$

$$\text{Percentage viability (\%)} = \left(\frac{\text{OD of test}}{\text{OD of control}}\right) \times 100$$

Acute Oral Toxicity study of Quinazolinone Derivative

An oral acute toxicity study was conducted as per the protocol based on OECD 423. The study involves six male mice in a group of four. Prior to administration the animals were fasted for 3hrs. Test compounds were dissolved in 2% acacia. Mice in groups II, III, IV were given 300, 2000 and 5000 mg/kg of the synthesized compounds and provided with 2% acacia for control group with a maximum administration dose volume of 1mL/kg body weight. The mice should be provided with food after administration for 2 hours. General clinical indications like alertness, grooming, touch response, pain response, tremor, convulsions, pupil, urination, salivation, skin color, hyperactivity, lacrimation, sedation, coma, morbidity and mortality were observed after dosing during the first 30 min, periodically during the first 24 hrs with special attention given during the first 4 hrs, and daily thereafter, for a total of 14 days. Subsequently the animals were sacrificed by CO_2 inhalation.[22, 23]

Physicochemical Properties

Organoleptic Characters & Melting Point

Organoleptic characters like color, odor and the nature of the compound were carried out by visual assessment. Open capillary method was used to determine the melting point.

Solubility

Solubility of the compound was determined in different solvents like hydrochloric acid, sulfuric acid, sodium hydroxide, methanol, ethanol, acetone, ethyl acetate, chloroform, DMSO, glacial acetic acid, n-hexane, cyclohexane, n-octanol, diethyl ether, benzene, toluene and water, followed by the preparation of saturated solution with those solvents which shows good solubility with the synthesized compound and then plotted a linear calibration curve to determine the concentration. The solubility determination was based on the USP Criteria.[24, 25]

pH

10 mg of the synthesized compound was weighed accurately and placed into three separate volumetric flasks containing suitable solvents and made up to 100 ml with distilled water. The pH meter was standardized with distilled water & prepared buffer solution of pH 4, 7 & 9.5 and determined the pH of the synthesized compound.

$$\text{Partition Co-efficient} = \frac{\text{Concentration of the compound in organic phase}}{\text{Concentration of the compound in aqueous phase}}$$

Dissociation Constant

Transferred 10 mg of the synthesized compound was transferred into a standard flask to attain a concentration of 100µg/mL using prepared buffer solution of pH 4, 7 & 9.2. The absorbance of each solution was determined using a UV spectrophotometer.

Preparation & Characterization of Solid Dispersion by Melting-Fusion Method

Polaxamer (PXM)-407 was melted at 60ºC with constant stirring prior to the incorporation of synthesized compound in the ratio of 1:1, 1:3, and 1:5 to form a homogeneous melt[28]. The prepared solid dispersion was characterized by FT-IR, DSC & SEM. FT-IR studies.[29, 30]

Evaluation of Solid Dispersions

Phase solubility studies

In a conical flask containing 25 ml phosphate buffer of pH 6.8 added excess amount of solid dispersions and placed in an orbital shaker for 48 hrs to achieve uniformity. 2 ml of the solution was pipetted out at 1hr time interval and by using whatsman filter paper no.45 the solution was filtered which was then analysed by UV spectrophotometer. The data's were used in phase solubility analysis to calculate various thermodynamic parameters such as ΔH, ΔS and ΔG.[31, 32]

$K_a = \frac{\text{slope}}{\text{Intercept (1-slope)}}$

Gibbs energy (ΔG) was calculated using the formula

$$\Delta G = -RT \ln Ka$$

$$\text{Enthalpy change} \quad \Delta H = -\frac{RT \ln Ka)}{dT\ (K)}$$

$$\text{Entropy change} \quad \Delta S = \frac{\Delta H - \Delta G}{T}$$

Where, R = universal gas constant (8.313 J/mole K), T =the temperature, Ka = stability constant, dT = difference in temperature (Kelvin), ΔH = enthalpy & ΔG= entropy.

