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Original Article

AN ADDUCT: 5-FLUOROURACIL INCORPORATED β-CD INCLUSION COMPLEX FOR SOLUBILITY AND STABILITY ENHANCEMENT IN DOSAGE FORMS

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ABSTRACT

Objective: Current work aimed to enhance solubility and stability of 5-fluorouracil drugs by preparing inclusion complex with β-cyclodextrin.

Methods: In this study, inclusion complex preparation ratio selected on the basis of slope and Kc (binding constant) value in between 5-fluorouracil-β-cyclodextrin and best method out of the physical mixture, kneading method, and co-evaporation method for solubility and stability enhancement is selected on the basis of % yield, drug content, dissolution rate study and stability study.

Results: Based on the phase solubility graph, a 1:1 ratio was selected for complex formation by Kc value which decided a quite stable form of a complex. The characterization of all three types of inclusion complex was performed by DSC and SEM. It proved that different moiety of the complex was formed, but all are quite stable with negligible interaction. The kneading method as the best inclusion complex at ratio 1:1 was selected after evaluating by performing percent yield and drug content and dissolution rate study for solubility profile and physical appearance, drug content, and drug release study for stability profile.

Conclusion: We finally conclude that the Kc value proved that the inclusion complex is quite stable and ready to convert in any dosage form of choice. Inclusion complex formed by kneading method is one of the best options among all three techniques for solubility and stability enhancement of drug, which definitely help for a 5-fluorouracil drug to convert into a better dosage form to treat carcinoma.

Keywords: 5-fluorouracil, β-cyclodextrin, Inclusion complex, Kneading method, Stability study

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INTRODUCTION

5-Fluorouracil (5-FU) is a drug that behaves as antimicrobial and broad-spectrum antineoplastic agents. It is basically a derivative of pyrimidine [1]. Among major antimetabolites, 5-fluorouracil is one of the best drugs, presented as potent and highly capable to work against breast, colon, skin, throat, and mouth cancer [2]. The 5-fluorouracil drug is very common when provided through intravenous route; this is because this drug is having few disadvantages when given through oral route like it is a sparingly soluble drug and having only 12 mg/ml solubility, cause gastrointestinal irritation during absorption, have a half-life of 10-20 min and cause severe level side effects [3, 4]. That's why this is necessary to increase the drug solubility if we want to decrease the drug dose and dose-related side effects and to solve a problem; first of all we have to increase the solubility of the drug, which is a very common process for poorly soluble drugs. There are many alternatives to solve the problem of low solubility, like structure modification [5], co-solvency, the addition of surfactant [6, 7], drug encapsulation in carrier [8], reduction in particle size [9], drug complexation [10] and many more.

Among all other methods; complex formation in presence of β-cyclodextrin was selected because cyclodextrin and its derivatives are the oligosaccharides which have a hydrophobic central cavity where drug encapsulated and easily dissolve in a media [11-14] because its hydrophilic outer surface help to dissolve poorly soluble substance which finally increase the solubility, dissolution rate and improve the overall bioavailability of poorly soluble drug [15-17] not only this cyclodextrin also play a great role in increasing the stability of the drug, which is must require factor in case of potent drug [18-21]. Among all three types, β-cyclodextrin is the most common variant for preparation of inclusion complex because of its ease and wide availability with a high percent of biocompatibility and non-reacting nature with almost all drugs with the least cost of manufacturing among all three types [22, 23].

In this study, we perform different techniques of complex formation, out of which three methods named as a physical mixture, kneading method, co-evaporation technique is selected because they guaranteed solubility enhancement, provide desired particle size with the help of sieving, easy to perform in a laboratory and high yield of inclusion complex. All these factors help to select the best and easy way to prepare inclusion complex for overall solubility and stability enhancement of 5-fluorouracil drug, which definitely help to prepare the formulation that targets through the oral route as this is one of the simplest route in case of ease design methods and dose administration with cost-effectiveness [24].

MATERIALS AND METHODS

Materials

5-fluorouracil was purchased from Balaji Enterprise, thakordwar society, B/H spinning mill, Varcha road, Surat-395011(Gujarat). β-Cyclodextrin and ethanol were purchased from Ranbaxy fine chemical Ltd., New Delhi-110002, and all other chemicals that may be used are of analytical grade.

