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Research Article

The use of hydrophilic carrier derived from eggshell and novel gel forming technique in a solid dispersion system

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ABSTRACT

The study aimed to prepare calcium acetate (CaA) from the eggshell (ES) by the chemical reaction with aqueous acetic acid and to improve the dissolution properties of poorly soluble model drug, chloroxylenol (CXN), by solid dispersion (SD) system with CaA as a carrier. In the present study, SDs were prepared by two methods using various drug:carrier ratios. Fourier-transform infrared spectroscopy (FTIR), powder X-ray diffraction (PXRD), thermal analysis and scanning electron microscope (SEM) studies were carried out to characterize the prepared SDs in comparison with pure drug and physical mixtures (PMs). FTIR analysis indicated that there was an intermolecular hydrogen bonding between the terminal hydroxyl group of drug and water molecules of CaA. Decrease in crystallinity of SDs was observed in PXRD and differential scanning calorimetry (DSC) studies. Moreover, thermogravimetric analysis (TGA) study showed the drug protected within SD. SEM images revealed the morphology of SDs prepared by the gel forming technique as a rod-like microstructure in which the drug was occupied. The rough surface of SD prepared by the wet granulation method was due to the adherence of drug particles on the surface of CaA. SDs prepared by the gel method exhibited superior performance for the dissolution of CXN with a release of 93-113% at 60 min compared to PMs and pure CXN, which could be due to its transformation from crystalline to amorphous form as well as the improved wettability. Therefore, CaA might be the potential candidate for the dissolution enhancement of poorly soluble drugs.

Keywords:
Eggshell, Calcium acetate, Gel forming technique, Chloroxylenol, Solid dispersion, Drug release

1. INTRODUCTION

The continued popularity of solid dosage forms leads to a demand for newer excipients with functional property to address issues such as disintegration, dissolution and bioavailability of solid dosage forms[1]. The effects of pharmaceutical excipients contribute unique functionalities to formulations; thereby determine the quality of medicinal products[2]. Pharmaceutical formulation development includes various components in addition to the active pharmaceutical ingredients (APIs). Nature provides a wide variety of substances either directly or indirectly[3]. The waste-to-wealth concept aims to raise the value of wastes not only for their intrinsic benefits to the environment, but also for the development of new valuable substances[4]. Eggshell (ES) is generally considered as a solid waste with the production of several tons a day. One of the major advantages of the ES is the absence of toxic elements in its composition[5]. Therefore, it is necessary to look for an alternative method, which transforms the ES wastes into a valuable substance. Many studies have shown that the applications of the ES waste can be seen in different areas, for example, using the ES powder as a fertilizer or soil conditioner[5-6], as coating pigments for ink-jet printing paper[7], as food supplement and animal feeds for human and animals, respectively[8]. The major applications of the ES wastes include the preparation of a bone substitute, hydroxyl apatite (Hap), bone mineralization and growth[9-11], and a good adsorbent for ionic pollutants from the aqueous solution[5,9]. As a major constituent of the ES is

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calcium carbonate, it can be used to replace calcium carbonate as a pharmaceutical excipient in solid dosage forms. Some researchers demonstrated that the ES powder can be used as a diluent and/or drug release controlling agent in tablet formulation[12].

Solid dispersion (SD) technique is regarded as one of the most promising strategies to enhance the dissolution profile of poorly soluble drugs[13]. The pharmaceutical application of SD of drugs in water-soluble carriers to enhance their dissolution rates, and finally improve bioavailability was first revealed by Sekiguchi and Obi[14]. The term "solid dispersions" is a dispersion of one or more API in an inert carrier or matrix at the solid state[15]. The API in SDs can be dispersed as separate molecules, amorphous particles, or crystalline particles, while the carrier can be in the crystalline or amorphous state. Based on the physical nature of the carrier, which is crystalline or amorphous, the SDs can be categorized into crystalline SDs and amorphous SDs, respectively. The SDs can be classified into four generations depending on their composition[16].