Drug content & Saturation solubility studies

10 mg of solid dispersions was taken into a conical flask containing 25 ml phosphate buffer solution of pH 6.8 and placed in an orbital shaker at 37ºC for 24 hrs and the solutions were filtered through whatsman filter paper. The concentrations of solid dispersion and drug content were analyzed by UV spectrophotometer. Actual drug content was calculated using equation.[30, 33]

$$\text{\% Drug Content} = \frac{\text{Actual QD content in weight quantity SD}}{\text{Theoretical amount of QD in SD}} \times 100$$

Micromeritic properties

10g of the granules were poured into a 10 ml measuring cylinder. Initial volume was observed prior to 100 times tapping. The final volume was noted. Bulk density, tapped density, compressibility index (Carr's index) and Hausner's ratio were calculated using equation.

$$\text{Bulk density} = \text{Mass / Bulk volume}$$

$$\text{Tapped density} = \text{Mass / Tapped volume}$$

$$\text{Hausner's ratio} = \text{Tapped density/Bulk density}$$

$$\text{Carr's index} = \frac{\text{Tapped density-bulk density}}{\text{Bulk density}} \times 100$$

The angle of repose was measured using the funnel method. Approximately 10 g of powder was placed in a funnel. The height of the funnel was adjusted to a point where the tip of the funnel was just above the apex of the heap of powder and calculated by equation.[34]

$$\theta = \tan^{-1} h/r$$

Where θ - Angle of repose, h - height, r - radius

Wettability study

Samples were placed in a Buchner glass funnel placed below the surface of a liquid in a beaker so that the beaker and the powder stay parallel. The Methylene red powder was layered uniformly on the surface of the powder. The time required for wetting the methylene red powder was measured.[34]

***In-Vitro* Dissolution Studies for Solid Dispersions**

In-vitro release profile for solid dispersions was performed using USP type I basket type dissolution apparatus. 200 mg of solid dispersions was used for the study. Phosphate buffer pH 6.8 was used as a medium. 5ml samples were withdrawn at various time intervals at 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 min and absorbance was measured.[35, 36]

Mathematical modeling of kinetics

The release kinetics were studied by fitting the data in different kinetic models.[37]

RESULTS & DISCUSSION

Synthesis

6-substituted anthranilic acid undergoes nucleophilic addition reaction with benzoyl chloride, yielding 6-substituted 2-phenyl benzoxazinone followed by the formation of carbenolamine intermediate through aromatic amine to a carbonyl carbon resulting in dehydration along with cyclisation gives the corresponding benzoxazinone. In the second step nitrogen lone pair on the amino group of substituted anilines attacks the carbonyl carbon of benzoxazinone and loses a molecule of water to form 3-aryl 2-phenyl quinazolinone.[38, 39]