Methods

Phase solubility study

A phase solubility study was performed for 5-fluorouracil drugs in which excess amount of 5-fluorouracil (100 mg) in a conical flask of 50 ml, which already contain β-cyclodextrin different concentration range from 0.25 to 2 mol in distilled water as a media [25, 26]. Containers were shaken non-stop for 3 d (24 h) at 30 rpm at 37 °C to attain an equilibrium state [27, 28]. Finally, the solution was filtered and the filtrate was analyzed for 5-fluorouracil content with the help of the UV spectroscopy method at 266 nm. Lastly, we constructed the phase solubility diagram based on the solubility of a drug against the concentration of β-cyclodextrin in moles also called the isothermal saturation method. With the help of slope and intercept, values were calculated for binding constant (Kc values) [29-31].

$$Kc\ (1:1) = Slope/So(1\text{-}slope)\ [32]$$

Where,

Kc is apparent stability constant,

So = intrinsic drug solubility

M = molar concentration

Method of preparation of inclusion complex

Physical mixture

A physical mixture in between 5-fluorouracil and β-cyclodextrin is generally prepared by transferring a respective weighed amount of both substances to the mortar where proper triturating takes place for one hour and transferring the prepared physical mixture from the sieve no. 80 [33, 34].

Kneading method

First weighed a respective amount of 5-fluorouracil and a β-cyclodextrin for the preparation of complex and transfer both the substance to the mortar, where proper mixing takes place in the presence of ethanol and water (2:8) mixture, which is regularly transferred to the mortar during their mixing process and finally end when slurry-like consistency will obtain. The dried form of slurry was obtained after 24h, which further pass through sieve no.80 [35-37].

Co-evaporation method

First weighed β-cyclodextrin and 5-fluorouracil drugs according to their given ratio (1:1), then β-cyclodextrin is dissolved in distilled water, and fluorouracil is dissolved in an ethanolic solution. Then mix both the solution and transfer it to the beaker which was kept over a magnetic stirrer with maintaining the temperature at 37 °C and stirring speed of 300 rpm for one day. Finally, solvents were vaporized while maintaining the temperature of 45-50 °C over water-bath in the end we obtained a crystalline powder which was finally pulverized and pass through sieve no.60 [34, 38].

Characterization of inclusion complexes

Scanning electron microscopy

Scanning electron micrographs were performed for four different samples. This included complex formed in between 5-fluorouracil and β-cyclodextrin by physical mixture, kneading method, and co-evaporation method. All samples were analyzed under SEM, JEOL JSM 6390, England. All sample particles were coated with a thin layer of gold and preparation was dispersed over a carbon tab to render electrical conductivity [39].

Differential scanning calorimetry analysis

Differential scanning calorimetry was performed for the 5-fluorouracil-β-cyclodextrin inclusion complex by using (PerkinElmer Pyris 1 DSC). Here 5 mg samples were tested by a heating rate of 10 °C/min in a range of 0-300 °C and an inert atmosphere was maintained with nitrogen which flows through the rate of 10 ml/min throughout the runs, by using an empty sealed pan as a reference. Finally, Temperature and heat flow calibrations were performed by using indium as a standard [39, 40].

Evaluation parameter of inclusion complexes

Percent yield

The Percent yield was calculated with the help of the given formula. In which we calculated the exact weight of molecular inclusion complex against the theoretical given weight of drug and excipients. Here complex was dried for 4-5 h in a desiccator for further loss of moisture which was helpful to provide the exact value of their weight. This method was performed in triplicate.

Percent yield= (weight of prepared solid dispersion/weight of drug+carriers) x 100 [39].

Drug content

In this method first, we weighed 20 mg of the complex which is dissolved in 10 ml of media containing distilled water and ethanol as a ratio of (9.2:0.8). The final solution obtained was further diluted and noticed the absorbance at 266 nm with the help of a UV/Visible spectrophotometer. Then calculate the drug content by the regression equation [41].

Dissolution rate study

Dissolution behavior of 100 mg of drug was noticed with the help of USP XXII rotating basket, in which respected amount of complex added to the dissolution basket which should contain 100 mg of drug and should present inside hard gelatin shell of the capsule. Other necessary conditions that should maintain for the study; 900 ml distilled water as a media, temperature 37 °C±1 °C, and rotating speed 75 rpm in which we drew a 5 ml of a sample at different time duration and analysis through UV spectrophotometer at 266 nm, for the actual value of drug dissolution rate [42].

