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Abstract
Inclusion complex can enhance the drug solubility, stability, and masking smell. Cyclodextrin (CD) is a commonly used host molecule for inclusion, with three main types of CD, exist, α, β, and γ. They are classified based on their chemical structure. The derivative of β-CD, (Hydroxypropyl-β-Cyclodextrin; HP-β-CD), is widely used in pharmaceutical formulations. HP-β-CD works by a dynamic equilibrium process for inclusion. Nifedipine is a Class II drug (Biopharmaceutical Classification System) that has poor solubility in water, and high permeability through the cellular membrane. The Phase-solubility profile of nifedipine/HP-β-CD complex showed an A type (according to Higuchi and Connors' method) indicating a complex ratio of 1:1. The objective of this study was to investigate the pH effect on the inclusion process of nifedipine/HP-β-CD. The results of this study showed that ionization pH conditions improved nifedipine solubility in water to a limited extent however it was not as efficient as the unionization pH conditions since unionization conditions produced higher Complexation Efficiency (CE) and the stability constant (K_{1:1}) value. Therefore, maintaining the drug in a unionized form is perhaps a more efficient way of producing inclusion complex with HP-β-CD. In order to characterize the inclusion complex thus formed between the drug and HP-β-CD, a solvent evaporation method was used to prepare the complex which was compared to a physical mixture, pure drug, and pure HP-β-CD. Significant differences were found to exist between the inclusion complex and all the other groups based on the TGA-DSC, ATR, and PXRD analysis.

Keywords
Biopharmaceutical Classification System; Complex Characterization; Complexation Efficiency; Cyclodextrin; Hydroxypropyl-β-Cyclodextrin; Inclusion Complex; Nifedipine; Phase-Solubility Profile; Stability Constant

Introduction
Nifedipine is a circulatory system agent that is a calcium channel blocker that can specifically block Ca^{2+} entering the membrane to reduce the calcium concentration in the cell [1]. It has asymmetric pyridine ring perpendiculars with the benzene ring; such structure facilitates the calcium antagonism [2]. The structure modification of the 3, 5-substituent group can make the carbon atom connect with the benzene ring to be chiral, and the steric hindrance will improve the drug potency. The dihydropyridine structure of the calcium channel blocker has high specificity and potency in angiectasis, which is used for treating coronary spasm, high blood pressure, and myocardial infarction without suppressing heart activity [3]. The pure nifedipine is a yellow crystalline powder, soluble in many organic solvents.

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Cyclodextrin (CD) is the product of starch cultivated with bacillus alcalophilus, which is composed of 6 to 12 D-dextrose molecules and connected by a 1,4-glucosidic bond between the D-dextrose forming a cyclic oligosaccharide structure [3]. Three main CD types (α, β, γ) are classified based on their chemical structure, which has 6, 7, 8 D-dextrose, respectively. The β-CD has relatively poorly water-soluble due to its structure being optimal for formatting a ring of intramolecular hydrogen bonds that counteract the hydration of β-CD; therefore, β-CD is not suitable for parenteral administration [5]. Due to their cyclic structure, CD types are three to five times more resistant to non-enzymatic hydrolysis as compared to linear dextrins [6]. CD has lipophilic groups inside the cavity binding with drug molecules, and hydrophilic groups outside that can form a hydrogen bond with water molecules so that to increase the drug solubility [7]. Another reason for solubility enhancement is that the substitution transforms the crystalline cyclodextrins into amorphous mixtures of isomeric derivatives [8]. The derivatives of β-CD are widely used for a pharmaceutical manufacturer based on the formulation desired characteristics by the placement of the alkyl groups on the CD chain [4]. For instance, CD-containing ethyl group is used to decrease the solubility of drugs and thus to prepare controlled release dosage forms for highly soluble drugs, while hydroxypropyl group (HP-β-CD) is used for increasing solubility of poorly soluble drugs [9]. HP-β-CD has been used for research, which was the first CD derivative approved by the FDA. HP-β-CD has been widely applied in the food, agriculture, and the pharmaceutical fields [10]. Some of the advantages of HP-β-CD are high solubility at room temperature, does not undergo metabolic degradation, is excreted in the urine, and demonstrates excellent shelf life stability [11].

