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Development and validation of a discriminative dissolution medium for poorly soluble nutraceutical tetrahydrocurcumin

Habibur Rahman Shiek Abdul Kadhar Mohamed Ebrahim[1], Telny thomas Chungath[2], Karthik Sridhar[1], Karthik Siram[1], MANOGARAN Elumalai[3], hariprasad ranganathan[1], Sivaselvakumar Muthusamy[3]
[1]PSG College of Pharmacy, Coimbatore, India 641004.
[2]Chemists College of Pharmaceutical Sciences and Research, Ernakulum, India,
[3]Faculty of Pharmaceutical Sciences, UCSI University, Kuala Lumpur, Malaysia 56000

Corresponding Author Information
Habibur Rahman Shiek Abdul Kadhar Mohamed Ebrahim
09894229078
hablet1@gmail.com
0000-0003-2609-4677
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Abstract
INTRODUCTION: The present study was aimed to develop and validate a discriminative dissolution method for tetrahydrocurcumin, a BCS class II drug, by simple UV spectrophotometric analysis. The final dissolution medium composition was selected based on the solubility and stability criteria of the drug.
METHODS: As a prerequisite for this, the solubility of the drug was assessed in media of different pH (1.2 to 7.4) and surfactant concentrations of 0.5 to 1.5% (w/v) sodium lauryl sulphate (SLS) in water and pH 7.4 phosphate buffer. The dissolved drug concentration in each medium was quantified by UV analysis at 280 nm wavelength.
RESULTS: The drug solubility was found to be high at a pH of 1.2 and 7.4. The media with surfactant enhanced solubility of the drug by approximately 17-fold and exhibited better sink conditions. The discriminative power of the developed dissolution medium (i.e. 1% w/v SLS in pH 7.4) was determined by performing the in vitro dissolution studies of the prepared tetrahydrocurcumin tablets and comparing their release profiles using fit factors (f1 and f2). The results of the fit factor comparisons made between the dissolution profiles of THC tablets proved the discriminative ability of the medium. The validation of the developed dissolution method was performed by international guidelines and the method showed specificity, linearity, accuracy and precision within the acceptable range.
DISCUSSION AND CONCLUSION: The proposed dissolution method was found to be adequate for the routine quality control analysis of tetrahydrocurcumin as there is no specified dissolution method for the drug in the pharmacopoeia.
Keywords: Tetrahydrocurcumin, Sodium lauryl sulphate, solubility, dissolution medium, dissolution comparison, fit factors

INTRODUCTION

In recent years, significant attention has been paid to nutraceuticals, as they are advantageous over the synthetic drugs in possessing many pharmacological actions with little or no toxic

effects. But the bioavailability of nutraceuticals is often compromised due to their poor aqueous solubility. Many formulation methods have been proposed to enhance the solubility and bioavailability of these hydrophobic entities.[1] Dissolution testing is an important quality control tool to identify the effects of manufacturing variability on product performance and to ensure batch to batch equivalence.[2] In this context, there is a utmost need for a suitable and validated dissolution method for analyzing the nutraceutical formulations which are in the pipeline of development.

The choice of medium for the dissolution studies of class II drugs of Biopharmaceutical classification system (BCS) is critical and developing a suitable dissolution media has always been a challenge because of the hydrophobicity exhibited by the drugs of this class. The solubility factor of a drug should be given paramount importance while designing a dissolution method. The medium used should be able to homogenously solubilise the drug for accurate quantification of released amount from the total drug dose of the dosage form at each time interval. On the other hand, solubility of a drug is directly or indirectly dependent on many variables like pH of the medium, temperature, log P, pK_a and ionic behaviour of the drug.[3] Maintaining of large aqueous sink conditions, alteration of pH and addition of co-solvents or surfactants are few approaches that have been adopted in the development of dissolution medium for the poorly soluble drugs.[4] Addition of surfactants is preferred widely as they enhance the solubility of hydrophobic drugs by reducing the surface tension of the medium, increasing the wetting of the drug and by micellar solubilisation (above CMC) in the media. Although bile salts in the GI system acts as the surfactants that help in solubilisation of the drug in vivo, use of these bile salts externally for routine in vitro analysis is not possible as they are expensive. Hence, the use of surfactants in the dissolution medium can serve well as a reliable alternative to mimic the GI conditions.[5] Among the different surfactants, anionic surfactant like sodium lauryl sulphate[6-8] and non-ionic surfactant like polysorbate 80were commonly used by the researchers.[9,10]