Stability study

Sample of all three kinds of inclusion complex and the pure drug selected for accelerated stability study, in which all selected samples filled in vials and covered with screw cap, finally stored in a universal oven for 6 mo time period on a temperature and humidity of 40 °C±2 °C and 75%±5% (RH). All the samples were checked at different time intervals for drug content, drug release, and physical appearance; if any significant change occurs in the resulting data after 6 mo then the time period will increase by 12 mo [42].

RESULTS AND DISCUSSION

Phase solubility study

A phase solubility study was conducted for 5-fluorouracil drugs in distilled water containing different concentrations of β-cyclodextrin in moles; the whole criteria for phase solubility calculation were conducted according to the method explained by Higuchi and Conner's. The graph was plotted in between the solubility of 5-fluorouracil mg/ml on the y-axis and concentration in moles of β-cyclodextrin on the x-axis. After seeing (fig. 1), which was framed in between solubility of fluorouracil and concentration of β-cyclodextrin plot, clearly observed that the aqueous solubility of 5-fluorouracil is directly proportional to the mole's concentration of β-cyclodextrin. This linear motion of graph between the solubility of a 5-fluorouracil and concentration of β-cyclodextrin is not fully linear which proven that solubility of a 5-FU drug increases up to a limit in the presence of β-cyclodextrin and graph classified as B (BS type) curve, which means a limited solubility enhancement is obtained through inclusion complex [43]. The host-guest correlation was found in which slope value is less than 1(0.964) suggested the formation of a stable complex, a 1:1 ratio is required in between 5-fluorouracil-β-cyclodextrin complex and value of K1:1 was found to be 120.5M^{-1}, which further proved that the complex formed was quite stable and takes some time to dissociate because dissociation kinetics is inversely proportional to Kc values. Based on the research, consider that the Kc values range between 100 to 1000M^{-1} are stable and applicable for all kinds of dosage forms. On the basis of phase solubility results, we can consider that 1:1 is the best ratio for the β-cyclodextrin and fluorouracil for complex formation. This is noticeable that B type phase solubility behavior is quite rare for solubility enhancers like natural β-cyclodextrin since the drug-CD complex is more soluble than the free drug itself, but then also solubility limit for the drug-CD complex is reached within the particular concentration range of the cyclodextrin [7].

Percent yield

The Percent yield for 5-fluorouracil encapsulated complex was determined for three different batches formed by different techniques (physical mixture, kneading method, and co-evaporation method), which was in ranged from 92.66±1.11–98.00±0.407 % as a mean±SD values are shown in table 1. Percent yield data confirmed that all three techniques used to prepare inclusion complex are able to provide acceptable reproducibility power for the preparation of complex and percent yield got after followed the physical mixture method (97.33%) is higher than the other two methods, which proven drug is uniformly dispersed in the β-cyclodextrin polymer by the following technique. Moreover, the drug loss in % yield depends on agglomeration and sticking of complex to the surface of the container during preparation and transfer from one to another container of solid dispersion. This may be the reason for losing the drug during percent yield.

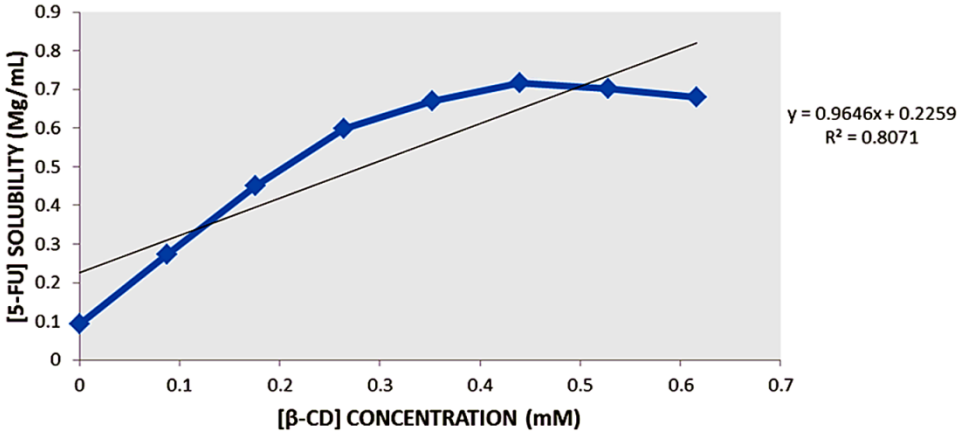

Fig. 1: Phase solubility diagram of inclusion complex formed in between 5-fluorouracil-β-cyclodextrin

