Original Article

Solubility Enhancement of Lawsone by Complexation with Beta Cyclodextrin

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ABSTRACT

Introduction: Lawsone is a phyto-constituent obtained from the leaves of the *Lawsonia inermis*. It has antibacterial, antifungal, antiviral, wound healing, antiparasitic, tuberculostatic, anti-fertility, analgesic, anti-inflammatory, enzyme inhibitory, nematicidal, anticoagulant, and protein glycation inhibitory activities. It has poor water solubility (hydrophobic), poor bioavailability, and poor dissolution characteristics. **Aim:** The purpose of this research is to improve the aqueous solubility of Lawsone by preparing the inclusion complexes of Lawsone with beta-cyclodextrin. **Materials and Methods:** Inclusion complexes of Lawsone were prepared by different methods such as physical mixture, kneading method and co-precipitation method. Characterization of the complexes were done by Fourier Transform Infrared (FTIR) spectroscopy and *in vitro* dissolution study. Differential scanning calorimetry (DSC), Nuclear Magnetic Resonance spectroscopy (NMR), and Powder X-Ray Diffraction (PXRD). were used to evaluate the co-precipitation technique inclusion complexes. Antimicrobial studies of complexes against *Escherichia coli (E. coli)*, *Staphylococcus aureus (S. aureus)*, *Bacillus subtilis (B. subtilis)*, and *Pseudomonas aeruginosa (P. aeruginosa)* were done by colony counting method. **Results:** Phase solubility study revealed the molar ratio of Lawsone to beta-cyclodextrin in the complex is 1:1. At the third hour, the drug release was 64%, 78%, 93%, and 97% for pure drug, physical mixture, inclusion complexes formed by kneading technique and co-precipitation technique respectively. DSC, NMR, and PXRD confirmed the formation of complexes of Lawsone. The order of antibacterial activity of inclusion complexes was *E. coli* > *B. subtilis* > *P. aeruginosa* > *S. aureus.*

Keywords: Lawsone, Beta-cyclodextrin, Inclusion complexes, Kneading method, Co-precipitation method.

INTRODUCTION

Lawsone (2-hydroxy-1,4-naphthoquinone)[1] is a red-orange dye derived from the *Lawsonia inermis* leaves (henna plant) as well as from the flowers of *Eichhornia crassipes* (water hyacinth). Lawsone is also known as hennotannic acid.[2] Lawsone possesses antibacterial,[3-4] antifungal, antiviral, antiparasitic, molluscicidal, tuberculostatic, antifertility, analgesic, anti-inflammatory, cytotoxic, antisickling, wound healing,[5] nematicidal,[6] hepatoprotective,[7] anticoagulant, and antidysentery properties.[6]

Lawsone has poor solubility[8] and low solubility in aqueous mediums resulting in low bioavailability,[9] poor permeability, and instability in biological environments.[2] Improvement of aqueous solubility will enhance the biological activities.

The formation of IC with cyclodextrins offers useful effects on solubility, dissolution rate, chemical stability, and absorption rate of a poorly water-soluble drug.[10-11] Cyclodextrins are a group of organic compounds formed by binding glucose units via 1,4- glycosidic linkages. Cyclodextrin has hydrophilic outer surfaces and lipophilic inner cavities. Through non-covalent interactions, the hydrophobic interior can interact with a number of guest drug molecules. This result in the formation of inclusion complexes that have modified physicochemical properties without the

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molecular modification of guest molecule. Thus, the solubility, rate of absorption, and bioavailability of poorly water-soluble drug molecules can be enhanced.[12-13] Among various cyclodextrins, the advantages of beta-cyclodextrin include improvement of solubility,[14] improvement of bioavailability,[15] improved stability,[16] decreased irritation, incompatibility prevention,[17] masking of taste and odour, and also material handling advantages.[18] Reduced dosing and improved stability of drug molecules improving patient compliance were also observed. [2,19] To increase the dissolution characteristics, IC of Lawsone is prepared with beta-cyclodextrin.

MATERIALS AND METHODS

Materials

Lawsone (YUCCA enterprises, Maharashtra), Beta-cyclodextrin (Chemdyes Co-operation, Gujarat), 0.5 Mc Farland turbidity standard (Chemdyes Co-operation, Gujarat), Nutrient broth (HiMedia laboratories private Ltd, Karnataka), Agar (Research lab fine chem industries, Maharashtra), Methanol (Nice chemicals, Kochi). *E. coli* (ATCC 8739), *P. aeruginosa* (ATCC 9027), *B. subtilis* (ATCC 6633), and *S. aureus* (ATCC 6538P) (NCIM, CSIR, Pune, Maharashtra).

