Palestinian Medical and Pharmaceutical Journal

The Inclusion Complex of Allicin with B-Cyclodextrin as A Drug-Delivery System for Enhanced Solubility in the Treatment of Breast Cancer

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Type: Full Article. Received: 13rd Nov. 2024, Accepted: 13rd Mar. 2025, Published: 1st Jun. 2026,
DOI: https://doi.org/10.59049/2790-0231.11.2.2515

Abstract: The goal of this study was to enhance the aqueous solubility, stability, and therapeutic efficacy of allicin, a bioactive organosulfur compound found in garlic (*Allium* species), through the formation of a β-cyclodextrin (β-CD) inclusion complex without altering the chemical structure of allicin. Allicin is widely recognized for its pharmacological potential but suffers from poor water solubility, instability, and strong odor, which limit its bioavailability and therapeutic application. In this study, β-CD was used to form an inclusion complex with allicin using the co-precipitation method. Various ratios of allicin to β-CD (1:1, 1:2, and 2:1) were evaluated, with the optimal complex obtained at a 1:1 weight ratio. The optimized complex achieved a drug loading of 50% ± 0.63 and an entrapment efficiency of 86.1% ± 0.37. Characterization of the inclusion complex was carried out using Fourier-transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), and scanning electron microscopy (SEM), confirming successful complex formation. Solubility studies demonstrated a significant increase in allicin's solubility from 0.52±0.03 mg/ml to 4.82±0.08 mg/ml after complexation. *In-vitro* dissolution testing revealed an improved release profile, with the inclusion complex achieving 94.10±0.05 % release within 2 hours, compared to 48.79±0.16 % for free allicin. Additionally, cytotoxicity studies against the MCF-7 breast cancer cell line showed that the allicin/β-CD complex exhibited a higher inhibition rate (147.54± 2.10 µg/ml) compared to allicin alone (69.66±8.58 µg/ml). These results indicate that β-cyclodextrin inclusion complexation is a promising strategy for enhancing the solubility, stability, and therapeutic performance of allicin.

Keywords: Allicin, β cyclodextrin, inclusion complex, MCF-7 cell line, breast cancer.

Introduction

Asia has a long history of human contact with the environment, leading to the use of herbal medicines. Many compounds used to treat infectious and chronic disorders are found in traditional medicinal plants. Plants are useful in medicine as they contain compounds that affect human physiology. Plants contain alkaloids, flavonoids, tannins, and phenols, which are the most significant bioactive chemicals [1].

Phytopharmaceuticals are bioactive compounds derived from plants that exhibit various pharmacological effects. They constitute a significant portion of medications sourced from natural rather than synthetic molecules. Phytopharmaceuticals are available in various forms, including whole fruits, purees, vegetables, prepackaged products, and supplements, and play crucial roles in supporting optimal human bodily functions [2].

Garlic is an edible bulb that belongs to the family Liliaceae. Historically, it has been used as a spice to enhance the flavor and texture of food, and as a popular home treatment to treat a variety of ailments.

The therapeutic and medicinal properties of garlic have been studied in various human ailments. Numerous bioactive components, including polysaccharides, phenolic compounds, saponins, and organosulfur compounds, are found in garlic [3].

Allicin is one of the first produced bioactive organosulfur compounds made by some species of Allium due to its predominant flavor and fragrance. Along with garlic, Allicin is found in high concentration among vegetables because it is present in almost 70 % of the total thiosulfate found in fresh garlic. A single fresh garlic clove carries about 4 to 5 milligrams of Allicin, a substance that can be easily spotted by its unique smell [4]. Despite its medicinal value, Allicin has some, quite unreasonable limitations such as instability, excessive taste and smell, and low water solubility [5]. These obstacles lower bioavailability and cause difficulty with the formulation of effective oral dosage forms

There are many approaches including the use of surfactants or co-solvent, lipid systems, and solid dispersions that have been tried to enhance the solubility and stability of Allicin. However, none of the strategies have been successful for solving the challenge of solubility and stability for such poorly water-soluble compounds. Among all of these strategies, cyclodextrin inclusion complexation has attracted significant interest due to its dual ability to enhance solubility and protect labile compounds from environmental degradation by encapsulating hydrophobic molecules within its cavity [6]. Cyclodextrins are cyclic oligosaccharides formed of glucopyranose units linked by alpha - (1, 4) bonds. Their production occurs via the enzymatic hydrolysis of starch carried out by cyclodextrin glucanotransferase (CGTase) [7]. They have a unique molecular structure with a hydrophobic cavity and a hydrophilic surrounding. This enables the formation of inclusion complexes with wide range of guest molecules [7].

The assembly of such host-guest type inclusion complexes is a non-covalent association process that is mainly mediated by the cyclodextrin cavity, whose water is replaced by guest

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molecules. This replacement results in favorable apolar-apolar contact with hydrophobic guest substances [8]. This specific feature enables the use of cyclodextrins for the stabilization of some labile compounds such as allicin, whose degradation is affected by temperature and the type of solvent. Allicin is more stable in the presence of polar solvents such as 20% ethanol and is rapidly degraded in water, and low polarity solvents such as hexane and vegetable oil [9]. Additionally, allicin's taste and odor together with chemical structure instability restrict its medicinal use. Inclusion complex with cyclodextrins offers a promising strategy to mask its sensory properties while enhancing its stability and solubility.