Inclusion complexes formed between CD and drug molecules depend on attributes related to the drug molecules, the CD type being used, and other factors (pH, temperature, rate of agitation, incubation time) that affect the dynamic equilibrium process and influence the complexation efficiency. Among the physical and chemical characteristics that influence the complex formation are electrostatic interactions, van der Waals force, hydrophobic interactions, hydrogen bonding, the release of conformational strain, and charge-transfer interactions [12].

Generally, one drug molecule is entrapped in one cyclodextrin cavity, therefore for 1:1 complex is formed; [12].

\[
[CD] + [D] \leftrightarrow [D-CD]
\]

Where \( K_{1:1} \) is the stability constant at steady state. A phase solubility graph can be constructed between [D] versus [CD]; [13].

\[
K_{1:1} = \frac{[D-CD]}{[D][C]} \quad (1)
\]

\[
S_0 \cdot K_{1:1} = \frac{\text{Slope}}{1 - \text{Slope}} \quad (2)
\]

Where CE is the complexation efficiency and the slope corresponds to the phase-solubility graph. When selecting cyclodextrin or complexation conditions during formulation work, it can frequently be more convenient to compare the CE than \( K_{1:1} \) values [12], because for very poorly soluble drugs the y-intercept often is a negative value which would produce an unrealistic value for \( K_{1:1} \). For this reason, the more accurate method for determining the combined effect is the Complexation Efficiency (CE), which is determined by the slope of the line that happens to be always positive in value.

Previous studies have shown that increasing temperature and amount of cyclodextrin will significantly enhance the complexation process [14]. In this study, the effect of pH on CD/Drug complex was examined, and CE values of the inclusion complex at pH 2, 3, 5, and 7 using phase-solubility profile methodology were estimated. Additionally, in order to verify the nifedipine entrapment within the HP-β-CD, inclusion powder was prepared for characterization using solvent evaporation and kneading methods. Thermo Gravimetric Analysis, Differential Scanning Calorimetry (TGA-DSC), Powder X-Ray Diffraction Analysis (PXRD), and Attenuated Total Reflection Analysis (ATR) were used for determining the physical properties of the complex, and Ultra-Fast Liquid Chromatography (UFLC) was used for phase-solubility study to determine the intrinsic solubility of nifedipine at different pH condition.

Materials and Methods

Materials

The materials and equipment for this study are summarized in table 1 and 2, respectively.

Methods

Buffer preparation: Four different pH buffers (2, 3, 5, 7) were prepared. Table 3 summarizes the volumes of 0.2 M Na<sub>2</sub>HPO<sub>4</sub> and 0.1 M citric acid that were needed to prepare 100 mL of buffer.
Material | Manufacturer
--- | ---
Hydroxypropyl-beta-cyclodextrin | ISP technology
Nifedipine | Sigma
Acetonitrile | Sigma
Citric acid | Fisher Scientific
Dibasic sodium phosphate | Fisher Scientific

Table 1: Materials.

| Equipment | Manufacturer
--- | ---
Analytical Balance | Mettler Toledo (Columbus, Ohio, USA)
Ultra-Fast Liquid Chromatography | Shimadzu
Particle X-ray Diffraction | Rigaku (the woodlands, Texas, USA)
Differential Scanning Calorimetry Q-200 | TA instrument (New Castle, Delaware, USA)
Fourier Transfer Infrared Spectroscopy | Perkin Elmer (Shelton, Connecticut, USA)
Thermal gravimetric analysis Q-500 | TA instrument (New Castle, Delaware, USA)
VWR centrifuge tube (0.22 µm) | VWR

Table 2: Equipment.

| pH | 0.2 M of Na₂HPO₄ (mL) | 0.1 M of Citric acid (mL)
--- | --- | ---
2 | 3.60 | 96.40
3 | 20.55 | 79.45
5 | 51.50 | 48.50
7 | 82.35 | 17.65

Table 3: Chemicals composition for buffer solutions.