Methods

Phase Solubility Study

Higuchi and Connors method was used for carrying out the phase solubility studies.[20] Excess amount of Lawsone (30 mg) was added to the 15 ml of an aqueous solution containing various concentrations of beta-cyclodextrin (0,4,8,12,16,20 mM/l) in a beaker and the mixtures were placed at room temperature on a rotary flask shaker for 24 hr. The solutions were tested for drug content using a UV-Visible spectrophotometer (SHIMADZU) at 451.6 nm once they had reached equilibrium.[21]

Preparation of Lawsone inclusion complexes

Physical Mixture

Lawsone and beta-cyclodextrin were taken in the molar ratio 1:1 and mixed thoroughly in a mortar with vigorous trituration for about 30-45 min. Then it was sieved through sieve number 100 and stored in a desiccator.[22]

Kneading Method

First, a homogenous mass of beta-cyclodextrin was made by adding the solvent containing equal amounts of water and methanol. Then, with continued kneading, Lawsone is gradually added to the paste. The wet mass was then kneaded thoroughly with vigorous trituration using a pestle for 1 to 3 hr to obtain a paste-like consistency. The bulk was then dried for 24-48 hr in a hot air oven at 45°C-50°C. After that, the powdered complexes were sieved through sieve number 100 and kept in a desiccator.[23-25]

Co-precipitation Method

Lawsone and beta-cyclodextrin were taken in the molar ratio 1:1 and separately dissolved in the solvent containing equal amounts of water and methanol. The Lawsone solution was then added drop by drop to the cyclodextrin solution. A magnetic stirrer was used to mix the solution continuously for 5-6 hr. The bulk was then dried for 24-48 hr in a hot air oven at 45°C-50°C. After that, the powdered complexes were sieved through sieve number 100 and kept in a desiccator.[23-24]

Percentage yield

The complexes were weighed, and the following calculation was used to obtain the percentage yield:[26]

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Percent Drug Content Estimation

In phosphate buffer at pH 7.4, complexes containing 10 mg of Lawsone were dissolved. After appropriate dilution, the drug content was measured using a UV-visible spectrophotometer at 453.2 nm.[26-27]

$$\text{Percent drug content} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

In vitro Dissolution Studies

Lawsone and the inclusion complexes were undergone *in vitro* dissolution using USP type 2 dissolution test apparatus. Inclusion complexes equivalent to 50 mg of Lawsone were placed in dissolution vessels containing 900 ml pH 7.4 phosphate buffer maintained at 37± 0.5°C and stirred at an rpm of 50 for 3 hr. At fixed time intervals 5 mL of aliquots were taken out and replaced with fresh buffer. The samples were filtered through Whatman filter paper and absorbance was determined spectrometrically at 453.2 nm using a UV-Visible spectrophotometer. Dissolution studies were performed in triplicate and calculated the mean values. At various time intervals, the % drug released was determined and plotted against time.[28]

Fourier- transform Infrared Spectroscopy (FTIR)

FTIR spectrums of Lawsone, physical mixture, and inclusion complexes were obtained by using FTIR spectrophotometer. The materials were combined 1:100

with IR grade potassium bromide, and pellets were made by pressing them in a Shimadzu hydrophilic press. The pellets were then scanned in an FTIR spectrometer between 4000 and 400 cm^{-1}.

DSC Analysis (differential scanning calorimetric)

Lawsone, beta-cyclodextrin, and co-precipitation technique preparation of inclusion complexes were subjected to DSC analysis using DSC analyzer (NETZSCH DSC 204F1 Phoenix). Under nitrogen atmosphere, samples were sealed in an aluminium pan and heated to a temperature of 20°C / 10 (K/minutes) / 300°C.

PXRD (Powder X-Ray Diffraction)

A BRUKER D8 ADVANCE powder diffractometer with Cu as anode material was used to record powder X-ray diffractograms at a voltage of 40 kV and a current of 35 mA. The samples were examined at a scan rate of 1° min^{-1} in the 2θ angle.

Nuclear magnetic resonance spectroscopy (NMR)

Determination of the ^{1}H NMR spectra of Lawsone, Beta-cyclodextrin, and inclusion complex were done using a 400 MHz Bruker AVANCE III NMR spectrometer.

Antibacterial Study

Bacterial Strains and Cultures

The study included four bacterial strains: *E. coli* and *P. aeruginosa*, which represent Gram-negative bacteria, and *S. aureus* and *B. subtilis*, which represent Gram-positive bacteria. 24 hr nutrient broth culture of all bacterial strains were used.