Considering allicin's demonstrated anticancer effect, especially its ability for invasion and metastatization of MCF-7 breast cancer cells through suppression of TNF-alpha induced ERK1/2 and NF-kappa B signaling pathways [10,11], the allicin effectiveness could be enhanced by making it more bioavailable to the body.

Because one clove of garlic yields just about 2-4 mg of allicin, dosing effectively presents a challenge. Practically consuming allicin in the target dose would require one to ingest 6-7 garlic cloves on a daily basis which is not friendly towards tolerability [12]. Therefore, enhancing the aqueous solubility and stability of allicin is essential to achieve the desired therapeutic effect with reduced intake. Despite the known benefits of cyclodextrin inclusion complexes for improving drug solubility and stability, limited studies have specifically explored their application in enhancing allicin's solubility.

The present work investigates the potential of β-cyclodextrin inclusion complexation to enhance allicin's solubility and stability while reducing degradation and masking its strong taste and odor, ultimately improving its bioavailability and therapeutic action.

Materials and Methods

Allicin (purity) was received as a gift sample from Plants Genic PVT LTD, Andheri (W), Mumbai. β-cyclodextrin was purchased from Research Lab, Fine Chemicals Industries (Mumbai). All the other ingredients were of analytical grade.

Methods

Preparation of Inclusion Complex [13]

The cyclodextrin inclusion complex was prepared using three distinct techniques: solvent evaporation, kneading, and physical trituration at weight ratios of 1:1, 1:2, and 1:4.

Physical Mixture: The physical trituration approach involved weighing, sieving, and equal mixing of allicin and βCD. Using light trituration, allicin was gradually added to βCD in a mortar. The mixture was continuously mixed for up to an hour to ensure homogenization. The mixes were stored in a closed container after passing through a #65 mesh (0.211 mm) sieve.

Kneading Method: In the kneading procedure, βCD was placed in a mortar, soaked in sufficient ethanol to form a paste, and allicin was added gradually. The mixture was dried in an oven (Remi, Mumbai, India) at 50 °C for 24 h. A mortar and pestle was used to grind the dried complex. Once the inclusion complex was filtered through a sieve, it was stored in a well-packed bag.

Solvent evaporation Method: Using the solvent evaporation method, β-CD was solubilized in 50 ml ethanol, and allicin was dissolved in 25 ml ethanol. Both solutions were combined and agitated using a magnetic stirrer (Remi Equipment Ltd., Mumbai) for one hour. Acetone was removed by boiling the mixture at 50 0 C while stirring continuously.

Water was then extracted using a rotary evaporator (Rota Vap) at lower pressure. To remove the remaining solvent, the mixture was placed overnight in an oven at 50 °C (Prerana ent, Mumbai). The inclusion complexes were crushed using a mortar and pestle. The inclusion complex was sieved and stored in a sealed container.

Co-precipitation: A weighed quantity of drug and cyclodextrin, in a suitable molar ratio (1:1), was added separately in ethanol. The drug solution was added dropwise to the cyclodextrin solution. The resultant mixture was continuously stirred for 6 hours and then dried in a hot air oven at 45– 50°C for 2 days. The dry product was crushed, passed through a #65 mesh sieve, and placed in an airtight container until use.

Inclusion Complex Using Garlic

Using Ethanolic Extract: Garlic was finely crushed and mixed with a sufficient amount of pure ethanol to create an ethanolic extract. To obtain a dry powder of garlic, the resulting filtrate was centrifuged and vacuum-dried. Using the co-precipitation technique and physical mixing, this powder was employed to prepare the inclusion complex.

Using Aqueous Extract: Garlic aqueous extract was made into an inclusion complex by mixing freshly made extract with β-CD aqueous solution that was kept in an ice bath. After the liquid was removed, the crystals were separated, allowed to air dry, and then utilized in preparation of the complex.

All experiments were performed in triplicate (n = 3), and the results are presented as mean ± standard deviation (SD). Statistical analysis was conducted using Origin Pro 2022 (Version 9.9).

Evaluation Parameters

Phase Solubility studies [14,15]: Phase solubility study was conducted using the methodology described by Connors and Higuchi. After adding excess allicin (1gm) to phosphate buffer solutions (pH 7.4) containing β-CD at varying molar concentrations (0.01-0.1mM), the mixture was agitated at a steady temperature for a full 72 hours. Once the filtered solutions had been appropriately diluted with phosphate buffer, they were examined at 242 nm using a UV Spectrophotometer (Shimadzu UV-1900) to determine their solubility characteristics.

The solubility of allicin (Y-axis) was plotted against the concentration of β-CD (X-axis). The association constant (Ka) quantifies the stability of the allicin-β-CD complex and was calculated only from the linear (AL-type) portion of the phase solubility curve using the Higuchi and Connors equation.

According to Higuchi and Connors, the association constant can be calculated using the equation:

$$Ka = \text{Slope}/ S0 (1-\text{Slope}) \quad (1)$$

Where S0 is the aqueous solubility of allicin.

Solubility Study

Aqueous solubility: An excessive quantity of allicin inclusion complex was placed in 50 milliliters of distilled water to carry out a solubility analysis. The flasks were shaken at 120 rounds per minute for 72 hours after getting vortex-mixed for three minutes. 3ml samples were taken at fixed intervals of 30 min, filtered via a 0.45 µm nylon membrane filter, and their absorbance at 242 nm was measured using a UV Spectrophotometer (Shimadzu UV-1900). If necessary, the

filtrate was properly diluted. Each measurement was repeated thrice.

pH solubility profile: Solubility of inclusion complex obtained by co-precipitation method was studied in three different pH using shake flask method.