Preparation of HP-β-CD buffer solution: Buffer solutions (pH 2, 3, 5, 7) containing HP-β-CD (1400 Daltons) were prepared with the following concentrations 0, 5, 10, 15, 20, 25 mmol/L for each pH value.

Ultra-Fast Liquid Chromatography (UFLC) method: The amount of nifedipine dissolved in the solution was determined using UFLC. The UFLC parameters are summarized in table 4. The calibration curve done in triplicates was linear with R² of 0.9988 (p<0.0001).

| Parameter | Value |
--- | ---
Flow rate | 0.4 mL/min
Injection volume | 10 µL
Run time | 7 min
Wavelength | 235 nm
Temperature | Room temperature
Column | C18 (Waters)
Mobile phase | 0.1% formic acid in water and 0.1% formic acid in acetonitrile
Method | Gradient, 30% to 40% of acetonitrile from 0min to 5 min
Retention time | 5.12min

Table 4: Ultra-Fast Liquid Chromatography (UFLC) parameters for nifedipine analysis.
Phase solubility study: The purpose of this experiment was to evaluate the inclusion process of nifedipine with HP-β-CD under different pH conditions. From this experiment, the Complexation Efficiency (CE) and the stability constant (K_{i,1}) were determined using equations 2 and 3.

An excess amount of nifedipine powder (7 mg) was added to the above-prepared buffer solutions containing HP-β-CD. The resulting mixtures were stirred (300 rpm) using magnetic stirrer for 24 hours at room temperature. Due to nifedipine photosensitivity, each sample was covered by aluminum foil during stirring. After 24 hours of stirring, 200 µL of the solution was transferred into a centrifuge filter tube and centrifuged for 30 seconds at 6500 rpm. The filtered solution was used for UFLC analysis. For each pH value, the experiment was repeated four times.

Physical characterization of the nifedipine/HP-β-CD complex: The purpose of this experiment was to evaluate the physical profile of nifedipine/HP-β-CD complex. Solvent evaporation method and the kneading method were used to prepare two batches of the inclusion complex. Additionally, pure drug and pure HP-β-CD were also tested for comparison.

Sample Preparation: The inclusion complex was prepared by mixing the drug with HP-β-CD using a molar ratio strength of 1:1 in a solvent system consisted of 40% (v/v) of acetonitrile in water. The solvent was then completely removed under low pressure using a rotary evaporator under vacuum for 12 hours. A physical mixture (PM) of nifedipine and HP-β-CD was prepared in a molar ratio of 1:1 using porcelain mortar and pestle, triturated for 15 min to obtain homogenous blend. The resulting solid residue of the inclusion complex and PM powder were stored in the refrigerator protected from light until further analysis.

PXRD study: Powder X-Ray Diffraction (PXRD) is a useful instrument for analyzing the crystal structure of powder. The main principle is when the X-ray emitted from the instrument incident to the powder, the planes of powder will diffract the X-ray to another direction which will be absorbed by the X-ray detector, the angle of diffraction and density of the signal depends on the structure of the powder [15]. Crystal form material will present different sharp peaks from 2° to 50°, while amorphous form powder will present less and broaden peaks in the graph. The peaks of each complex will position in a specific angle, which can distinguish one material to another. Previous studies indicated that if the drug were entrapped into the cyclodextrin, the sharp peaks would disappear and turn into broadened peaks, which meant that the drug transformed from crystal form to amorphous form.

Procedure: The tube was preheated for one hour before loading the sample. The standby voltage and the amperage were set as 20.0 kV and 2.0 mA, respectively. The voltage and amperage increased gradually to 40 kV and 44 mA, respectively. Then the sample was placed on the glass slide. The analyze cycle was set from 2° to 38°.

ATR study: The aim of the study was to use the IR absorption curve for analyzing the inclusion complex. The range of the wavenumber is from 4000 to 400 cm\(^{-1}\). Each functional group will have specific vibration frequency which can help us to identify the chemical structure [16]. For the inclusion complex, the peak density will change if the guest molecule is entrapped into the cavity, the density may increase or decrease which depends on the chemical structure of the drug.