Drug content

In the case of the 5-fluorouracil-β-cyclodextrin inclusion complex, a range of drug content was found in between 86.491±0.016–95.769±0.617%, mention in given table 1. Which proven high drug content uniformity is present in all the given methods of preparations, Out of all the highest drug content in percent was noticed in the kneading method, which contains 95.76% of the drug, this proves that the drug is uniformly distributed in the network of β-cyclodextrin, all this happens because of the method utilized to encapsulate the drug in β-cyclodextrin. Also, the distribution of a drug in any polymer depends on the method utilized to form the complex and also the type of polymer used to encapsulate, as a result declaimed that the method selected for the preparation of complex is easy, unique, and best for drug loading in polymer out of all other methods.

Table 1: Percent yield and drug content of inclusion complex prepared by different methods

S. No.	Formulation name	Concentration (FU-β-CD)	Method of preparation	%Yield	Drug content
1	FPM-1	1:1	Physical Mixture	98.00±0.407	86.491±0.016
2	FKN-2	1:1	Kneading Method	95.66±0.47	95.76±0.617
3	FCE-3	1:1	Co-Evaporation Method	92.66±1.11	92.039±0.320

(Values represented mean±SD, n=3)

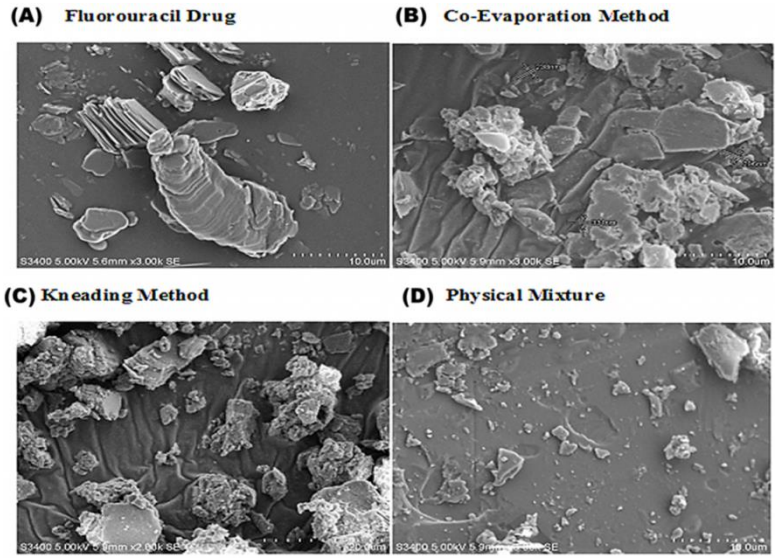

Fig. 2: SEM images of: 2(A) 5-fluorouracil drug and inclusion complex prepared by 2(B) Co-evaporation technique 2(C) Kneading method 2(D) Physical mixture

Scanning electron microscopy

With the help of different images of SEM, shown in fig. 2 we are able to study the structure and surface morphology of the 5-fluorouracil alone and in its complex state formed by different methods. Here micro graphical images of the drug prove that the 5-fluorouracil is having a 3-dimensional (3-D) layer structure which is almost irregular in shape, rough surface, and arranged like 3-D crystalline form. But the major morphological changes in 5-fluorouracil was noticed when the drug was encapsulated in β-cyclodextrin through the kneading and the co-precipitation method; here in the Co-precipitation method, after the process of complex formation is completed and the complex has dried

the surface of the complex become enlarged from the actual surface of 5-fluorouracil and β-cyclodextrin, and the two substance is impossible to differentiate with the help of an image, the only noticeable thing was found is crystalline drug form convert to amorphous form, similarly in case of kneading method drug and β-cyclodextrin actual surfaces are impossible to recognize and differentiate. The observable thing is a particleS of both components are combined well and can't be differentiated as they form agglomeration and more away to look like matrix particles. Finally, we can say that in both cases of complex formation in between (5-fluorouracil and β-cyclodextrin) it is impossible to differentiate while watching images; finally, we conclude that the drug lost its original morphology and crystalline nature and fully convert to a newly 3-dimensional form. Lastly, in the case of a physical mixture, the only noticeable thing to find is the drug's actual structure is changed and the layered form of the drug is lost, and few drug particles still maintain crystalline structure with irregular 3-D structure.