Moreover, the medium chosen to perform the in vitro dissolution studies of the drug formulations should be discriminative enough to differentiate drug release patterns and help in identifying the formulation and process variables that affect the release of the drug from the dosage form during the initial stages of product development.[11] For this purpose, the in vitro dissolution profiles of different batches of the formulation are often compared to understand the similarities or differences in the release pattern. Various statistical methods like ANOVA, model-dependent and model-independent approaches are being adopted to compare the dissolution profiles.[12,13] Determination of fit factors which includes difference factor *(f1)* and similarity factor *(f2)* is one of the effective and feasible model-independent methods of dissolution comparison. The FDA also recommends the use of these fit factors for dissolution comparison in the guides for industry.[14-16]

The natural herb curcumin ((1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) falls under the category of nutraceuticals and tetrahydrocurcumin (THC, 1,7-Bis(4-Hydroxy-3-Methoxyphenyl)Heptane-3,5-Dione) is one of the active metabolites of curcumin that exhibits similar pharmacological actions as that of its parent molecule curcumin.[17] THC showed potent anti-inflammatory and antioxidant effect while compared to other curcuminoids.[18] THC able to inhibit amyloid precursor protein based on the QSAR studies and has the potential to be utilized in treating Alzheimer's disease.[19] The bioavailability of THC is limited due to its poor solubility and absorption. The aqueous solubility of these drugs is low and hence classified into class II of BCS.[20] The role of pH and surfactants in the dissolution of curcumin was previously studied by Rahman et al., 2009.[21] In the present study, an attempt was made to develop a suitable dissolution medium for in vitro analysis of THC by employing a surfactant to enhance its solubility, and to maintain the sink conditions.

To the best of our knowledge, no previous study on the dissolution medium development for THC has been done. The solubility of THC in different pH media and in the presence of anionic surfactant sodium lauryl sulphate (SLS) was studied and the effect of different media on the solubility of the drug was assessed. The discriminative power of the developed dissolution medium was determined by comparing the dissolution profiles of the prepared THC tablets using fit factors (*f1* and *f2*).

MATERIALS AND METHODS

Materials

Tetrahydrocurcumin (THC) was a gift sample from Sami Labs (Bangalore, India). Sodium lauryl sulphate (SLS), Disodium hydrogen phosphate, Sodium dihydrogen phosphate, Sodium acetate trihydrate, hydrochloric acid and glacial acetic acid were purchased from Himedia (Mumbai, India). Microcrystalline cellulose (MCC, Avicel PH 102, FMC biopolymer, USA) and polyvinyl pyrrolidone (Povidone K 30, BASF, Ludwigshafen, Germany) were purchased from Signet chemical corporation Pvt. Ltd. (Mumbai, India). Sodium starch glycolate (SSG), magnesium stearate and talc were purchased from Amishi drugs and chemicals Pvt. Ltd. (Ahmedabad, India).

Media preparation for dissolution studies

The buffer solution of pH 1.2 (0.1 N Hydrochloric acid (HCl)) was prepared by dissolving 8.5 mL of concentrated HCl in 1000 ml of purified water. The acetate buffer of pH 4.0 was prepared by transferring 362 mL of 0.2 M glacial acetic acid and 148 mL of 0.2 M sodium acetate solution into a volumetric flask and the volume was made up to 1000 mL with purified water. For the preparation of phosphate buffers, 0.2 M sodium dihydrogen phosphate (NaH_2PO_4) and 0.2 M disodium hydrogen phosphate (Na_2HPO_4) solutions were prepared initially. Then 255 mL of 0.2 M NaH_2PO_4 + 245 mL of 0.2 M Na_2HPO_4 and 100 mL of 0.2 M NaH_2PO_4 + 400 mL of 0.2 M Na_2HPO_4 were mixed to obtain buffer solutions of pH 6.8 and 7.4 respectively. The pH of the solutions was adjusted with NaOH or acetic acid and finally, volume was made up to 1000 mL with purified water.

Formulation of THC tablets by direct compression method

THC tablets were prepared in our lab since no commercial THC tablets are available in the market yet. The tablets were prepared using microcrystalline cellulose (MCC, Avicel PH 102) as filler, polyvinyl pyrrolidone (Povidone K-30) as binder and sodium starch glycolate (SSG) as a disintegrating agent. Magnesium stearate and talc were used as antiadhesive agent and glidant. Three different tablet batches; TF1, TF2 and TF3 with three different concentrations of binder (5%, 7.5% and 10% w/w) were formulated to determine the effect of binding strength on the drug release and also to evaluate the discriminative ability of the dissolution medium in identifying the formulation changes among the three tablet batches. The amount of the drug was equivalent to 100 mg in all the tablets and the total tablet weight was 200 mg. (Table 1)

Before preparing the tableting mixtures all the materials were sieved manually through a sieve with a mesh size of 0.595 mm. The weighed quantities of the drug, filler, disintegrant and binder were mixed in a poly bag for 10 min. Finally, magnesium stearate and talc were added to the initial mixture and blended for 3 min. 100 tablets from each batch with 8.7 mm diameter and 4.6 mm thickness were produced by direct compression method using Rimek minipress II (Karnavati engineering Ltd., Ahmedabad, India). All tablets were compressed with a constant compression pressure of 12 kN.