Differential Scanning Calorimetry (DSC) and Thermal Gravitation (TGA) Study: DSC can investigate the thermal events including glass transition temperature, crystallization, and the melting point of the analyte [17]. The crystal form material has a melting point and a sharp endothermic peak which indicates the crystal form start melting, conversely, amorphous form don't have such endothermic peak but have a glass transition point, then the baseline will shift down or up in the DSC graph (depends on the definition of the heat flow direction in the y-axis varied by instrument brand).

Thermal Gravitation Analysis (TGA) is an important method analyzing the relationship between temperature and weight loss, giving the information to evaluate the thermo stability of the target material. With the help of TGA and DSC, we can get more details of the analyte. In this study, all the samples (pure HP-β-CD, nifedipine, sample K, and sample S) were tested in this section.

Procedure: Before DSC run, we used TGA to measure the decomposition temperature to ensure the temperature parameter is acceptable for a DSC instrument [18,19].

TGA: Select a platinum pan placed on the platform and select TARE from the TGA control menu touch screen. Place the 10 mg sample weighed by an analytical balance in the sample pan and position the pan on the sample platform. Touch the LOAD key on the menu. Position the thermocouple at the edge of the sample pan. Touch the FURNACE on the menu to close the furnace by moving it around the sample. Set the dynamic method start from 25 °C to 300 °C, 10 °C/min.

DSC: Select an aluminum pan putting on the balance and tare it, then place the sample in the pan to weigh about 5 mg for study. Remove the lids, carefully place the sample pan on
the front raised platform and the reference pan on the rear left platform, then cover the cell. Set the dynamic method start from 25 °C to 300 °C, 10 °C/min. After each cycle, collect the results of glass transition ($T_g$), melting points ($T_m$), and heats of fusion ($H_m$) of the samples.

**Statistical analysis:** The complexation parameters were compared at various pH conditions using a one-way analysis of variance test. A p-value of less than 0.05 was considered statistically significant.

**Results and Discussion**

**Phase-solubility study**

Higuchi and Connors [20], summarized the relationship between cyclodextrin and drug by phase-solubility profile. There are two main types of complexes A and B, in which A-type has three subtypes (A$_p$, A$_L$, A$_N$) and B-type has two subtypes (B$_s$, B$_I$) [20]. Figure 1 represents A$_L$ fit-line for the data collected from this study.

The phase-solubility profile is shown in figure 2, in which all four pH conditions (2, 3, 5, and 7) show a good linear relationship between the drug and cyclodextrin. The A($\gamma$) type suggests that the complexation ratio between the drug and cyclodextrin is 1:1. Based on the phase-solubility profile, the results were summarized in table 5 where the complexation efficiency and stability constant at various pH conditions were calculated. The CE and $K_{1:1}$ decreased as the pH became more and more acidic (Figure 3). Moreover, CE and $K_{1:1}$ demonstrated an inverse relationship with $S_0$. This suggests that as the drug became more and more ionized (i.e., in the case of nifedipine ionization this was enhanced with a decrease in the pH) would decrease the stability of cyclodextrin-drug complex and thus the tendency of the drug to be present as the free form in the solution. Therefore, it is not only an exterior hydrophilic group form hydrogen bond with water that improves the solubility, but also this system follows a dynamic equilibrium process that facilitates the drug solubility in water. The results from the analysis of the data also indicated that CE values and $K_{1:1}$ values were significantly different at various pH conditions ($P< 0.05$).
Table 5: Data analysis of phase-solubility profile.

<table>
<thead>
<tr>
<th>pH</th>
<th>$S_8$</th>
<th>$R^2$</th>
<th>$K_{L1}$</th>
<th>CE</th>
<th>Linear Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>9.49E-06</td>
<td>0.976941</td>
<td>55.93</td>
<td>0.000529</td>
<td>NI (M) = 9.4892e-6 + 0.0005288×HPβCD (M)</td>
</tr>
<tr>
<td>3</td>
<td>6.36E-06</td>
<td>0.973386</td>
<td>83.80</td>
<td>0.000532</td>
<td>NI (M) = 6.3596e-6 + 0.0005319×HPβCD (M)</td>
</tr>
<tr>
<td>5</td>
<td>2.21E-06</td>
<td>0.978292</td>
<td>316.68</td>
<td>0.000688</td>
<td>NI (M) = 2.2143e-6 + 0.0006836×HPβCD (M)</td>
</tr>
<tr>
<td>7</td>
<td>7.92E-07</td>
<td>0.990604</td>
<td>967.52</td>
<td>0.000736</td>
<td>NI (M) = 7.9231e-7 + 0.0007355×HPβCD (M)</td>
</tr>
</tbody>
</table>