There are three methods to prepare SDs, namely, melting method, solvent method and melting solvent or fusion solvent method. In the melting method, the physical mixture of a drug and a water-soluble carrier is heated directly to melt. Then the melted mixture is cooled and solidified in the ice bath agitation. The final solid mass is crushed, pulverized, and sieved to reduce the particle size[15]. In the solvent method, SDs can be obtained by dissolving a drug and a carrier in a common solvent, followed by evaporation of the solvent[15,17]. In the melting-solvent method, SD is prepared by dissolving a drug in a suitable solvent and incorporated into a molten carrier. After removing the solvent and carrying out solidification process, the product is formed[16].

Hen ES was successfully used in this study as a calcium source for the preparation of CaA with a high aqueous solubility. There is no report in the literature on the role of CaA as a carrier in SD technique. The anhydrous form of CaA is very hygroscopic; therefore the monohydrate form $(CH_3COO)_2Ca.H_2O$ is the common form[18]. CaA has unique characteristics, i.e., it can form gel with ethanol, and it shows exothermic property when dissolves in water[19-20]. Therefore, CaA may be used as an excipient of choice for solid dosage form to enhance drug dissolution. In this study, chloroxylenol (CXN), a halogenated phenol (Figure 1), commonly used as an antimicrobial agent was used as a model drug due to its low water solubility (0.3 g/L)[21]. SD of CXN in a hydrophilic carrier, CaA, was prepared. The aim of the present study was to investigate SD of CXN using CaA as a hydrophilic carrier for the enhanced and rapid dissolution of oral drug delivery system.

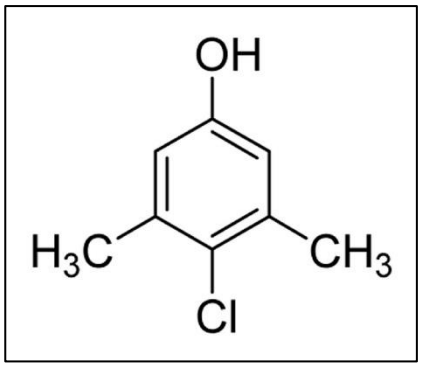


Figure 1. Molecular formula of CXN.

2. MATERIALS AND METHODS

Hen ES was collected from the cafeteria in a University in Thailand. CXN was purchased from Fluka Chemie (Sigma-Aldrich Chemie GmbH, Switzerland). Absolute ethanol and glacial acetic acid were obtained from Labscan, Bangkok, Thailand. The other chemicals were of analytical grade.

2.1. Preparation of calcium samples

2.1.1. Preparation of CaA powder

Raw ESs were processed within 24 h after collection. The ES samples were rinsed with tap water and the membranes were removed. After boiling in deionized (DI) water for 1 h, the ES pieces were dried in the oven (Mammert, Germany) at 60°C for 3 h[22]. The dry ES pieces of 3-10 mm size were immersed in 35% w/v acetic acid solution for 2 h to remove the brown outer coating layer of the ES. The ES pieces were collected by decanting method and the samples were washed thoroughly with DI water and dried in the oven at 60°C for 3 h. The dry ES pieces were ground by using a glass mortar and pestle to obtain fine powder. Then the eggshell powder (ESP) was passed through No.40 mesh sieve (Retsch, Germany) to obtain the uniform-sized ESP.

Thirty grams of ESP was used to react with dilute acetic acid (50 mL glacial acetic acid in 150 mL of DI water[23]). The mixture was stirred continuously by using the magnetic stirrer (LMS-1003, Daihan Labtech, Korea) at 500 rpm for 2 h. Then the mixture was kept overnight in the fume hood. The reaction mixture was undergone prefiltration by using the stainless-steel sieve, and then vacuum filtration by using Whatman filter paper No.1 to obtain a clear filtrate. The filtrate was dried in the oven at 50°C for 2 h. Then 30 g of the crude CaA powder was redissolved in 100 mL DI water and the solution was undergone the vacuum filtration using Whatman filter paper No.1. Afterwards, the filtrate was dried and ground using the method described above. The CaA powder obtained was passed through No.60 mesh sieve (Retsch, Germany) to get a uniform-sized powder.