Determination of mechanical strength of the tablet

The mechanical strength or crushing strength of the prepared tablets was determined using tablet testing apparatus (Model DHT-250, THERMONIK, Campbell electronics, Mumbai, India).

Determination of Critical Micellar Concentration

The micelle formation is an intrinsic property of a surfactant which plays a major role in the solubilisation of the drug. The two generally accepted models explaining the aggregation of surfactant monomers into micelles are the "mass-action model" and the "phase-separation model", and the concentration of surfactant at which the micelles are formed (CMC) is dependent on many factors.[22,23] The CMC of sodium lauryl sulphate in water and pH 7.4 phosphate buffers was determined by dye solubilisation and drop number methods prior to the solubility studies of the drug in these media. In the former method, eosin Y was added to various concentrations of SLS (0.25 - 2% w/v) prepared in water and pH 7.4 phosphate buffers and shaken for 24 hr at 37° C. The residue was filtered and the absorbance of the resultant solutions was measured at 542 nm (λ_{max} of eosin Y) using UV-Visible spectrophotometer (Shimadzu1650PC, Tokyo, Japan). The obtained absorbance values were plotted against the concentrations of surfactant expressed in millimoles (0.009 to 0.069 mM/ml equivalent to 0.25 to 2% w/v).[24] The surface tension of the surfactant solutions prepared in water and pH 7.4 phosphate buffers was also determined by drop number method using the following equation

$$\frac{\gamma_l}{\gamma_w} = \frac{n_w}{n_l} \cdot \frac{d_l}{d_w}$$

Where γ_l and γ_w are surface tensions of the liquid in test and water (71.97 dynes/cm). n_w and n_l are number of drop counts for water and liquid. d_l and d_w are densities of liquid and water. A plot of surface tension versus log concentrations of surfactant in millimoles (-2.665 to -1.158 mM/ml equivalent to 0.25 to 2% w/v) was made to determine the point of micelle formation in both the media.[25]

Solubility study of pure THC

To assess the role of pH and the surfactant in the solubility of THC, the equilibrium solubility of the drug was determined in dissolution media of different pH (1.2 to 7.4) and 0.5-1.5% (w/v) SLS in water (pH 5.5) and phosphate buffer (pH 7.4). Excess of drug (approximately 50 mg) was poured into 20 ml of each of the medium and were kept over the shaker at 37° c and shaken for 2 hr. Then, 2ml of samples were withdrawn at the end of 1 and 2 hr, filtered (0.45µ pore size cellulose esters membrane filter, Millipore Corp., Billerica, MA, USA) and diluted in methanol. The concentration of the dissolved drug in each medium was determined by measuring the absorbance and correlating with the standard concentration curve of THC built using UV-Visible spectrophotometer (Shimadzu1650PC, Tokyo, Japan) at a corresponding wavelength of 280nm (λ_{max} of THC). The solubility parameter was kept as one of the main criteria for selecting the composition of the dissolution medium.

Dissolution method validation for the analysis of THC tablets

The developed dissolution method was validated by evaluating specificity, accuracy, precision, linearity, filter suitability and stability.

Specificity

Specificity test was done to demonstrate the absence of interference of excipients or dissolution medium with the drug response at 280 nm. For this purpose, the solutions of placebo (excipients without drug), placebo with drug and reference standard was prepared and analysed in UV spectrophotometer (Shimadzu1650PC, Tokyo, Japan).

Accuracy and precision

The accuracy of the method was determined by the recovery test of known amount of THC added to the placebo. An initial stock solution was prepared in methanol and the final concentrations of 4.8, 6 and 7.2 µg ml^{-1} in dissolution medium were obtained corresponding

to 80%, 100% and 120% of the nominal assay concentration. The samples were analysed in triplicate on different days. The same solutions were used in evaluating the precision of the method. Repeatability and inter day precision were evaluated based on the relative standard deviation (RSD) of the results.

Linearity

Aliquot of a stock solution containing 100 µg/ml of THC was prepared in methanol. It was then transferred into 25 ml volumetric flask and diluted with dissolution medium to obtain final concentrations of 2, 4, 6, 8 and 10 µg ml^{-1} and analyzed in UV spectrophotometer (Shimadzu1650PC, Tokyo, Japan). The solutions were analysed in triplicate and the linearity was evaluated by linear regression analysis and calculated by least square regression method and analysis of variance (ANOVA).