Figure 2: Phase-solubility profile of Nifedipine.

Figure 3: The relationship between Stability constant with pH and Complexation efficiency with pH.
From this study, pH condition is an important factor affecting the complexation tendency, for a basic compound, the acidic condition will undermine the complexation trend. Table 6 and 7 is the comparison of solubility increment when the Conc(HP-β-CD) go up to 25 mM compared to 0 mM in each pH condition, and the following two graphs (figures 9 and 10) clearly show how pH affects this system. From figure 9, we can see the solubility increment increases when increasing the pH (ionization trend), however, the intrinsic solubility (solubility in absence of HP-β-CD the word intrinsic here refers to the actual observed solubility of the dug in the absence of CD) decreases when increasing the pH (ionization trend) as shown in Figure 10. Even pH 2 batch has a higher starting point (S0), the ionization pH condition will prevent the complexation process and finally, the ionization pH condition will have a higher solubility than ionization pH condition as the concentration of cyclodextrin increase.

<table>
<thead>
<tr>
<th>Pure HPβCD</th>
<th>300°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Nifedipine</td>
<td>188°C</td>
</tr>
<tr>
<td>Inclusion complex</td>
<td>225°C</td>
</tr>
<tr>
<td>Physical mixture</td>
<td>212°C</td>
</tr>
</tbody>
</table>

Table 6: Decomposition temperature of 4 samples.

<table>
<thead>
<tr>
<th>pH</th>
<th>Intercept (S0)</th>
<th>S increment (2θdiff)</th>
<th>CE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>9.49E-06 M</td>
<td>0.00001322</td>
<td>0.000529</td>
</tr>
<tr>
<td>3</td>
<td>6.36E-06 M</td>
<td>0.000013298</td>
<td>0.000532</td>
</tr>
<tr>
<td>5</td>
<td>2.21E-06 M</td>
<td>0.00001709</td>
<td>0.000688</td>
</tr>
<tr>
<td>7</td>
<td>7.92E-07 M</td>
<td>0.000018388</td>
<td>0.000736</td>
</tr>
</tbody>
</table>

Table 7: Solubility improvement analysis based on pH factor.

Many other techniques have been demonstrated to enhance the solubility of nifedipine by using techniques such as formulation of microwave-generated bio nanocomposites [21], use of spray-dried solid dispersions with fusion method, a solvent evaporation method, an extruding method [22], use of various carriers [23], and many others [24]. For drugs with high intrinsic solubility, increasing the ionization tendency is sufficient to render the drug soluble in water. On the other hand, for drugs that have low intrinsic solubility in water, such as nifedipine, their solubility is enhanced greatly by complexation with HP-β-CD. When compared to the control sample (no HP-β-CD present) the solubility of nifedipine increased incrementally with HP-β-CD concentration. This implies that the solubility of nifedipine may be enhanced at higher pH condition in the presence of a higher concentration of HP-β-CD even though the degree of ionization of the drug at these higher pH conditions is low for nifedipine. As the data show, the solubility of nifedipine at pH 7 in the presence of 25 mmol of HP-β-CD as compared to no cyclodextrin was slightly less than that at pH 2 and at the same HP-β-CD concentration. However, the lines shown in figure 2 for pH 2 and pH 7 can intersect if they are extended beyond the HP-β-CD concentrations that were used in this study, and the solubility of the drug even becomes greater at pH 7 than that at pH 2 if the line is extended further. It has been said that one of the mechanisms of action of nifedipine involves pH-dependent inhibition hence, the difference in the solubility of nifedipine in case of higher concentration of HP-β-CD at higher pH condition might lead to enhancement of the activity of nifedipine [25]. This indicates that the use of cyclodextrin as a solubility enhancer for poorly water-soluble drugs such as nifedipine can overcome the low intrinsic solubility of these drugs in water and improve their dissolution profile beyond that expected from ionization influence alone. This relationship can be reflected by the Complexation Efficiency (CE) shown in figure 3. Therefore, maintaining unionization complexation condition is an important factor if we want to get high volume production of inclusion complex.