1 gm of inclusion complex was dissolved in conical flask each containing 50 ml solution of different pH (pH 4, 7 and 9) and rotated at 100 rpm. At fixed interval of time 5 ml of sample was withdrawn, filtered and solubility was determined using a UV Spectrophotometer (Shimadzu UV-1900).

Drug Loading and Entrapment Efficiency [16]

The amount of drug present per unit weight of the complex generated is known as drug loading. The amount of drug encapsulated or entrapped inside the inclusion complex is indicated by the entrapment efficiency. 10 mg drug and complex equivalent to 10 mg of the drug is dissolved in 10 ml water, filtered, and analyzed using UV-1900 at 242 nm.

The drug loading (DL) and entrapment efficiency (EE) are determined using the formulae:

$DL= \frac{M}{M1} x100$

$\%EE=\frac{M}{M2} x100$

Where, M= Amount of drug present in the complex

M1= Weight of inclusion complex

M2= Total weight of drug added

Fourier transform infrared spectroscopy (FT IR) [17]

The spectra of allicin, β-CD, and inclusion complexes were recorded on an FT-IR Spectrometer (Bruker Spectrometer equipped with ATR Diamond) between 4000 and 400 cm−1 using the potassium bromide compression method (2 mg sample and 600 mg KBr). It is difficult to accurately detect the presence of a guest molecule in β-CD. Still, variations in the distinctive peaks' form, position, and intensity before and after the inclusion could be used to evaluate the presence of allicin in the complex.

X-ray diffraction studies (XRD) [18]

The crystallinity of compounds, including raw allicin and its inclusion complex, was characterized using XRD. The diffractogram was obtained with a Bruker D8 Advance diffractometer (Bruker, USA) employing a locked coupled scan type. Both the compounds were irradiated with monochromatized Cu Kα radiation and analyzed over a 2θ angle range of 3–40°. The XRD patterns were recovered with a tube voltage of 40 kV, a tube current of 40 mA, and a scan speed of 0.1 s per step.

In-vitro Dissolution Study [19]

In-vitro dissolution studies of the inclusion complex and allicin were carried out using a USP dissolution tester (Basket Method) equipped with a Lab India dissolution apparatus. The dissolution medium was made up of 900 ml of phosphate buffer that was kept at 37.0 ± 0.5 °C while the basket rotated at 100 revolutions per minute. Each vessel was filled with an exact quantity of both the drug and the complex. At prearranged intervals, 5 ml samples were taken out and filtered. The amount of drug in the sample was determined by a UV spectrophotometer (Shimadzu (UV-1900), Japan) set at 242 nm.

Scanning electron microscopy (SEM) [20]

The surface morphology of the allicin and allicin/β-CD inclusion complex was examined by Scanning electron microscope, Philips 500. Using double-sided tape, each sample was secured to a brass stub. The samples were then vacuum-coated with gold using a sputter coater Polaron E5100. Images were captured with a 20 kV excitation voltage.

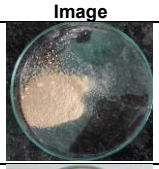
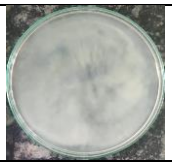
Cell Line Study [21,22]

The MCF-7 Human Breast Cancer Cell Line was supplied by the National Centre for Cell Sciences (NCCS), Pune. It was cultured in DMEM media with 10 % fetal bovine serum added. Cells were cultivated in culture media at a concentration of 1 ×104 cells/ml for 24 hours at 37°C and 5 % CO2. Cells were introduced at a concentration of (70 µl) 104 cells/well in 100µl culture media and 100 µl Sample-A and A1 (10-100 µg/ml) into tissue culture grade microplates with 96 wells. In control wells, DMSO (0.2 % in PBS) and cell lines were grown. Every sample was incubated for three rounds. Controls were maintained to determine the percentage of living cells following culture as well as the survival rate of control cells. Cell cultures were cultured for 24 hours at 37 °C and 5% CO2 in a Thermo Scientific BB150 CO2 incubator. 20 µl of MTT reagent (5 mg/min PBS) was added after the medium was withdrawn.

Following the addition of MTT, the cells were incubated for 4 hours at 37°C in the CO2 incubator. A microscope was used to examine the wells for the growth of formazan crystals. The yellowish MTT could only be changed into a dark-colored formazan by cells that were alive. Following the full removal of the medium. Later 200 µl of DMSO was added, let sit for ten minutes, and then covered with aluminium foil, incubated at 37 0C. Three samples were examined in triplicate, and each sample's absorbance was determined with the help of an Elisa microplate reader (Benesphera E21) set to a 570 nm wavelength.

Table (1): Optimization of Method.

Sr. No.	Method of Preparation	Solubility (mg/ml)	Observation	Image
1	Using Allicin			
1	Allicin	0.52±0.03	Amorphous powder with a strong and irritating smell	
A	Physical Mixture	2.91±0.06	Reduced odor and color	
B	Kneading	2.57±0.05	Requires 2-3 days for complete evaporation of solvent. Has reduced taste and odor.	