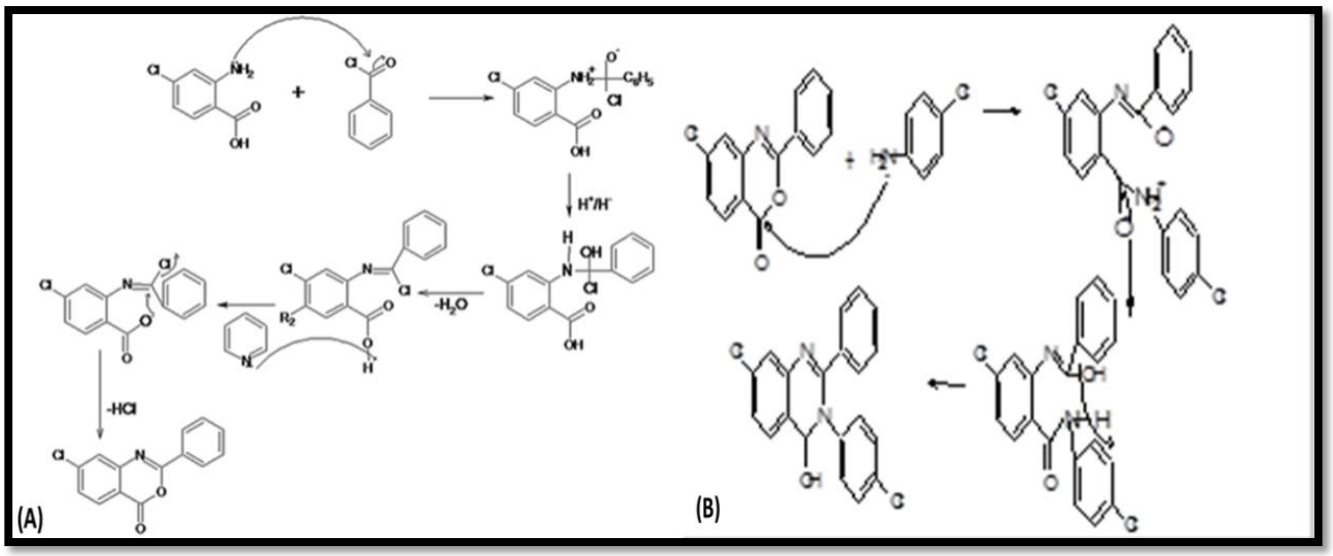

Figure 1: A) Synthesis of 2-phenyl-4(3H)-3, 1-benzoxazinoneB) Synthesis of 7-chloro-3-(4-chlorophenyl)-2-phenyl quinazoline-4(3H)-one

Method Development

From the spectral analysis, the λ max of the synthesized compound was found to be 237nm. The calibration curve was found to be linear and hence suitable for the estimation of the compound.

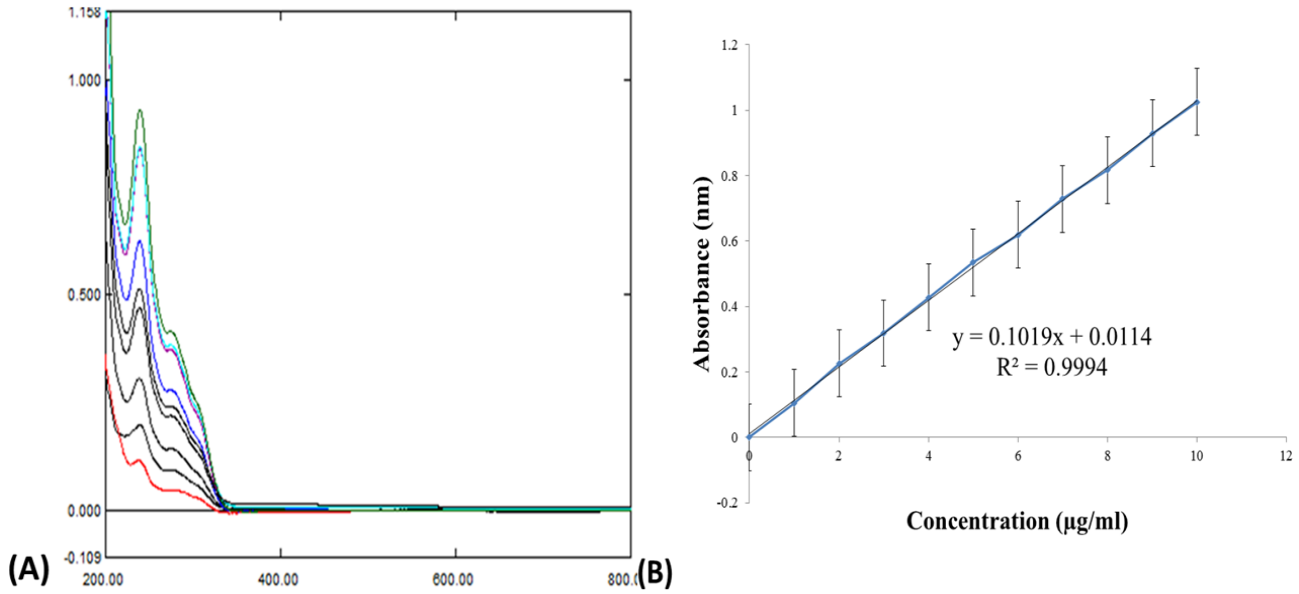

Figure 2: A) Observed peak of synthesized compound for absorption maxima B) Standard graph for the compound