Colony Counting Method

The antibacterial activity of Lawsone and its inclusion complex was evaluated by the colony counting method. Bacterial cells of *E. coli*, *S. aureus, B. subtilis*, and *P. aeruginosa* were grown 48 hr on an incubator at 37 °C. Inoculum was suspended to get a density of 1×10^{8} colony forming units (CFU/ml) in pH 7.4 phosphate buffer by matching its turbidity with 0.5 Mc Farland turbidity standard (about 1×10^{8} CFU/ml). Then diluted to get 1×10^{6} CFU/ml. 150 mg of UV sterilized inclusion complexes and equivalent amount of Lawsone and beta-cyclodextrin were then immersed in bacterial suspension, and incubated at 37°C for 24 hr. Colonies were counted after the culture was put on a nutrient agar plate and incubated at 37°C overnight. The following equation was used to calculate the antibacterial activity of the IC:

$$\text{Antibacterial activity (\%)} = (A\text{-}B)/A \times 100$$

Where A represents the number of colonies (CFU/ml) in the control group and B represents the number of colonies following the addition of Lawsone/cyclodextrin combination.[29]

RESULTS

Phase Solubility Study

Figure 1 represents the phase solubility diagram of Lawsone in solutions of different concentrations of beta-cyclodextrin in water and the results are given in Table 1.

Percentage Yield

The percentage yields of the inclusion complexes were found to be 86.32%, 90.67%, and 96.10%, for kneading technique, co-precipitation technique, and physical mixture respectively.

Percentage Drug Content

The percentage drug contents of the inclusion complexes are given in the Table 2.

In vitro Dissolution Studies

The percentage drug release of Lawsone from inclusion complexes of kneading technique, co-precipitation

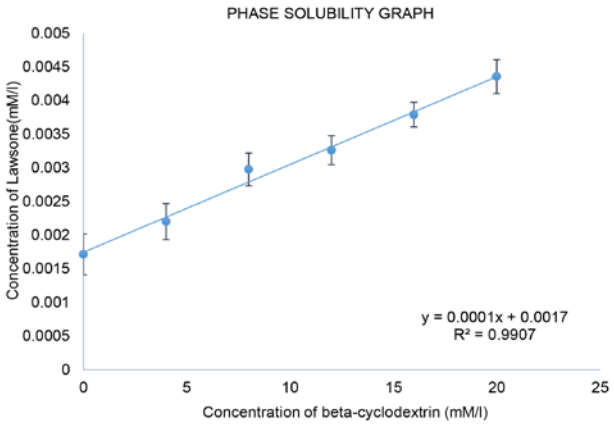

Figure 1: Phase solubility diagram.
MM - millimolar

Table 1: Phase solubility study.

Concentration of Beta-cyclodextrin (mm/l)	Concentration of lawsone (mm/l)
0	0.001714± 0.000304
4	0.002202± 0.000268
8	0.002978± 0.000243
12	0.003264± 0.000215
16	0.003792± 0.000184
20	0.004356± 0.000253

mM/l- millimolar per litre

Table 2: Drug content estimation.	
Method of preparation	**Drug content (%)**
Kneading method	87.71± 0.0375
Co-precipitation method	95.32± 0.0416
Physical mixture	66.08± 0.0901

%- Percentage

Table 3: *In vitro* dissolution studies.				
Time (min)	**Pure drug (% C.R)**	**physical mixture (% C.R)**	**kneading method (% C.R)**	**co-precipitation method (% C.R)**
5	19.052± 0.59	25.726± 1.79	70.215± 1.52	73.176± 1.79
10	28.053± 1.41	33.276± 2.02	78.767± 2.37	82.805± 2.33
15	36.419± 0.82	43.257± 1.24	82.623± 2.97	87.681± 1.25
30	44.988±0.16	52.416± 2.15	88.119± 1.63	90.759± 3.21
45	49.498± 0.68	62.819± 0.02	90.521± 0.79	93.354± 4.32
60	56.033± 2.07	72.244± 1.10	91.435± 0.13	93.808± 0.99
90	62.180± 1.11	76.222± 2.16	92.590± 0.11	95.144± 0.60
120	62.466± 0.45	77.354± 1.83	93.509± 0.20	96.099± 0.49
180	64.541± 0.53	78.171± 1.05	93.891± 0.98	97.221± 0.88

Min- minutes
% C.R – percentage cumulative release

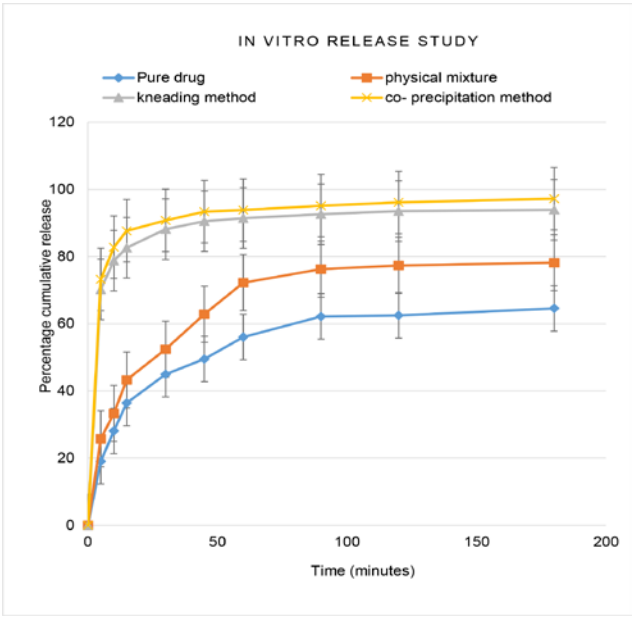

Figure 2: *In vitro* drug release study.

technique, physical mixture, and also from the pure drug are given in Table 3 and depicted in the Figure 2.