Sr. No.	Method of Preparation	Solubility (mg/ml)	Observation	Image
C	Co-precipitation	4.82±0.08	Slightly crystalline powder with reduced odor and color. Shows highest solubility.	
D	Solvent Evaporation (at 60°C)	2.42±0.04	Crystalline powder, difficult to recover, low yield	
E	Solvent Evaporation (at 80°C)	0.68±0.04	Due to temperature, allicin degrades and turns into a solid, sticky mass	
2	Using Garlic			
	Inclusion complex of Aqueous Extract	0.94±0.04	Crystals observed in solution. Requires 2-3 days for drying. Very little yield.	
	Ethanolic Extract	0.34±0.01		
A	Physical Mixture	1.88±0.05	White, amorphous powder with reduced smell. Low yield, sticky.	

Results

Phase solubility studies

As per the classification system of phase solubility diagram, presented by Higuchi and Connors [23,24], The phase-solubility diagram predicts how higher-order compounds will form with CD., Fig.1 represents a phase solubility study of allicin with β-cyclodextrin (β-CD), which is a typical method to evaluate the solubility enhancement and complex formation between a guest molecule (allicin) and a host (β-CD). The plot shows the concentration of dissolved allicin (y-axis) against the concentration of β-CD (x-axis). The linear increase in allicin concentration with increasing β-CD concentration indicates the formation of an inclusion complex. The linearity of the graph (with an R^2 value of 0.9937, which is very close to 1 suggests an AL-type diagram, commonly associated with a 1:1 stoichiometric complex between allicin and β-CD.

The equation of the line is: y=0.0089x+0.1173y = 0.0089x + 0.1173

where: slope = 0.0089, intercept = 0.1173

For an AL-type diagram, the association constant ($K_{1:1}$) for a 1:1 complex can be calculated using the equation 1.

The phase solubility study indicates good complex formation between allicin and β-CD with a linear increase in solubility. The calculated association constant ($K_{1:1} \approx 766\ M^{-1}$) reflects a moderate to high affinity, indicating that β-CD significantly enhances the solubility of allicin through stable complex formation.

The CD solubility of the drug is unaffected in this area of the diagrams. The creation of allicin/cyclodextrin complexes with restricted solubility and depression of CD solubility due to drug presence is indicated by the plateau region in the diagram, where the solubility of the drug remains intact even as CD concentration increases.

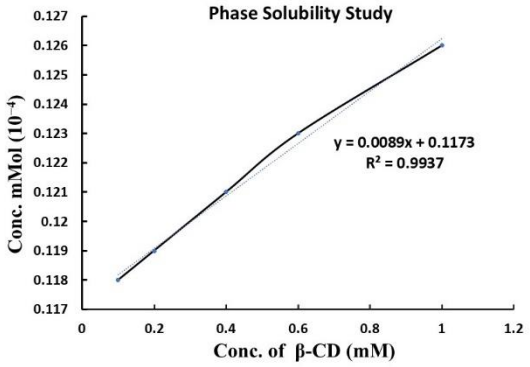


Figure (1): Phase solubility profile.

Preparation of β-CD inclusion complex

The maximum solubility is observed with allicin co-precipitation (4.82 ± 0.08 mg/ml, $p < 0.05$), which is 9 times higher than pure allicin (0.52 ± 0.03 mg/ml), proving the efficacy of β-CD inclusion. In the case of garlic extracts, co-precipitation (2.09 ± 0.07 mg/ml) was superior to the ethanolic extract (0.34 ± 0.01 mg/ml, $p < 0.05$) but was still lower than allicin complexes. The significant loss of solubility at 80°C highlights the temperature sensitivity of allicin. Low SD values indicate high reproducibility (n=3) in all techniques.

Solubility studies

Aqueous solubility profile*:* Table no.1 shows the solubility of allicin, and allicin/βCD inclusion complexes. Allicin solubility was significantly increased with the addition of βCD regardless of the method used for the formation of the complex. All the preparation methods employed could increase the boost of allicin but to varying extents.

The co-precipitation method produced the highest solubility of allicin in βCD, followed by physical mixture, kneading method,

and lastly solvent evaporation. A 1:1 ratio of allicin to βCD enhanced the solubility of allicin. Allicin solubility did not increase when cyclodextrin concentration increased from 1:1 to 1:2.

Table (2): Aqueous Solubility of Prepared Inclusion Complex.

Sr. No.	Sample Name	Solubility (mg/ml)
1	Allicin	0.52± 0.03
2	Physical Mixture	2.91± 0.06
3	Co-precipitation	4.82± 0.08
4	Kneading	2.57± 0.05
5	Solvent Evaporation (at 60°C)	2.42± 0.04

For complex prepared using co-precipitation, the solubility of allicin /βCD 1:1 ratio was 4.82±0.08 mg/ml, which, at a similar ratio, was around two times that produced using the physical combination method (2.91±0.06 mg/ml). Hence, the co-precipitation method was more effective than the physical mixture method in solubility enhancement.

The co-precipitation method was the most superior among the four methods employed, producing the greatest allicin solubility at the same ratios of allicin and βCD. Allicin Significantly greater solubility was observed from 0.52 ±0.03 mg/ml to 4.82±0.08 mg/ml. Nevertheless, there was not a noticeable improvement in allicin solubility upon increasing the βCD quantity from 1:2 to 1:4. This finding indicates that the ideal allicin/βCD ratio was 1:1.

pH solubility profile

The study concluded that the allicin/β-CD complex exhibited the highest solubility at pH 7, followed by pH 4, and the lowest solubility at pH 9. Hence, further studies on drug release was conducted using buffer of pH 7.4.