Filter suitability

Generally, the filters used in the dissolution sample preparation must be evaluated to verify that it does not adsorb the drug and is adequate enough to filter the excipients that would otherwise interfere with the drug analysis. The standard and sample drug solutions with a final concentration of 6 µg ml^{-1} prepared in the dissolution medium were used to carry out the test. The sample solutions were prepared using the placebo. Both reference and sample solutions were subjected to filtration through 0.45µ pore size cellulose esters membrane filter (Millipore Corp., Billerica, MA, USA) and recovery of the drug after passing through the filters was assessed. For a filter to be acceptable the recovery must be within 98-102% range.[26,27]

Standard and sample solution stability

The stability of THC in the dissolution medium was evaluated to demonstrate that the drug solution created during the dissolution test is stable over the period of dissolution and analysis of the samples. For this purpose both standard and sample were subjected to dissolution in the developed dissolution medium at 37±0.5 ºC for 2 hr to obtain a final concentration of 10 µg ml^{-1} solution. The samples were assayed at 0 hr at room temperature (25±2 ºC) and after 24 and 48 hr at both room and refrigerator (8±2 ºC). The assay was performed in triplicate and observed for any change in the absorbance values as indicative of degradation of the drug. The acceptable assay range to confirm the sample and standard stability is 98 % to 102 %.[28]

In vitro dissolution study of pure THC and tablet formulations

The dissolution of pure THC and the in vitro release studies of the prepared THC tablets in the developed dissolution medium were performed using USP II rotating paddle apparatus (Electrolab TDT-08L, Mumbai, India). The dissolution study of pure drug was carried out by filling 100mg of pure THC into the hard gelatin capsules (capsule size 2) and allowing it to sink in each of the dissolution medium with the help of sinkers. The dissolution flask was filled with 900 ml of each dissolution medium and the temperature was maintained at 37ºc. The speed of paddles was set at 50 rpm, and the distance between the paddle and bottom of the flask was 25 mm. The same conditions were maintained to study the dissolution of prepared THC tablets in the developed dissolution medium. Six tablets of uniform weight were chosen from each batch (TF1, TF2 and TF3) and the study was carried out for 2 hours. The samples were collected at 5, 10, 15, 30, 45, 60, 90 and 120 min. 5 ml of sample was withdrawn at each time interval and replaced with fresh dissolution medium to maintain the sink condition. The collected samples were filtered (0.45µ pore size cellulose esters membrane filter, Millipore Corp., Billerica, MA, USA) and the amount of drug released, and the cumulative percentage of drug released were determined by UV spectrophotometric analysis at 280 nm. The method is repeated for three times (n=3).

Discriminating test on developed dissolution medium

The discriminative power of the developed dissolution medium was determined by comparing the dissolution profiles of the prepared THC tablets. Model independent approach of dissolution comparison was used to differentiate the in vitro release profiles of the tablets. The difference factor (f1) and similarity factor (f2) were determined using the following equations[29]

Difference factor *(f1)* = $\frac{\sum_{t=1}^{n}(R_t - T_t)}{\sum R_t} \times 100$

Similarity factor (*f2*) = $50 \log\{[\,1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2]^{-0.5} \times 100\}$

Where *n* is the number of time points and R_t and T_t are the dissolution values of the reference and test formulations at time *t*.

RESULTS

Determination of mechanical strength of the tablet

The hardness of the tablets increased with increase in binder concentration due to increased interparticular bonding. The tablets with lowest binder concentration (5% w/w) exhibited an average crushing strength of 31.30 N, whereas those of intermediate (7.5% w/w) and highest (10% w/w) concentrations exhibited 46.06 N and 61.74 N respectively.

Determination of critical micellar concentration

From the dye solubilisation study, it was observed that the solubility of the dye gradually increased below CMC and a sharp increase was observed at 0.017 mM (0.5% w/v) of SLS in water media whereas, the maximum solubility of the dye was at 0.035 mM (1% w/v) of SLS in pH 7.4 buffer (Figure. 1A). The concentration of the surfactant showing maximum solubility of the dye indicated CMC. The surfactants tend to reduce the surface tension of the solutions. The surface tensions of the aqueous and buffer solutions gradually decreased with increasing concentration of SLS below CMC. A sudden drop in surface tension was seen at -1.76 mM (0.5% w/v) in aqueous solution. On the other hand, this drop in surface tension was observed at -1.46 mM (1% w/v) of SLS in phosphate buffer of pH 7.4, indicating the CMC (Figure. 1B).