Structural Elucidation

From the NMR spectroscopic data revealed that 1H NMR (DMSO, 400 MHz) 7.978 (d, 1H J=7.2), 7.803 (d, 2H, J=8), 7.596 (d, 1H, J=8.8), 7.462 (t, 3H, J=7.2) δ 7.392 (s, 1H), 6.975 (d, 2H, J=6), 6.762 (d, 2H, J=7.2) 13C NMR (DMSO, 100MHz) δ 149.437, 148.237144.117, 143.128, 139.229, 137.845, 128.845, 128.507, 127.081, 126.470, 122.587, 120.459 Mass spectroscopic data were recorded for the synthesized compound in positive mode. The synthesized compound showed molecular ion (M+) peak at (m/z 368) denoted the presence of chlorine in the compound.[42]

The synthesized compound was found to be more potent against HT-29 cell lines with IC_{50} of <25µg/ml. The synthesized compound showed better anticancer activity with IC_{50} values of 16.3 µg/ml. The synthesized compound as per ISO 10993/5 guidelines in L929

fibroblasts has shown moderate toxicity in-vitro. The predicted LD_{50} value comes in the range of 163.6 µg/ml. The percentage inhibition & viability[42] of the compound in HT-29 & L929 were graphically represented in (Fig. 3.).

***In-Vitro* Cytotoxicity of Quinazolinone Derivative**

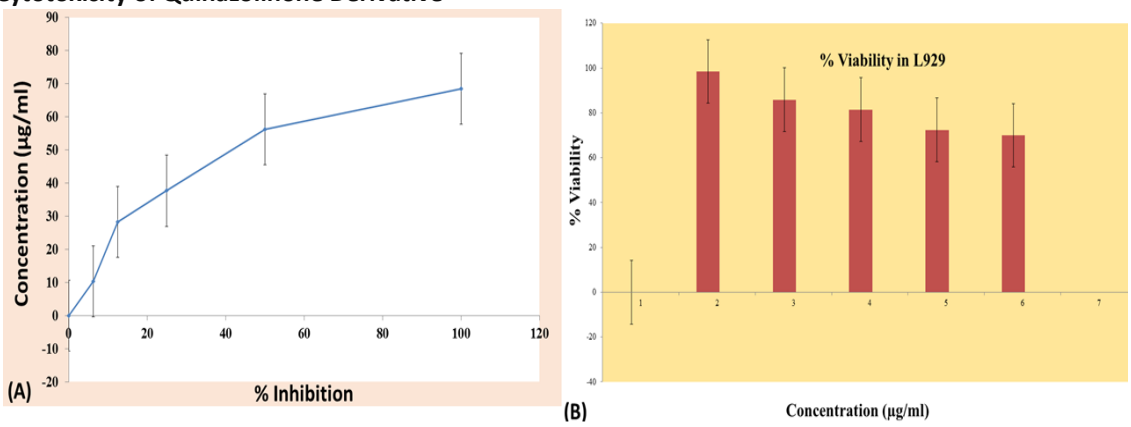

Figure 3: A) Graphical representation of % inhibition of synthesized compound B) Representation of % viability of cells in synthesized compound

Acute oral toxicity study of QD (OECD 423)

Mortality: The present study conducted revealed that the even after a period of 14days no mortality[43] was found in animals when observed at 300, 2000 mg/Kg. However, the animals were observed with certain clinical symptoms when administered 5000 mg/Kg.