The table given below provides the solubility data about inclusion complex in different pH levels and different time points.

Table (3): Solubility of complex in different pH.

pH	Solubility (mg/ml)
4	3.94 ±0.04
7	4.28± 0.03
9	3.07±0.02

Drug Loading and Entrapment Efficiency

In the present study, the inclusion of allicin and β-cyclodextrin was successfully prepared by co-precipitation procedure. This method involves mixing separately prepared allicin and β-CD solutions using a magnetic stirrer. Finally, the DL and EE were determined to be 50% ± 0.63 and 86.1% ± 0.37 respectively.

Fourier transform infrared spectroscopy (FT IR)

The FTIR spectra of allicin, β-Cyclodextrin, and Allicin/βCD [Co-precipitation] are represented in Fig.no.2. All of the functional groups like —O-H, C-H, H-O-H, C-O-C, along with C-H—are visible in the β-cyclodextrin's FT IR spectra, which are located at 3398.92, 2927.89, 1645.46, 1159.01, and 855.27 cm-1, respectively. The FT IR spectra of allicin, a pure drug shows all the functional groups O-H, C-H, C=C, S=O, and C-S at 3306.87 cm^{1}, 2927.89 cm^{-1}, 1651.73 $cm^{-1,}$ 1078.5 cm^{-1} and 713.05 cm^{-1} respectively.

The FT IR spectrum of the allicin/β-cyclodextrin inclusion complex, which was prepared by physical mixture, displays all of the characteristic peaks of O-H, C-H, C=O, C-H, C-O-C, and S=O at 3423.99 cm^{-1}, 2927.89 cm^{-1}, 1645.46 cm^{-1}, 1413.08 cm^{-1}, 1159.01 cm^{-1}, and 1041.37 cm^{-1}, respectively. This indicates that all of the functional groups are unchanged.

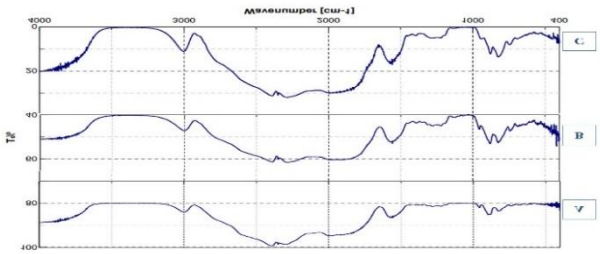


Figure (2): FTIR spectra of: (A) β Cyclodextrin; (B) Allicin βCyclodextrin inclusion complex and (C) Allicin.

X-ray diffraction studies (XRD)

According to the diffraction pattern, allicin does not exhibit any prominent characteristic peaks in the 10–30° range; yet, those peaks did emerge in the inclusion complex (Fig. No.3) The appearance of intense, sharp peaks in the X-ray diffraction pattern of inclusion complex indicate to the growth of crystallinity. This structural transformation could greatly impact the drug's stability, solubility, and bioavailability, which can be examined in future studies.

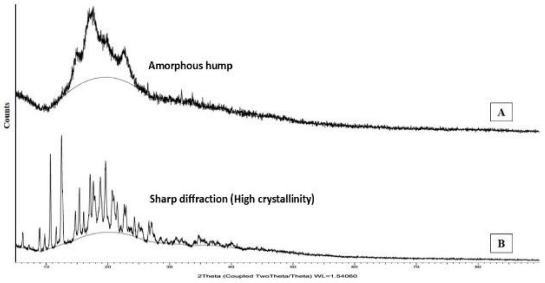


Figure (3): XRD patterns of A: Allicin and B: Allicin/β Cyclodextrin inclusion complex.

In-vitro Drug Release Study

The allicin/β-cd complex and allicin *in-vitro* release rates are displayed in Fig no.4. Based on the findings, allicin/β-CD released at a faster rate than allicin itself. Within one hour, the total amount of free allicin released was 41.26 ±0.01 %. As a result, in an hour, the total amount of allicin released from the inclusion complex was 65.13%. After two hours, the combined release of free allicin was 48.79±0.16 %, whereas the release of allicin/β-CD was 94.10±0.05 %.

Thus, it is evident that the release of allicin was facilitated by β-CD and allicin. According to a dissolution research profile (Fig. No.4), the cumulative release of allicin was significantly increased by the β-CD when compared to free allicin.

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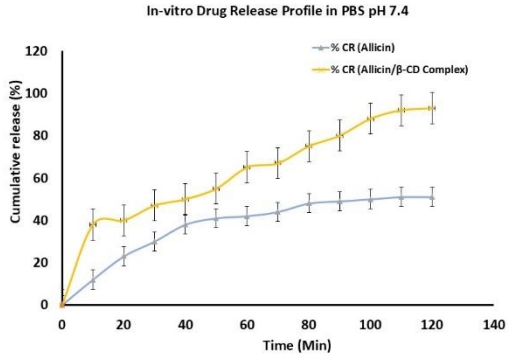

Figure (4): *In-vitro* Drug Release Profile of Allicin and Allicin/β-CD Complex in Phosphate Buffer Solution pH 7.4.