Solubility study of pure THC

The solubility of the drug was high at acidic pH of 1.2 (113.4±3.41 µg/ml) and basic pH of 7.4 (88.54±4.5 µg/ml) and the drug exhibited less solubility at intermediate pH of 6.8 which was only 66.7±5.05 µg/ml after 2 hr of study (Figure. 2a). A previous stability study on curcumin and its metabolites in various pH conditions indicated that THC was more stable in basic pH conditions than acidic.[30-32] On basis of the available stability information, further evaluation of drug dissolution in presence of surfactants was carried out in phosphate buffer of pH 7.4, though the solubility of drug seems to be slightly higher in acidic pH of 1.2 than pH 7.4. A substantial increase in solubility of the drug was observed when tested in media with surfactant. The dissolution of drug increased with increase in surfactant concentrations in both media e.g. from 617.67± 6.55 to 808.33± 5.31 µg ml^{-1}, 933.0±4.08 to 998.33± 6.94 µg ml^{-1} and 1746.33± 5.31 to 1944.70± 4.92 µg ml^{-1} in water, and from 589.33± 4.50 to 714.00± 5.35 µg ml^{-1}, 1228.33± 4.92 to 1271.67± 5.73 µg ml^{-1} and 1421.00± 5.35 to 1572.33± 5.79 µg ml^{-1} in pH 7.4 PBS (Figure.2b). The high initial solubility of THC at 0.5% w/v SLS in aqueous surfactant medium relative to pH 7.4 PB was due to the micelle formation and attainment of CMC at low levels of surfactant in water. Whereas, CMC of SLS in pH 7.4 PB was attained only at 1% w/v, that led to high solubilisation of the drug at this surfactant concentration in phosphate buffer than water. The increase in solubility of the drug above CMC in both the media is due to increase in the micelle aggregation with an increase in the amounts of surfactant (20). From the experimentally determined CMC and solubility data, it was observed that the CMC of SLS was reached below 1.5%. Thus, 1% SLS in pH 7.4 PB

was chosen as a suitable dissolution medium. The results are subjected to statistical analysis of ANOVA and standard error is incorporated in the solubility graph. (Figure 2)

Dissolution method validation

Specificity

The specificity test demonstrated that there was no interference of the excipients and the dissolution medium in the quantitative determination of THC at 280 nm. There was no change in the UV spectrum of the drug in presence of the excipients used for the tablet preparation. This indicates that the developed UV spectrophotometric method can be used for the assay of THC in the dissolution studies.

Accuracy and precision

The accuracy of the method is evaluated by the recovery of the known amount of drug added to the placebo. The recommended recoveries in the dissolution tests should be in the range of 95 to 105 %. The mean recoveries of the drug for three different concentrations on different days were in the range 99.50 to 99.78 %, which demonstrated the accuracy of the method. The recovery results are presented in the Table 2. The interday and intraday precisions were also evaluated for three different concentrations (4.8, 6.0, 7.2 μg ml^{-1}) in two days. For a method to be precise, the RSD should be low i.e. ≤ 2%. From the results, the RSD was found to be less than 2%, which demonstrated good precision of the method (Table 3).

Linearity

The linearity of the THC calibration curve for the concentration range of 2 to 10 μg ml^{-1} was evaluated by least square regression method and analysis by ANOVA. The results showed good linearity and the regression equation obtained was $y = 0.031x+0.028$. The correlation coefficient was found to be 0.9996. The analysis by ANOVA showed significant linear regression ($p < 0.05$) and no significant deviation from the linearity.

Filter suitability

The average percentage recoveries of the standard and sample solutions after filtration were found to be within the range of 98-102%. This showed the absence of any interference of the filter used in the analysis of the drug and that the filter used is suitable for the routine sample preparation in the dissolution test.

Standard and sample solution stability

The stability test of both standard and sample solutions showed that the drug is stable in the dissolution medium for a period of 48 hr at both 25±2 ºC (room temperature) and 8±2 ºC (refrigerated conditions). This indicated that the drug did not form any degradation products in the dissolution medium (1% SLS in pH 7.4 PBS). The results of the stability test are shown in the Table 2.

In vitro release study of pure THC and tablet formulations

The dissolution of pure THC in different dissolution media was found to be solubility dependent. The cumulative percentage of drug that released in pH 1.2, 4.0, 6.8 and 7.4 was 40±1.8, 30±4.7, 23.8±2.67 and 35.3±2.6 respectively at the end of 120 min (Figure.3a). The percentage drug dissolved was found to be linearly dependent on the surfactant concentration and a maximum drug release of 93±3.2 % and 92±2.8 % was seen in 1.5% w/v of SLS in water and pH 7.4 PBS respectively at the end of 120 min (Figure.3b).

The release study of three tablet formulations TF1, TF2 and TF3 in 1% w/v SLS in pH 7.4 PB showed varying drug release profiles. The prepared THC tablets differed in the amount of binder, the effect of which was reflected on their drug release pattern. Formulations TF1, TF2 and TF3 showed an initial drug release of 87.3±2.14%, 73.16±2.49% and 64.6±2.04% respectively in the first 5 min of the study (Figure.3c). The overall release of drug from the

tablet formulations was found to be retarded with an increase in binder concentrations. As it is evident from the hardness test, the crushing strength of the tablets increased as the amount of the binder increased. This caused the delay in disintegration of the tablets and hence the formulations with higher binder concentrations showed slow release rate of the drug.