Fourier-transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of beta-cyclodextrin, Lawsone, and inclusion complexes are represented in Figures 3-7.

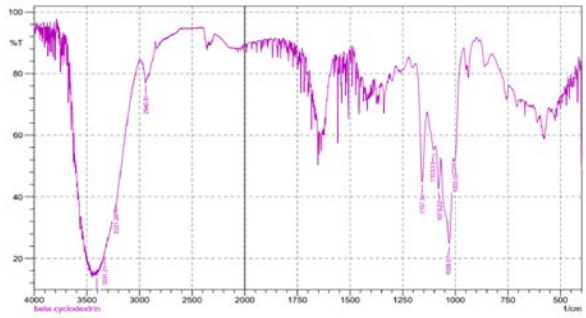

Figure 3: FTIR spectrum of beta-cyclodextrin.

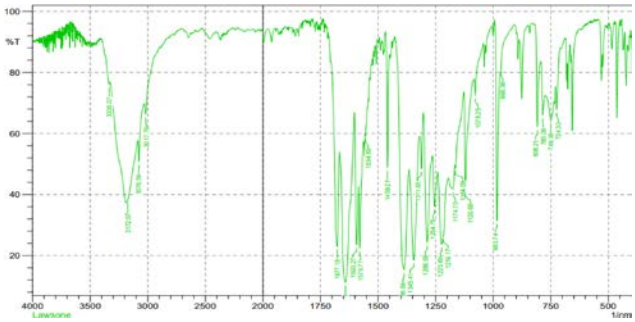

Figure 4: FTIR spectrum of Lawsone.

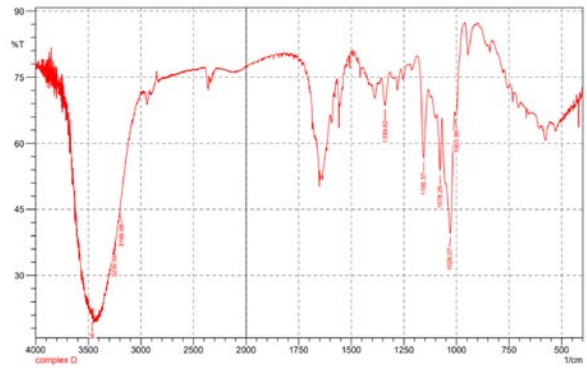

Figure 5: FTIR spectrum of physical mixture.

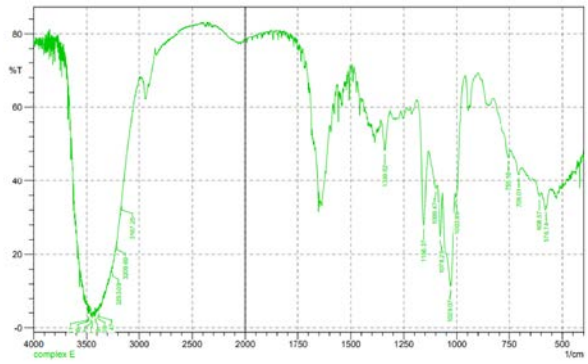

Figure 6: FTIR spectrum of inclusion complexes prepared by kneading method.

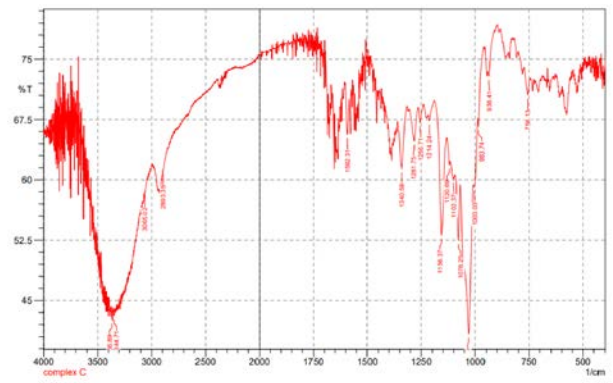

Figure 7: FTIR spectrum of inclusion complexes prepared by co-precipitation method.