Scanning electron microscopy (SEM)

The SEM analysis was carried out to compare the morphological characteristics of pure allicin and the allicin-beta-cyclodextrin complex prepared via the co-precipitation method. The aim was to observe any changes in particle size, shape, and distribution resulting from the complexation process. The SEM images were taken at a magnification of 5000 X with a scale bar indicating a length of 5 µm. The results are shown in Fig (5) given below:

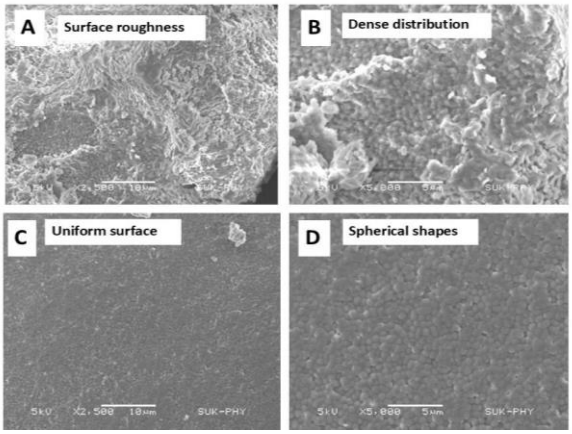

Figure (5): Scanning electron microscopy (SEM) morphology of A, B: Allicin and B, C: Allicin/ β-CD Complex.

The surface morphology of both images A and B represent a rough, densely packed structure with irregular surface. The texture appears to be consistent across both images. Whereas images C and D illustrate a more refined, uniform surface with distinct spherical particles. The differences suggest transition from rough, uneven morphology to a smooth, well-organized particle surface with consistent morphology.

Cell Line Study

The MTT assay was used to assess the cytotoxic effects of both free allicin and the allicin/β-cyclodextrin (β-CD) inclusion complex on the MCF-7 breast cancer cell line. Before treatment, cells were seeded into 96-well plates and given a full day to adhere. Cells were then treated to different doses of free allicin and allicin/β-CD complex (10, 20, 40, 80, and 100 µg/ml). Following a 24-hour treatment period, each well received 20 µL of MTT reagent (5 mg/ml), and the plates were incubated for an extra 4-hour period at 37°C to facilitate the production of formazan crystals. After dissolving the crystals in DMSO, a microplate reader was used to measure absorbance at 570 nm. The cytotoxic effects of the examined substances were compared using 5 Flurouracil, a common chemotherapeutic drug, as a positive control.

Free allicin was found to have an IC50 value of 147.54± 2.10 µg/ml (the concentration needed to inhibit 50 % of cell viability), but the allicin/β-CD complex had a substantially lower IC50 value of 69.66± 8.58 µg/ml, suggesting that complexation with β-CD increased anticancer action. These findings imply that, in comparison to free allicin, the β-cyclodextrin inclusion complex of allicin increases its cytotoxic effectiveness against MCF-7 cells, possibly because of improved stability and solubility.

The comparison between the cell density of culture treated with sample A and A1 is shown in Fig no.6 given below. The images show that cell density is higher in the case of sample A while in sample A1 cell density is relatively low.

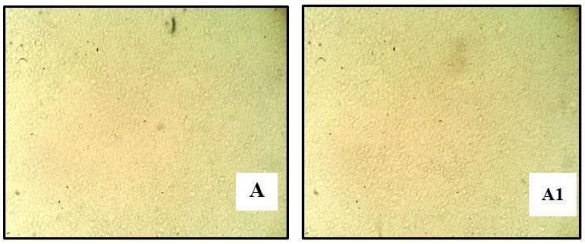

Figure (6): Comparative cell density of sample A (allicin) and A1(allicin/β-CD Inclusion complex).

In this study, β-CD was chosen to form an inclusion complex with allicin without altering the chemical structure of the guest (drug) molecule, cyclodextrin can modify its physical properties. Additionally, it contributes to increased biocompatibility, stability, and solubility. Furthermore, it provides a controlled release mechanism that can reduce adverse effects and improve therapeutic efficacy.

The inclusion complex containing allicin, ethanolic, and aqueous garlic extract was made using β-cyclodextrin. Various weight and molar ratios of allicin and β-CD were utilized during optimization, including 1:1, 1:2, and 2:1 ratios. To find the most effective formulation in terms of solubility, entrapment efficiency, physical appearance, and drug release, several formulations were created by varying the ratio.

Various methods of solubility enhancement such as the co-precipitation method, physical mixture, kneading method, and solvent evaporation were optimized. Out of which co-precipitation method produced the highest solubility of allicin in βCD.

In comparison the co-precipitation method offers better control over the formation of inclusion complexes, leading to uniform particle sizes and enhanced surface area, which significantly improves the solubility of the active compound. This method also ensures a higher degree of interaction between β-CD and the phytoconstituent, forming more stable inclusion complexes.

The results are by the findings reported by Kanni [25]. The optimal ratio for creating the allicin/β-CD complex is determined using the co-precipitation with a weight-to-drug ratio of 1:1. This resulted in a drug loading of 50 ± 0.63 % and an entrapment efficiency of 86.1 ± 0.37 %. The development of the inclusion complex altered several physical properties, including appearance and odor.