Discriminating test on developed dissolution medium

The release profiles of formulations TF1, TF2 and TF3 were compared choosing four time points (5, 15, 60 and 120 min) of drug release curve and the fit factors (f1 and f2) were determined. Two release profiles are said to be similar when the value of difference factor (f1) is between 0 to15 and of similarity factor (f2) 50 to 100.[14,33] From the fit values, the release profile of formulation TF3 was found to be different from TF1 and TF2 and a slight similarity was observed between TF1 and TF2
(Table 3). Though similarity was observed between formulations TF1 and TF2 in terms of f2 which was at a border value of 51.61, a noticeable difference existed in the cumulative percentages of drug released at the initial time points of the study. This showed that the medium was able to differentiate the release rates of the prepared THC tablets.

DISCUSSION

In the past decade, dissolution testing has undergone much transition in its application and value. Based on the FDA guidance dissolution test is applied as a surrogate marker for bioequivalence test along with quality control.[34] On the other hand, there is very little published on the application of dissolution in the development and testing of natural and nutraceutical products. In the recent past there is lot of attention given for the nutraceutical product development because of its proven pharmacological actions.[35-37] The present investigation was focused in the development of a discriminative dissolution medium and its validation that could serve as a reliable medium for quality assessment of THC formulations. The initial CMC determination was aimed to assess the contribution of the surfactant in the solubility of the THC. The results of CMC determination by drop number method were in agreement with those of the dye solubilisation method. The critical concentration for micelle formation of the surfactant was low in aqueous solutions whereas it had increased in pH 7.4. This is due to the presence of ions, which would have reduced the aggregation and formation of micelles at lower concentrations of SLS in 7.4 buffers.[38]

The increase in solubility of the drug in media with SLS was apparently due to the micellar solubilisation of the drug by the surfactant. Surfactants are generally employed in developing the dissolution media of low soluble class II drugs to maintain the sink conditions and the required surfactant concentration depends on its CMC to solubilise 85% of the drug. The usual concentrations of SLS being used in the dissolution media are between 0.1 and 3%.[39,40] Apart from solubility of the drug, the suitability of a dissolution medium depends on the stability of the drug in the medium. Considering the stability of the drug and on basis of solubility data, 1% SLS in pH 7.4 PBS had been chosen as a suitable dissolution medium for THC.

Further in vitro release test of the prepared THC tablets was performed in 900 ml of 1% w/v SLS in pH 7.4 phosphate buffer with a paddle speed of 50 rpm and the release profiles were compared. Generally, a paddle speed of 50 or 75 rpm is used to carry out the in vitro release studies of tablets by USP II paddle method. However, the dissolution medium may become indiscriminative when the paddles are rotated at higher agitation rate.[41,42] Hence the release studies were carried out at low agitation speed of 50 rpm to produce steepest drug release profiles. The rate of release of the drug from each tablet formulation differed owing to the effect of the binder on mechanical strength and disintegration of the tablets.

To elucidate the discriminative power of the medium the dissolution curves of THC tablets were compared using fit factors (f1 and f2). The release curves of THC tablets were compared at three different release time points before and, one point after 85% of drug dissolution from all the tablets, as per FDA guidelines.[43] The difference or similarity observed between the release profiles of THC tablets was due to the changes made in the formulation composition and the developed dissolution medium was able to discriminate this. Further plasma absorption studies of THC formulations are required to establish a reliable *in vitro in vivo* correlation (*IVIVC*) and confirm the bio relevance of the medium.

CONCLUSION

Dissolution is crucial for the low water-soluble compounds because the absorption of these molecules is dissolution rate limited. In the case of THC, dissolution is the important rate limiting step for absorption, so developing a suitable dissolution medium is necessary to predict the differences in bioavailability of THC formulations.[43] In the present study, dissolution method for THC by UV spectroscopy was developed and validated. The results are evident that, the pH of the medium and micellar aggregation of surfactant in aqueous and ionic solutions influenced the solubility of the drug. The final conditions for the dissolution test are 900 ml of 1% SLS in pH 7.4 PBS at 37±0.5ºC as dissolution medium, using USP apparatus II with a paddle speed of 50 rpm. The validation showed that the developed dissolution method is appropriate for quantification of THC. The release profiles comparison of prepared THC tablets by model independent approach and determination of fit factors showed that 1% SLS in pH 7.4 to be a discriminative dissolution medium. Considering this, it can be concluded that the developed dissolution method is simple, cost effective and adequate for the routine quality control analysis of THC, since there is no official monograph and validated method available.

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CONFLICT OF INTEREST

No conflict of interest has been declared by the authors.

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Table 1. Composition of prepared THC tablets showing weight in mg of excipients per tablet for a total average tablet weight of 200 mg.