Characterization

PXRD test: A sharp contrast was observed between the inclusion complex and pure drug. The pure drug showed a typical crystalline form that had many sharp peaks; for inclusion, the crystal form almost disappeared and the position of the two main broad peaks at the same angle compared with pure cyclodextrin suggests that nifedipine was included into the HP-β-CD. For the physical mixture, it has few sharp peaks as compared to pure drug and the general shape of the graph is more similar to pure HP-β-CD (Figure 4).

ATR test: Figure 5 shows the comparison of inclusion complex and HP-β-CD. The inclusion complex has a strong peak in 1678 cm⁻¹, this peak specifically reflects -C=O group, where nifedipine has two ester groups which are lipophilic that can be captured inside of the cavity of cyclodextrin, the outside of the cavity is hydrophilic that have more -OH groups, it has more peak intensity than pure drug in 3327 cm⁻¹. Additionally, for
HP-β-CD, hydroxypropyl group connects the cyclodextrin by an oxygen atom which forms an ether group. Once the drug is included inside, the inclusion complex has a stronger peak compared to the pure drug in 1022 cm$^{-1}$ (Figure 6). This was specifically demonstrated for the ether (C-O-C) group as seen in the pure HP-β-CD spectra.

**Figure 4:** PXRD profile for inclusion complex, physical mixture, pure drug, and pure HP-β-CD.

**Figure 5:** IR spectra of inclusion complex, HPβCD.
Figure 6: Fingerprint zone of Nifedipine, inclusion complex, and HPβCD.

TGA-DSC test: After TGA run (Figure 7), the four samples were subjected to decomposition temperatures as shown in table 7. Based on the temperature data the DSC maximum temperature should not be set higher than the decomposition temperature.

From figure 8, the inclusion complex had completely turned to amorphous form which was similar to that of pure HP-β-CD, whereas the DSC curve for pure nifedipine is a typical crystalline form curve which had a sharp endothermic peak at 170°C. The curve of the physical mixture had a smaller endothermic peak which indicated the drug was partially included into the HP-β-CD.

From TGA-DSC, ATR, PXRD studies, all the results indicate nifedipine molecule can form an inclusion complex with cyclodextrin using solvent evaporation method in the presence of 40% acetonitrile as the solvent.

Figure 7: TGA profile of Pure HPβCD, Pure Nifedipine, Inclusion complex, and Physical mixture.
Figure 8: DSC profile of Pure HPβCD, Pure Nifedipine, Inclusion complex, and Physical mixture.

Figure 9: Solubility increment versus pH.
Figure 10: Intrinsic solubility versus pH.

Figure 11: Complexation efficiency versus pH.
Conclusion

The Complexation Efficiency (CE) and stability constant ($K_{\text{1:1}}$) decreased when the pH became more acidic, which showed an inverse relationship to the $S_0$. Therefore, maintaining pH conditions that favor the drug in its unionized form along with a higher concentration of HP-β-CD would produce better solubility profile for the drug in water. Solvent evaporation method using 40% acetonitrile as the solvent was shown in this study to be an efficient way of producing inclusion nifedipine/HP-β-CD complexes. Kneading method could also be used to prepare this complex, but it was not as efficient as the solvent evaporation method. A significant difference in the characterization profiles was found between the inclusion complex with the control group. The spectra of DSC and PXRD of the inclusion complex are almost the same as the pure HP-β-CD that was amorphous in nature. The ATR spectra showed C=O group peak got weaker compared with pure nifedipine. This indicates that the drug was entrapped within the cyclodextrin cavity because the vibration of the C=O group was constrained by this reaction.

References