Table (4): Effects of Sample A and A1 against MCF7 by MTT

Sample	Sr. No.	Concentration (µg/ml)	Absorbance (OD) (Mean ± SD)	Cell Viability (%)	Percent Inhibition (%)	IC50 (µg/mL)
5-Fluorouracil (Control)	–	–	1.176 ± 0.010	100 ± 0	–	–
A (Free Allicin)	1	10	0.418 ± 0.006	35.54 ± 2.10	64.45 ± 2.15	147.54 ± 2.10
	2	20	0.409 ± 0.004	34.78 ± 2.10	65.22 ± 2.15	–
	3	40	0.387 ± 0.010	32.94 ± 2.10	67.06 ± 2.15	–
	4	80	0.381 ± 0.003	32.39 ± 2.10	67.60 ± 2.15	–
	5	100	0.355 ± 0.004	30.22 ± 2.10	69.78 ± 2.15	–
A1 (Allicin/β-CD Complex)	6	10	0.783 ± 0.004	66.58 ± 8.58	33.42 ± 8.58	69.66 ± 8.58
	7	20	0.674 ± 0.008	57.31 ± 8.58	42.69 ± 8.58	–
	8	40	0.627 ± 0.006	53.32 ± 8.58	46.68 ± 8.58	–
	9	80	0.577 ± 0.010	49.06 ± 8.58	50.93 ± 8.58	–
	10	100	0.518 ± 0.005	44.05 ± 8.58	55.95 ± 8.58	–

According to X-ray diffraction, the produced allicin/β-CD inclusion complex has a crystalline surface and reduced odor. Allicin's solubility increased 9-fold from 0.52± 0.03 to 4.82±0.08 mg/ml after forming the inclusion complex via the co-precipitation method. The biological activity of allicin is mainly attributed to its reactive –S(O)–S– group, which, contribute to antimicrobial and antioxidant activities, causing poor stability. Complexation with β-cyclodextrin (β-CD) enhances allicin's solubility and stability by protecting this reactive moiety without affecting activity. The increased and prolonged drug release of 94.10 ±0.05 % after a 2-hour *in-vitro* release experiment, nearly twice as high as the release rate of allicin alone (48.79±0.16 %) indicating enhanced stability and controlled release. Therefore, β-CD complexation maximizes allicin's pharmacokinetics to increase its therapeutic efficacy.

In the MCF-7 cell line experiment, the allicin/β-CD complex was more effective against cancer than free allicin. The allicin/β-CD complex's IC50 (69.66±8.58 µg/ml) was substantially lower than free allicin's IC50 (147.54± 2.10 µg/ml), showing higher potency. In addition, although allicin had a maximum inhibition rate of 69.78± 8.58 % at the highest concentration tested (100 µg/ml), the allicin/β-CD complex inhibited to 55.95± 8.58 % at the same concentration, which implies increased efficacy at reduced doses.

Discussion

Allicin has great potenial as a cancer treatment, but its use is limited by its low water solubility, rapid degradation and poor bioavailability. To overcome this limitation, researchers have investigated a number of different approaches including nanoparticle-based systems, liposomes and chemical modification to improve the solubility and stability of allicin, these tend to require complex synthesis, increased cost and potential scalability problems [26,27].

Our work, on the other hand, shows that β-cyclodextrin (β-CD) inclusion complexation offers a low-cost, easy, and effective approach, enhancing the solubility of allicin by almost 9-fold. In contrast to other systems, β-CD complexes are simple to prepare, contain approved drug excipients, and are perfect for the formulation of stable and high-solubility allicin preparations [3].

In contrast, the current study demonstrates that the β-cyclodextrin (β-CD) inclusion complex method is a simple, cost-effective, and efficient alternative to these advanced delivery systems.

β-CD inclusion complexes is a relatively simple and cost-effective approach compared to nanotechnology-based drug delivery systems. Additionally, it can be tailored to control the release profile of the active compound by varying the polymer-to-drug ratio or the nature of the co-precipitating agents. The use of β-CDs has emerged as a promising strategy to enhance the solubility of phytoconstituents. Several relevant studies and findings on the solubility enhancement of phytoconstituents using β-CDs have been reported [28,29,30].

Cyclodextrins (CDs) are used to enhance the stability of drugs by providing a protective environment. They achieve this by encapsulating sensitive drug molecules within their hydrophobic cavities, effectively shielding them from environmental factors like oxidation, light, and heat. This encapsulation helps maintain the integrity of the pharmaceutical compounds, potentially increasing their efficacy and shelf life [5]

In this work, we utilized the β-CD co-precipitation approach to investigate the solubility enhancement of allicin. Our results show that the solubility of allicin was greatly boosted by the β-CD co-precipitation technique. When β-CD and allicin created an inclusion complex, the mixture became more soluble when allicin was used alone. These findings are consistent with prior studies that have demonstrated how β-CD forms inclusion complexes to increase the solubility of hydrophobic substances, such as quercetin, curcumin, celastrol, chrysin and, resveratrol, and other phytoconstituents with potential therapeutic applications [29,31,32,33,34]. The developed inclusion complexes have reportedly been examined using a range of spectroscopic methods, such as X-ray diffraction, infrared spectroscopy, and UV-visible spectroscopy.

Furthermore, although β-CD inclusion complexes have been widely recognized for improving the solubility and stability of poorly water-soluble compounds, detailed investigations focusing on the preparation methods, optimization parameters, and comparative efficiencies of these techniques remain relatively limited in existing literature. Most studies mention inclusion complex formation but do not extensively evaluate different preparation methods that influence water solubility enhancement, or yield.