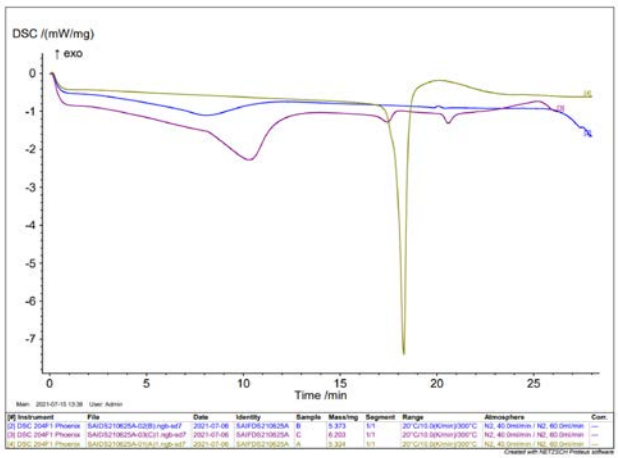

Figure 8: DSC of Lawsone (4), Beta- cyclodextrin (2) and Inclusion complex prepared by co-precipitation method (3).

DSC Analysis (differential scanning calorimetric)

The endothermic peaks of Lawsone, beta-cyclodextrin, and IC are represented in Figure 8. The endothermic peak for Lawsone is observed at 202.9°C. The peak of beta-cyclodextrin is observed at 100.8°C. IC formed by co-precipitation technique showed peaks at 123.3°C, 194.4°C and 225.8°C.

PXRD (Powder X-Ray Diffraction)

The PXRD patterns of Lawsone, beta-cyclodextrin, and Lawsone: beta-cyclodextrin systems are represented in Figures 9-11 respectively. The diffractograms of Lawsone and beta-cyclodextrin exhibited intense peaks, which indicate the crystalline nature. X-ray diffraction of Lawsone: beta-cyclodextrin at 1:1 molar ratio has shown peaks with less intensity compared to peaks in Lawsone and beta-cyclodextrin indicating that the complexation has been confirmed.

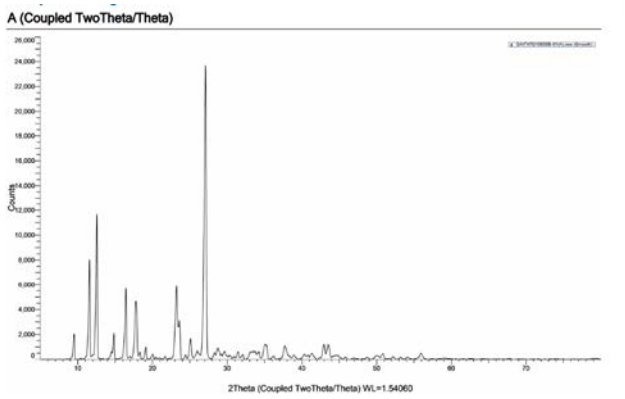

Figure 9: X-ray diffraction pattern of Lawsone.

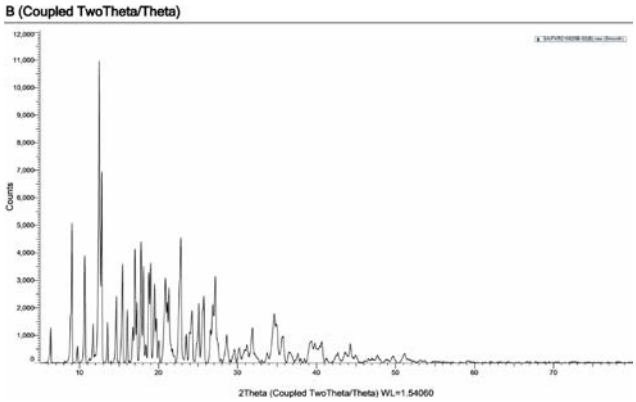

Figure 10: X-ray diffraction pattern of beta-cyclodextrin.

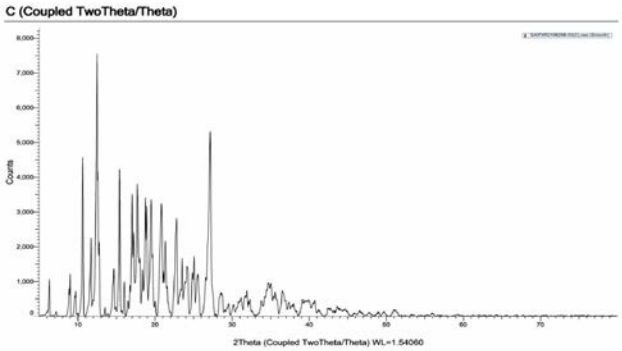

Figure 11: X-ray diffraction pattern of Inclusion complex prepared by co-precipitation method.

Nuclear Magnetic Resonance Spectroscopy (NMR)

Figures 12-14 are the ^{1}H-NMR spectrums of the characteristic peaks of Lawsone, beta-cyclodextrin, and inclusion complex respectively.