Therapeutic dose: The LD_{50} value was found to be greater than 5000 mg/kg and the dose for the formulation was fixed at 200 mg.[44-46]

Physicochemical Properties

The synthesized compound was found to be off white crystalline, odourless in nature. The solubility of the synthesized compound had been determined by parts per solute method. The concentrations of the compound were found in the range from 10-30 parts categorized under soluble class based on USP criteria.[47, 48, 60]

The melting point of the compound was found to be 229-230°C. The pH 6.46 of the synthesized compound was found to be very close to the pH of the colon. This indicates the modification of compound for formulation. From the partition coefficient result, the compound confirms its lipophilicity with coefficient value 2.17. The synthesized compound can belong to BCS class II because for any compound which shows log P value between 2-3 then the compound shows high permeability and low solubility, thereby achieving between permeability and first pass clearance.[49] The compound has been weakly acidic with dissociation constant value 5.75 since the pH >peak, which falls under the category of weekly acidic.[50]

Preparation & characterization of solid dispersion by melting-fusion method

Solid dispersions of the synthesized compound were prepared using the Polaxamer-407 carrier in the ratio of 1:1, 1:3, 1:5 using the melting - fusion method. The prepared solid dispersion was characterized by DSC & SEM.

Differential scanning calorimetry (DSC)

The DSC of pure compound and solid dispersion were represented in Figure 7. Differential scanning calorimetry technique detects the endothermic, exothermic re-crystallization reactions. The DSC thermograph of synthesized compounds formed a sharp endothermic peak at 239°C which corresponds to its melting point where as the prepared solid dispersion showed two endothermic peak at 59.30°C and 205.77°C indicating absence of interaction between compound and polymer resulting into amorphous form.[51, 52]

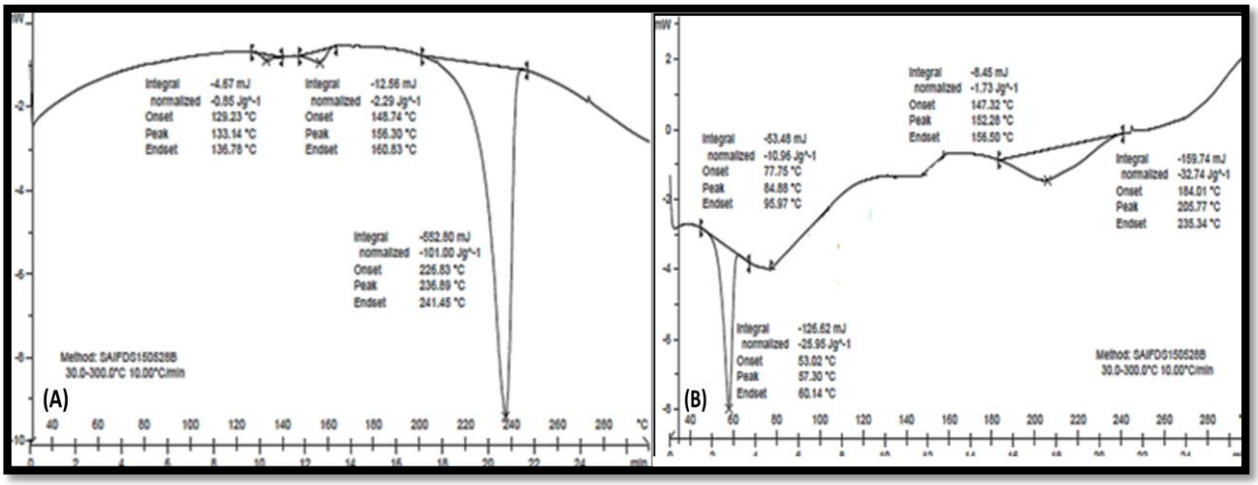

Figure 4: A) DSC of synthesized compound B) DSC of quinazolinone solid dispersion

Scanning Electron Microscopy (SEM)