	TF1		**TF2**		**TF3**	
Ingredients	**Weight per tablet (mg)**	**% w/w**	**Weight per tablet (mg)**	**% w/w**	**Weight per tablet (mg)**	**% w/w**
THC	100	50%	100	50%	100	50%
Avicel PH 102	80	40%	75	37.5%	70	35%
Sodium Starch Glycolate	6	3%	6	3%	6	3%
Povidone K-30	10	5%	15	7.5%	20	10%
Magnesium Stearate	2	1%	2	1%	2	1%
Talc	2	1%	2	1%	2	1%

Table 2. Stability data of THC showing assay concentration of the drug in percentage at different storage temperatures and time periods.

	At 0 hr Initial= 10 µg ml^{-1}		**At 24 hr**		**At 48 hr**	
	Std	**Sample**	**Std**	**Sample**	**Std**	**Sample**
Room temperature (25±2^{o} c)	100.0 %	100.0%	100.0 %	99.76%	99.75 %	99.65%

Refrigerator (8±2 º c)	100.0 %	100.0%	100.21 %	100.0%	100.14 %	99.75%

Table 3. Fit factor values obtained from the drug release comparison of THC tablets.

Fit Factors	**TF1/TF2**	**TF2/TF3**	**TF1/TF3**
f1	12.50	15.18	26.74
f2	51.61	41.29	33.52

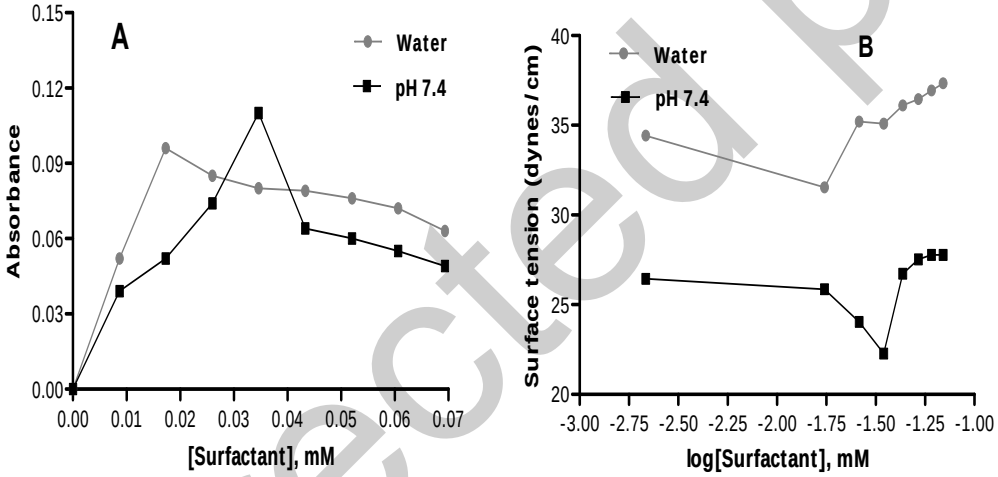

Figure 1. Experimental CMC values of SLS in water and pH 7.4 phosphate buffer determined by, (A) dye solubilisation method and (B) surface tension method.

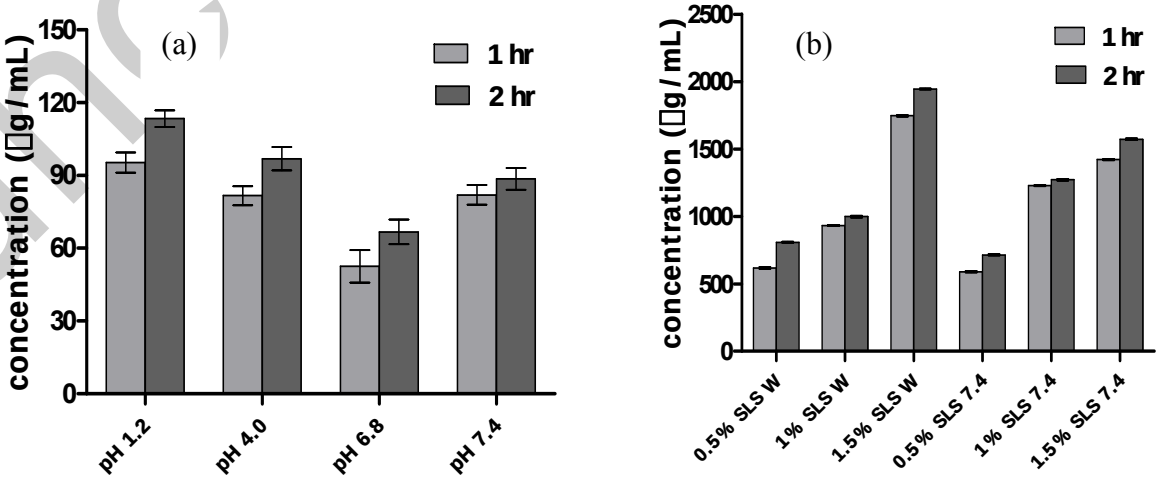

Figure 2. (a) Solubility profiles of pure Tetrahydrocurcumin at 1 hr and 2 hr in different pH solutions of 1.2 to 7.4. (b) Solubility profiles of pure Tetrahydrocurcumin at 1 hr and 2 hr in water and pH 7.4 containing 0.5-1.5% w/v sodium lauryl sulphate.