Differential scanning calorimetry analysis

With the help of exothermic and endothermic peaks in case of DSC, we can able to notify drug excipient interaction which is obvious for any kind of formulation [23]. Here in the case of 5-fluorouracil and β-cyclodextrin complex formation shown in fig. 3, that 5-fluorouracil drugs have a sharp endothermic peak which proves that the drug is melted down at 290 °C and second endothermic peak at 340 °C proven that the drug is degraded due to high temperature. Similarly, β-cyclodextrin is also having an endothermic peak at 78 °C, which proves that stored water in cyclodextrin starts releasing out from the cavity [44]. Also, its decomposition occurs at a temperature of 320 °C as in the case of a physical mixture of 5-fluorouracil and β-cyclodextrin, it was noticed that both the endothermic peak of 5-fluorouracil and β-cyclodextrin was present at their respective places, which mean there was no variation or interaction found in physical mixture preparation, also drugs were not encapsulated in a cavity of β-cyclodextrin. As in the case of the kneading method, a minor size change in endothermic peak at 290 °C but the peak present at its original position, also the second endothermic peak lost which proves that drugs encapsulated in β-cyclodextrin and the structure of 5-fluorouracil is almost changed. In the case of the co-evaporation method; Again change in the size of the endothermic peak observed and a small shift of the second endothermic peak was noticed at 300 °C from 320 °C, this proves that the drug melting point is changed which is because of encapsulation of drug in the cavity of β-cyclodextrin.

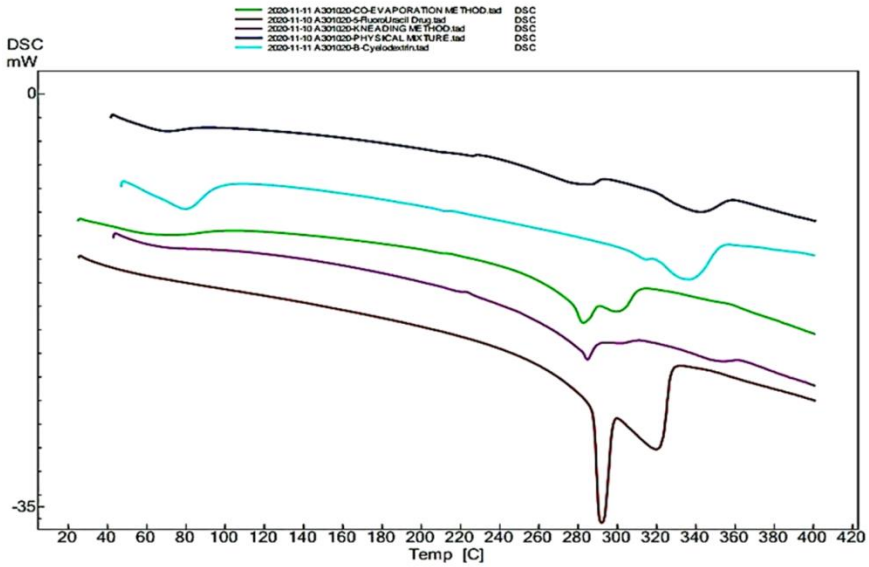

Fig. 3: Differential scanning calorimetry study overlay of physical mixture, β-cyclodextrin, co-evaporation method, kneading method, and 5-fluorouracil

Dissolution rate studies

The dissolution profile of a drug (5-fluorouracil) and its different complex are reported in a given fig. 4. The release rate of 5-fluorouracil was drawn against time in minutes. This study actually shows the dissolution time of the drug and its complex in a media, which totally depends on the nature of the drugs, the inclusion complex preparation method, and media used for dissolution.

According to the data shown, it proves that the highest rate of drug dissolution is shown by the kneading method in which the drug dissolution rate is increased up to 2.276 times in comparison with pure drug. An inclusion complex formed by the kneading method released up to 80.328 % of the drugs in total time duration of 30 min [45], whereas 43% of the drug was released within 10 min and almost 65 % drug released within 15 min but the pure drug was able to released only 35.282 % of drug within 30 min and only14 % of drug released within 15 min. All three different kinds of inclusion complex, including physical mixture, show a much better drug dissolution than pure drug (5-fluorouracil). The drug dissolution rate shown by a different kind of inclusion complex is arranged in decreasing order as kneading method, co-evaporation method, physical mixture, and pure drugs (5-fluorouracil). This proves that the kneading method is a kind of preparation method which can show the best dissolution rate for a 5-fluorouracil drug when encapsulated in a cavity of β-cyclodextrin, all this because during encapsulation, the drug crystalline nature changed to amorphous, which is already proven in SEM result that's why the wetting capability of a drug is increased in the presence of β-cyclodextrin and this effect is highly evident in case of kneading method then all other methods.