The pure compound indicates that the irregular shaped particles, while the solid dispersion shows globular form indicating the physical absorption and complete dispersion of compound with the carrier particles resulting in the formation of new structure when the compound melted with Polaxamer-407.[59]

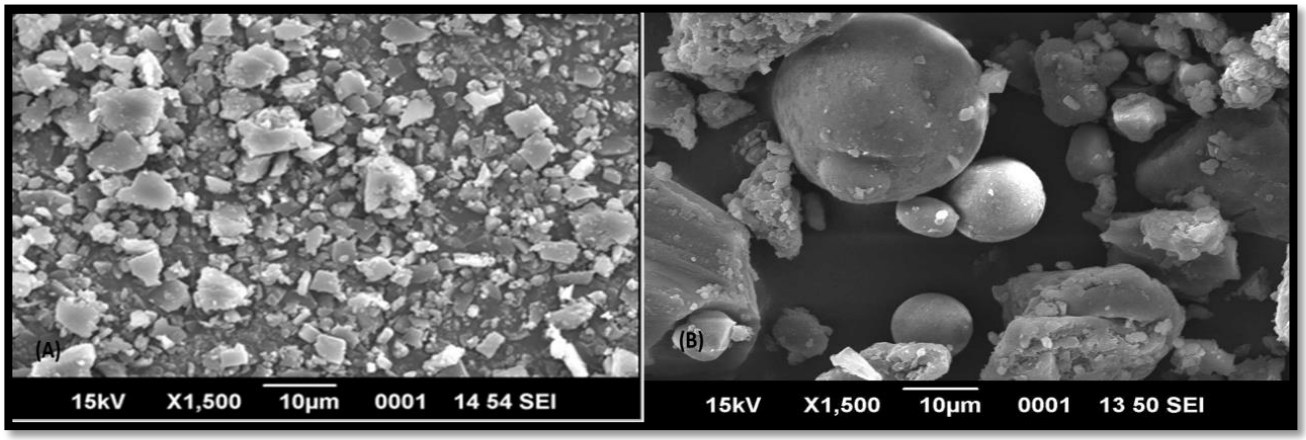

Figure 5: A) SEM image of synthesized compound B) SEM image of solid dispersion

Evaluation of solid dispersions

Phase solubility studies

The phase solubility diagram observed to be the AL type indicating a linear increase as a function of concentration of Polaxamer 407 with slope <1 resulting an increase in concentration, indicating an increase in solubility of Polaxamer (1:5) represented according to Higuchi and Connor's.

The linear slope from the graph indicate its apparent stability constant which was found to be 552.14M^{-1}, 602.148M^{-1}, 749.9 1M^{-1}. The increased value of Ka indicates that the solid dispersion formed was quite stable. Phase-solubility studies showed a linear increase with the increase in the weight fraction of surface-active carrier resulted in an increase in the solubility of all dispersions.

Thermodynamic parameters

The carrier efficiency was confirmed from the negative ΔG, ΔH and positive ΔS. ΔG decreased when the increase in carrier concentration resulting more favorable reaction. The higher Ka value signifies strengthened binding efficiency between the compound and solid dispersion. The greater the value of slope greater is the capacity of the carrier to solubilise the compound.

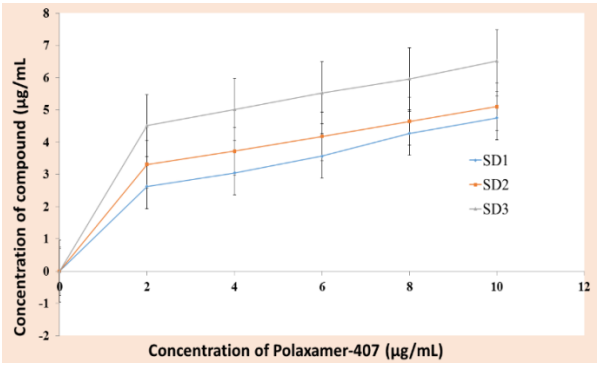

Figure 6: Phase solubility graph for compound and carrier at different ratios

Table 1: Effect of ΔG, ΔH, ΔS and Ka on QD-SD

S.No	Formulation Code	Ratios of dispersions	Ka (M^{-1})	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/molK)
1	SD1	1:1	552.14	-4.937	-0.589	0.1493
2	SD2	1:3	602.148	-4.117	-0.585	0.1031
3	SD3	1:5	749.913	-2.402	-0.503	0.1003