Antibacterial Study

The comparison of antibacterial activity of IC and pure Lawsone is represented in log CFU/ml and is depicted

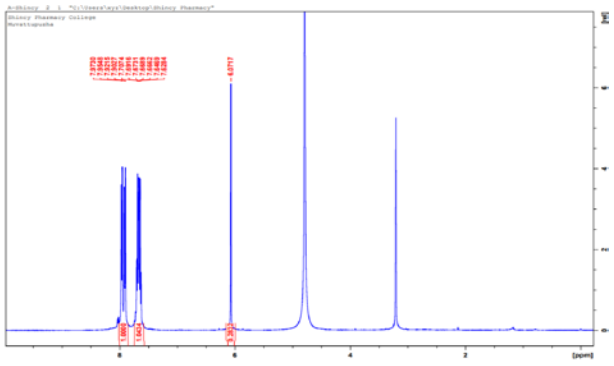

Figure 12: NMR spectrum of Lawsone.

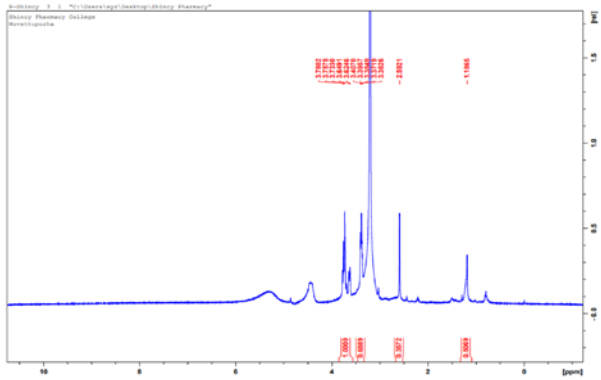

Figure 13: NMR spectrum of beta-cyclodextrin.

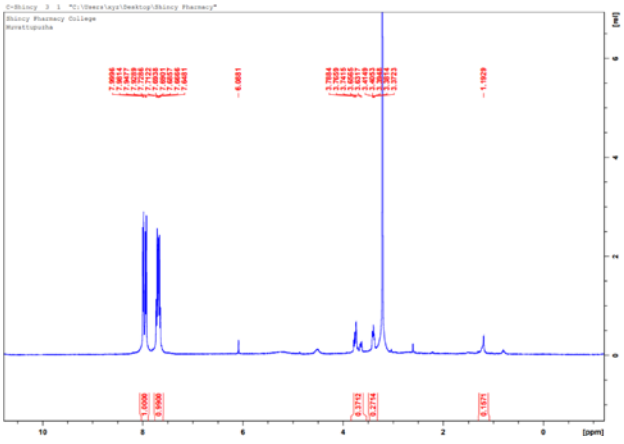

Figure 14: NMR spectrum of inclusion complex prepared by co-precipitation method.

in the Figure 15 and percent antibacterial activity is also calculated and given in Table 4.

DISCUSSION

According to Higuchi and Connors (Higuchi and Connors, 1965), the phase solubility profile of Lawsone with beta-cyclodextrin was classified as Higuchi's A_L

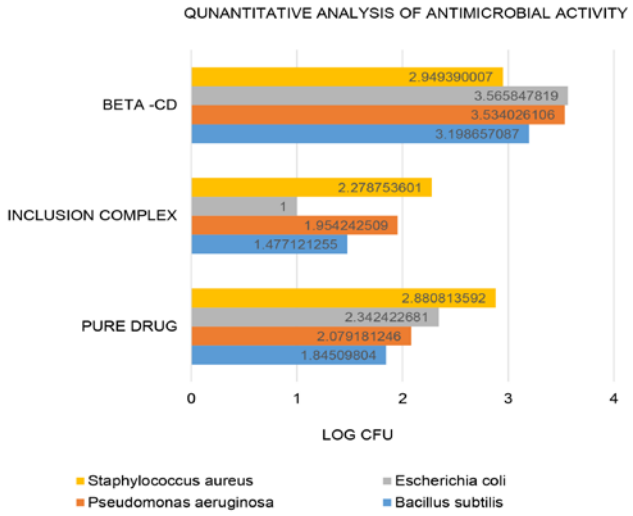

Figure 15: Antibacterial study.

Table 4: Percent antibacterial activity.

Organisms	Percent antibacterial activity (%)
Bacillus subtilis	57.14
Pseudomonas aeruginosa	25
Escherichia coli	95.45
Staphylococcus aureus	75

%- Percentage

type. In this, an obvious linear relationship could be found between water solubility of Lawsone and molarity of beta-cyclodextrin in aqueous solution till the highest beta-cyclodextrin concentration of 20 mM/l. So the A_L type configuration indicated that the molar ratio of Lawsone to the beta-cyclodextrin was 1:1.[20,21,28,30]

The IC of Lawsone with beta-cyclodextrin could produce significant improvement in the solubility of Lawsone. The time to release 60% of Lawsone was 90 min for pure drug, but it takes only 5 min for IC of kneading method. In 5 min 70% drug released in IC of co-precipitation. The physical mixture showed 60% drug release at 45 min. IC of co-precipitation method exhibited 90% drug release within 30 min. IC of kneading method exhibited t_{90} value for Lawsone as 45 min. So the result showed that the enhancement in the dissolution rate is related to the complexation process. The complexes could achieve improvement in the wettability of Lawsone due to the high aqueous solubility. At 180 min the drug releases were 64%, 78%, 93% and 97% for pure drug, physical mixture, IC of kneading method and co-precipitation technique respectively.