Stability study

The purpose of the stability study is to verify that the required formulation quality varies or not under the various storage factors such as light, moisture and temperature. In the following accelerated stability study for pure drugs and its different complex forms proves that all the complex forms, including physical mixture, were able to stabilize the pure drug and it was depend on physical appearance and drug content with drug released, results are shown in table 2 proved that kneading method is the best method to prepare a most stable inclusion complex among all four forms including drug and a best way for storage or stabilize drug because kneading method provides 94% of drug content and 92 % of drug release after 6 mo of accelerated study and a minor change was noticed in physical appearance which is acceptable. So, based on observation data of

accelerated stability study of different solid dispersion indicated that all formulation shows acceptable stability of the drug and all formulation can have the negligible effect of environmental condition accept pure drug forms.

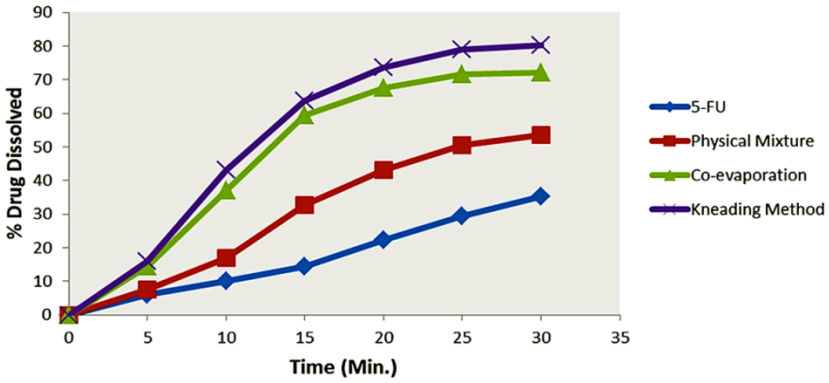

Fig. 4: Dissolution profiles of 5-fluorouracil, Physical mixture method, Co-evaporation method and Kneading method

Table 2: Evaluation parameter and stability study data of 5-fluorouracil, and β-cyclodextrin prepared by different methods

S. No.	Evaluation parameters	Time in days			
		0	30 d	45 d	60 d
1	Colour	Off-white	Off-white	Off-white	Dark white
2	Odour	Sweet smell	odourless	odourless	odourless
3	Physical appearance	Amorphous rough surface	Amorphous rough surface	Amorphous rough surface	Amorphous rough surface
4	% Drug Content	95.72±0.251	95.22±0.311	93.58±0.592	90.03±0.622
5	%Drug Dissolution (after 60 min.)	96.31±0.354	94.55±0.067	91.37±0.216	86.58±0.432

(Values represented mean±SD, n=3)

CONCLUSION

A confirm change in physicochemical properties of the drug was noticed after the formation of an adduct-Inclusion complex between 5-fluorouracil and β-cyclodextrin. Therefore, observed a dramatic enhancement in the solubility of the drugs and notified in all three types of inclusion complexes formations. After seeing the Bs type curve and the host-guest correlation value of 0.964, it is confirmed that the 1:1 ratio is the best-suited ratio for inclusion complex formation with enough stability confirmed by a molar concentration of K1:1 which was found to be 120.5M^{-1}. Lastly, on the basis of drug content, percent yield, drug dissolution study, and stability study, the kneading method is showing the overall best result, that's why the kneading method is selected for the preparation of complex through which the solubility and stability problem is going to be solved. In the future,5-fluorouracil drugs can also be used through the oral route as commonly as now-a-days through the I. V route.

ABBREVIATIONS

RH: Relative humidity, Rpm: Revolution per minute, ml: mililitre, FPM: Formulation physical mixture, FKN: Formulation kneading method, FCE: Formulation Co-evaporation method, SEM: Scanning electron micrscopy, DSC: Diffrential scanning calorimetry.

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Nil

AUTHORS CONTRIBUTIONS

Both authors have contributed substantially to the experimental design, performance, analysis, and reporting of work. Dr. Anirudh Singh Deora has contributed to conceptualization, proofreading, and correspondence. Rahul Kumar Singh has conducted all related bench-work with a literature survey, draft writing, manuscript writing and formal analysis.

CONFLICT OF INTERESTS

The authors declare no conflict of interest in this article

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