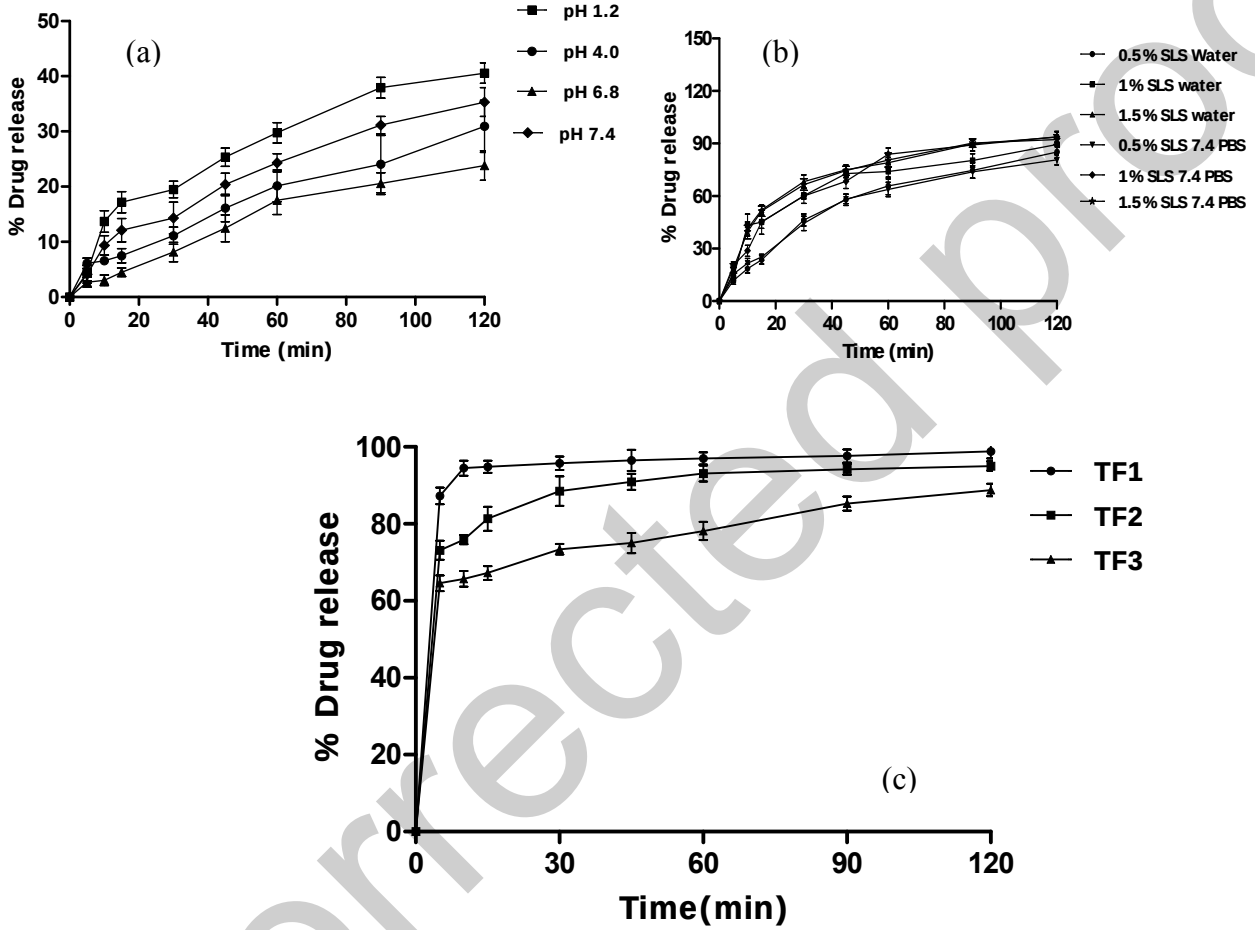

Figure 3. (a) Dissolution profiles of THC filled capsules in solutions of pH 1.2 to 7.4. (n=3). (b) Dissolution profiles of THC filled capsules in water and pH 7.4 containing 0.5-1.5 % w/v sodium lauryl sulphate. (n=3). (c) Cumulative percent drug release profiles of the prepared THC tablets in 900 ml of 1% w/v SLS in pH 7.4 PBS at 50 rpm. (n=3)