Drug content & Saturation solubility studies

The results showed the uniform compound distribution with low standard deviation in drug content of compound and solid dispersion with no significant loss of the drug during the preparation of solid dispersion. Maximum solubility in phosphate buffer solution was observed in 1:5 ratio 19.536±0.004714µg/ml.

Micromeritic properties

From the obtained results when compared with the IP criteria mentioned. It was found that the powder flow properties was found to be excellent with better compressibility.[53]

Table 2: Drug Content

Sl.No	Batch code	% drug content
1	Pure compound	94.61±0.0154
2	SD1	94.05±0.0162
3	SD2	96.43±0.0193
4	SD3	97.58±0.0214

Table 3: Micromeritic properties of compound-solid dispersion

Parameters	Compound	SD1	SD2	SD3	Inference
Bulk density (g/cc)	0.440±0.011	0.468±0.012	0.476±0.020	0.696±0.031	Increased
Tapped density (g/cc)	0.461±0.012	0.513±0.0115	0.525±0.027	0.790±0.0369	Increased
Carr's index	4.77	9.61	10.29	10.6	Excellent
Hausner's ratio	1.047	1.096	1.102	1.106	Excellent
Angle of repose	34.05±1.680	17.596±0.761	20.63±1.26	23.83±0.808	Excellent

Wettability Study

The wettability study for synthesized compound and solid dispersion had been carried out. From the result obtained, it was found that the wetting time in pure compound was 80 min but for the prepared solid dispersion, the wetting time decreased due to the increase in carrier concentration.[54]Thus the wettability property for SD3 was found to more efficient than compared to other.

***In-Vitro* Dissolution Studies**

SD3 (1:5) showed 47.893% drug release in 30 min, whereas a 64.786 % drug release in 60 min and the other two preparations had a decrease in the release at 30min and 60min as shown in Figure 12. When the solid dispersion comes in contact with the carrier it forms weak soluble complexes due to electrostatic forces and hydrogen bonds.[58] As a result polymer configuration change resulting in the formation of aggregates which solubilizes the compound by forming monomolecular micelles.[55] The better dissolution rate of solid dispersion may be due to an improved wettability, particle size reduction and dissolution due to increase in the surface area of compound, proper dispersion and increase in the amorphicity of a drug by adsorption on the surface of adsorbent.[56, 57]

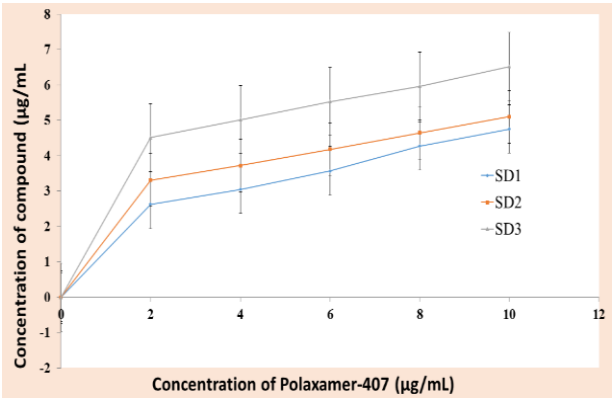

Figure 7: *In-vitro* release data of solid dispersion

Mathematical modeling of kinetics

The compound solubility, diffusion rate and dissolution of the polymer are important factors that affect the drug release kinetics. The data obtained from the *in-vitro* dissolution experiments were fitted to different mathematical model i.e. zero order, first order, Higuchi and korsmeyer-peppas to predict the kinetics and

release mechanisms of the solid dispersions. The regression coefficient obtained showed the optimized solid dispersions SD3 follow zero order kinetics with R2 value 0.9901 indicating drug release independent of drug concentration within the system followed by Fickian diffusion. Thus the results point out the diffusion phenomena.