The current study has investigated diverse techniques used to prepare β-CDs inclusion complex, with an emphasis on its water solubility and percent yield. In this research, there was an impressive enhancement in the solubility of allicin through β-cyclodextrin inclusion complexation, where there was an enhancement from 0.52± 0.03 mg/mL (native allicin) to 4.82 ± 0.08 mg/mL for the β-CD complex. This is an increase in more than 9 times the solubility, which is significant for ensuring therapeutic effectiveness of allicin. The significant increase in allicin's water solubility and release rate is due to the formation of a β-cyclodextrin (β-CD) inclusion complex. β-CD contains a hydrophobic interior that entraps the allicin molecule but has a hydrophilic outside that remains water compatible.

oxidation, heat, and degradation, problems often encountered in free allicin.

The rate of release is enhanced, as the complex disperses more efficiently in aqueous media. Stability is provided by protecting sensitive groups of allicin. This mechanism is responsible for the better performance of the allicin–β-CD

complex for both solubility improvement and anticancer activity, indicating it as a potential for use in pharmaceuticals.

The in-vitro release study shows a remarkable improvement in the release profile of allicin as a β-CD inclusion complex. The complex reached a release rate of 94.10±0.05 % in 2 hours, which is nearly double that of free allicin (48.79±0.16 %), showing the increased dissolution and bioavailability of allicin by β-CD complex.

In the MCF-7 breast cancer cell line assay, the inhibition rate of allicin/β-CD inclusion complex was 69.66± 8.58 %, whereas that of free allicin was 47.54 %. This shows that the increase solubility and stability of allicin in the inclusion complex were directly translated into higher cytotoxicity against cancer cells. In addition, the allicin/β-CD complex's lower IC50 value also indicates its higher efficacy, as a lower IC50 indicates that lower concentrations of the complex are needed to inhibit 50 % of cancer cell growth, which can reduce possible side effects and enhance therapeutic selectivity.

The *in-vitro* experiments conducted on MCF-7 breast cancer cell lines additionally proved that the allicin-β-CD complex was more cytotoxic than free allicin. The improved solubility and consequent bioavailability of allicin in the presence of β-CD are responsible for this increase in efficacy. Through the β-CD inclusion complex, allicin's solubility is enhanced, which indicates a major improvement in its potential use in breast cancer therapy. These results support the future potential of allicin as an anticancer drug, especially in the treatment of breast cancer.

In conclusion, compared to other solubility enhancement techniques, β-CD inclusion complexation stands out as a practical, scalable, and safe approach for improving allicin delivery, supporting its development into viable pharmaceutical formulations such as oral tablets or injectable solutions for breast cancer treatment

The cytotoxicity of the allicin-β-CD combination was evaluated in this work mainly using *in- vitro* models. Although these findings are positive, more *in-vivo* research is required to evaluate the pharmacokinetics, biodistribution, and real therapeutic efficacy in trials involving animals or humans. Furthermore, the best way to administer the allicin-β-CD combination is still unknown. Studying various β-CD derivatives, such as hydroxypropyl-β-CD or methyl-β-CD, might also be beneficial as they may improve solubility and controlled release characteristics.

Additionally, combining allicin-β-CD complexes with other therapeutic drugs may result in synergistic effects, especially when using combination therapy approaches to treat breast cancer [35].

Conclusion

To sum up, the work offers a thorough examination of how phytochemicals, cyclodextrins, and nutraceuticals can improve drug distribution and therapeutic effectiveness. Cyclodextrins are useful in pharmaceutical formulations because of their adaptability in enhancing drug solubility and bioavailability.

Given the rising prevalence of drug-resistant illnesses, more research is necessary to confirm the safety and efficacy of phytochemicals, as their therapeutic potential makes clear. In the fight against breast cancer, the significance of early identification and creative preventative techniques is also underlined.

Moreover, the increasing popularity of nutraceuticals represents a change in the direction of combining medicine and nutrition to enhance overall wellness and health. This study highlights how modern scientific techniques can be combined with traditional knowledge to create safer and more effective alternatives to therapy.

Future research should focus on exploring new cyclodextrin derivatives, validating the therapeutic potential of additional phytochemicals, and expanding the applications of nutraceuticals as well as cyclodextrin and its derivatives for various issues regarding health.

Thus, the current study lays a solid groundwork for advancing allicin-based treatments through demonstrating a scalable, practical, and biocompatible β-CD inclusion complexes, with great potential to extend to the treatment of breast cancer.

Disclosure Statement

- **Ethics approval and consent to participate:** Not applicable
- **Consent for publication:** Not applicable
- **Availability of data and materials:** The raw data required to reproduce these findings are available in the body and illustrations of this manuscript.
- **Author's contribution:** Dr. Harshada I Patil, study conception and design, Gandhali Koshti, interpreted the results and contributed to manuscript writing, Shital Doijad, writing review & editing, Dr. Kiran A. Wadkar, supervised the project and critically reviewed the manuscript
- **Funding:** This study was not funded
- **Conflicts of interest:** The authors declare that there is no conflict of interest regarding the publication of this article
- **Acknowledgements:** The author would like to thank Dr. Shivajirao Kadam College of Pharmacy, Kasabe Digraj, Sangli, for providing research facilities.

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