In FTIR spectra, the peaks of beta-cyclodextrin (Figure 3) were recorded at 3331 cm^{-1}, 3221 cm^{-1} (O-H stretching), 1157 cm^{-1}, 1079 cm^{-1}, 1029 cm^{-1}, (C- O- C stretch), 1103 cm^{-1} (C-C-C bending).[31-32] The IR spectra of Lawsone (Figure 4) showed the characteristic of the stretching vibration of H-bonded hydroxyl of phenol at 3335 $cm^{-1}$and 3172 cm^{-1}. Lawsone showed peaks at 1677 cm^{-1} and 1641 cm^{-1} due to vibration of benzene ring skeleton. Peaks at 983, 966, and 808 cm^{-1} were due to the bending vibration of the C–H bond. Peaks at 1459, 1345, and 1386 cm^{-1} were attributed to the C=C of the naphthalene ring. The small peaks at 3017 and 3076 cm^{-1} were due to an aliphatic and aromatic C–H group. Peaks at 1593 and 1579 cm^{-1} were due to the stretching vibration of C=O and C=C bonds.[33-36] Physical mixture (Figure 5) shows peaks at 1156, 1078, 1029, 3463, 3239, 3199, 1339 and 1003 cm^{-1}. Physical mixture shows shift in 1345 cm^{-1} to 1339 $cm^{-1}$and peaks at 3076, 3172, 3335 cm^{-1} shift to 3199, 3239, 3463 cm^{-1}. FTIR spectra of IC of kneading method (Figure 6) shows peaks at 1029, 1156, 576, 608, 705, 755, 1003, 1078, 1099, 1339, 3167, 3209, 3253 cm^{-1}. IC of kneading technique showed a shift in 1345 cm^{-1} to 1339 cm^{-1} and peaks at 3076, 3172, 3335 cm^{-1} shifted to 3167, 3209, 3253 cm^{-1}. FTIR spectra of complexes prepared by co-precipitation method shows peaks at 3366, 3065, 3344, 1592, 1340, 1281, 1156, 1078, 1030, 983, 938 and 756 cm^{-1}. IC of co-precipitation method (Figure 7) showed a shift in peaks at 1345 cm^{-1} to 1340 cm^{-1} and peaks at 3076, 3172, 3335 cm^{-1} shifted to 3065, 3344, 3366 cm^{-1}. The spectra of complexes showed changes in the position of peaks and a significant decrease in intensity when compared to the spectra of pure Lawsone. The peaks representing the vibration of the benzene ring skeleton and the C=C of the naphthalene ring in Lawsone were absent in the spectra of IC. This indicates an encapsulation of the naphthoquinone ring of Lawsone in the beta-cyclodextrin cavity.

DSC is a thermodynamical instrument that can be used to measure the direct heat energy intake that occurs in a sample during a controlled drop or increase in temperature, producing exothermic and endothermic peaks. It is used to identify changes in phase transitions.[37] In DSC spectra (Figure 8), Lawsone shows a broad endothermal peak at its melting point was 202.9°C. Beta-cyclodextrin shows an endothermal peak at 100.8°C corresponding to its melting point. Inclusion complexes prepared by co-precipitation technique showed three endothermic peaks at 123.3°C, 194.4°C and 225.8°C. The characteristic endothermic peak of Lawsone has disappeared in the IC which indicates that the Lawsone may be encapsulated in the beta-cyclodextrin.

X-Ray diffraction is used for the measurement of crystalline and non-crystalline materials to gather information relating to the structure of a compound. It is an important technique for the structure determination of crystalline compounds because it can reveal the information about the atomic arrangement in crystal.[38] PXRD indicates the formulation of the new crystalline phase by co-precipitation method. The results showed a remarkable decrease in crystallinity. The diffractograms of Lawsone (Figure 9) and beta-cyclodextrin (Figure 10) displayed high peaks, indicating that it was crystalline. The peaks observed at 27.107°, 12.545°, 11.569°, 23.221°, 16.442°, 17.769° for pure Lawsone and 12.490°, 12.808°, 9.030°, 22.816°, 17.784°, 17.019°, 10.652°, 19.002°, 18.084° in beta-cyclodextrin. Peaks at 12.464°, 27.169°, 10.634°, 15.402°, 17.727°, 17.033°, 19.498°, 20.836°, 22.786°, 17.223°, 11.691° observed for inclusion complex by co-precipitation method. The peaks observed for IC (Figure 11) were less intense and showed a shift in comparison with pure drug. There was a reduction in crystallinity of Lawsone in comparison to the sample. This shows Lawsone is dispersed in beta-cyclodextrin.