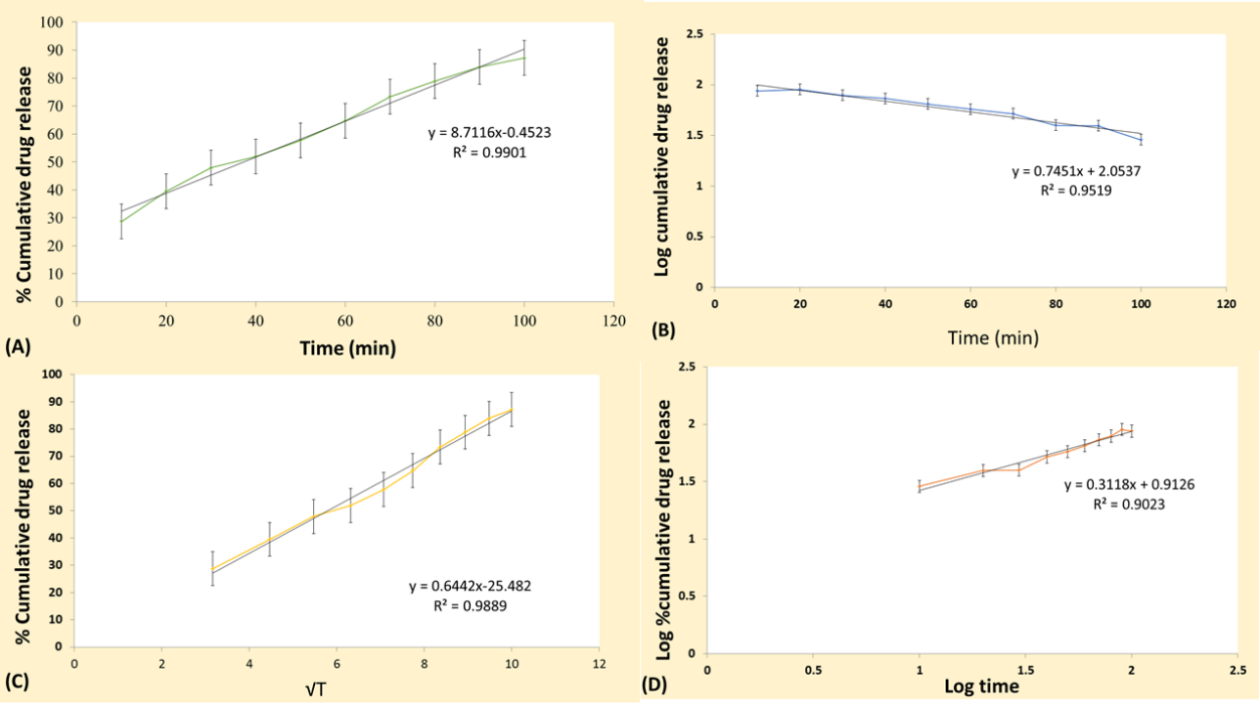

Figure 8: A) Zero order model B) First order model C) Higuchi model D) Korsmeyer-peppas model

CONCLUSION

7-chloro-3-(4-chlorophenyl)-2-phenylquinazolin-4(3H)-one, was selected as it showed good anti-colorectal cancer activity. The compound has an effective dose of 200 mg with minimum toxicity. This study showed the enhance dissolution rate by formulating solid dispersion from hydrophilic polymer using the melt fusion technique. The dissolution rate of compound depends on the concentration of polymer. A high proportion of polymer in the solid dispersion significantly increases the dissolution rate by inhibiting crystallization, reduction of particle size or by enhancing wettability of compound and thereby retarding the release into the stomach.

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