The ^{1}H-NMR spectrum of Lawsone (Figure 12) shows the whole spectrum 1-10 ppm and the spectrum in the 6-8 ppm range indicating Lawsone. The peaks at Lawsone spectrum in the 6-8 ppm range indicate H-3, H-8, H-9, H-7, H-10 aromatic protons.[39] The intensity of peak of aromatic protons at 7.5 - 8 ppm of Lawsone (Figure 12) was reduced in inclusion complex (Figure 14). The peaks at 6 ppm of Lawsone is almost disappeared in the inclusion complex (Figure 14). Similarly peak present between 2 - 3 ppm of beta-cyclodextrin (Figure 13) is absent in inclusion complex (Figure 14). The larger peaks present between 2 - 4 ppm of beta-cyclodextrin (Figure 13) have no change in the inclusion complex (Figure 14). The NMR spectra of Lawsone, beta-cyclodextrin, and its inclusion complex showed that there is no noticeable shifts. Although, there is a slight downfield shift of the aromatic protons peaks of Lawsone at 7.62 - 7.97 ppm to 7.64 - 7.99 ppm in the inclusion complex. The shift and reduction in the intensity of peaks confirm the complexation of drug.[40-41]

The antibacterial activity was calculated in log CFU/ml units (Figure 15). The growth inhibition was in the range from 1 to 2.27 log CFU/ml in IC and 1.84 to 2.88 log CFU/ml in pure drug. Relative to the population in control (pure drug), a population reduction occurred in the case of inclusion complexes. The values are 1, 1.47, 1.97, 2.27 log CFU/ml for *E. coli, B. subtilis, P. aeruginosa* and *S. aureus* respectively. IC always

shows more antibacterial activity than pure drug. The complexes of Lawsone exhibited more reduction in the viability of all bacterial strains in comparison with pure Lawsone. Among the various strains tested, IC shows the most pronounced antibacterial activity against *E. coli*. The pure drug exhibited pronounced antibacterial activity against *B. subtilis*. The order of antibacterial activity in IC was *E. coli* > *B. subtilis* > *P. aeruginosa* > *S. aureus* and in case of pure drug it was *B. subtilis* > *P. aeruginosa* > *E. coli* > *S. aureus*. Pure Lawsone and IC has least antibacterial in *S. aureus*. IC shows more than 1 log reduction when it comes to *E. coli* as compared to Lawsone. The results indicated that the IC exhibited a satisfactory antibacterial effect in all bacterial strains. There is an enhancement in antibacterial activity after complexation with beta-cyclodextrin. So this may be due to the enhanced aqueous solubility of Lawsone after complexation.

CONCLUSION

From the phase solubility study conducted the molar ratio was found to be 1:1 according to Higuchi's A_L type. IC of Lawsone was prepared by different methods and the complex formation was confirmed by FTIR, DSC, PXRD, and NMR. After *in vitro* studies, complexes prepared by the co-precipitation technique were selected because of their highest dissolution characteristics. Also, antimicrobial studies showed that IC has pronounced antibacterial activity compared to pure Lawsone.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

IC: inclusion complex; **°C:** Degree centigrade; **min:** minutes; **α:** Alpha; **β:** Beta; **γ:** Gamma; **ml:** Milliliter; **mM/l:** Millimole per liter; **µg:** Microgram; **mg:** Milligram; **rpm:** Revolutions per minute; **min:** Minute; **nm:** Nanometer; **cm:** Centimeter; **kV:** Kilovolt; CFU/ **ml:** Colony-forming unit per milliliter; **CD:** Cyclodextrin; **log:** Logarithm; **mA:** Milliampere.

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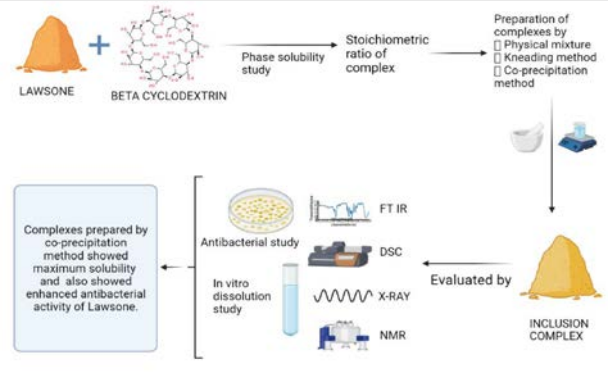
PICTORIAL ABSTRACT



SUMMARY

From the phase solubility study conducted the molar ratio was found to be 1:1 which is according to Higuchi's AL type. IC of Lawsone was prepared by different methods and the complex formation was confirmed by FTIR, DSC, PXRD, and NMR. After in vitro studies, complexes prepared by the co-precipitation technique were selected because of their highest dissolution characteristics. Also, antimicrobial studies showed that IC has pronounced antibacterial activity compared to pure Lawsone.

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