2.1.2. Preparation of SDs and PMs

2.1.2.1. Preparation of SDs by the gel forming technique

Fifty percent weight by volume of CXN-ethanolic solution and 30% w/v CaA aqueous solution were prepared. Then, CaA aqueous solution was used to form the gel formation with an organic solvent, ethanol. Fifty milliliters of ethanol were added dropwise (at the rate of 80 drops/min) by using a glass dropper to 100 mL of CaA solution under magnetic stirring (at the rate of 500 rpm) at controlled room temperature (25±2ºC). When the gel formation was occurred, 0.10 mL of CXN-ethanolic solution was added dropwise (at the rate of 80 drops/min) into the resulting gel by using glass dropper under magnetic stirring (at the rate of 500 rpm). The final gel was poured into flat and leveled petri dishes to get a thin layer with uniform thickness. After complete evaporation of ethanol (10 h), the samples were held in the oven at 40°C for 4 h. Then the sample was scraped out from the petri dishes, pulverized in a glass mortar, and sieved through No.60 mesh sieve to obtain SD of 1:1 w/w of drug to carrier. SDs of 1:2, 1:3 and 1:4 w/w of drug to carrier were also prepared.

2.1.2.2. Preparation of SDs by the wet granulation method

Firstly, preformed CaA powder was prepared as described in section 2.1.2.1. The resulting gel was placed in a petri dish. After the removal of ethanol, the petri dish was dried in the oven at 40°C for 4 h. Then the CaA powder was scraped out with a spatula, ground in a mortar and passed through No.60 mesh sieve to get a uniform-sized preformed CaA powder.

Afterwards, the previously prepared CXN-ethanolic solution was added dropwise into the preformed CaA powder in the mortar. After trituration for a few min, the resulting damp mass was transferred into the flat and leveled petri dishes to obtain a thin layer with uniform thickness. After complete evaporation of ethanol for 2 h at room temperature, the petri dishes were held in the oven at 40°C for 4 h. Then the dry samples were scraped out from the petri dish, ground in the mortar, and sieved through No.60 mesh to obtain powder. SDs of 1:1, 1:2, 1:3 and 1:4 w/w of drug to carrier were prepared.

2.2. Physicochemical studies of CaA powder

Physicochemical studies of CaA powder was carried out according to the USP 43 monograph[24]. The tests included chemical identification test for calcium, assay of CaA, impurity test (heavy metals, the limit of barium, the limit of nitrate and readily oxidizable substances) and pH analysis.

2.3. Solid-state characterization of SDs

2.3.1. Differential scanning calorimetry (DSC)[21]

Thermal analysis of each sample was conducted by using a differential scanning calorimeter (DSC8000, Perkin Elmer Inc., MA, USA). Each sample (about 2-5 mg) was placed in a sealed aluminum pan. The measurements were performed under a nitrogen flow (20 mL/min) at a heating rate of 10°C/min from 25°C to 200°C.

2.3.2. Thermogravimetric analysis (TGA)[21]

The weight loss profiles of CXN, CaA, SDs and PMs were analysed by using a thermogravimetric analyzer (TGA4000, Perkin Elmer Inc., MA, USA). Each sample (10-15 mg) was accurately weighed into the ceramic crucibles and placed in the TGA furnace. TGA and derivative thermogravimetric (DTG) measurements were carried out under the nitrogen atmosphere with 20 mL/min at the heating rate of 20°C/min.

2.3.3. Fourier transform infrared (FTIR) spectroscopy[25]

FTIR spectroscopic analysis was carried out on the SDs to ascertain for any possible interactions between the drug and the CaA. The spectrum was obtained at 4,000 to 400 cm^{-1} wavenumbers with 4 cm^{-1} resolution using a FTIR spectrometer (Nicolet iS5, Thermo Scientific, MA, USA) equipped with a diamond attenuated total reflectance (ATR) cell. Samples (5-10 mg) were kept on an ATR plate and FTIR measurements were done in transmittance mode. The spectrum analysis was conducted using an OMNIC Software. Pure drug, CaA and different ratios of PMs were run as controls.

2.3.4. Powder X-ray diffractometry (PXRD)[26]

Powder X-ray diffractograms of individual components, SDs and PMs were obtained using an X-ray diffractometer (Miniflex 600, Rigaku, Japan). The instrument produced monochromatic Cu-Kα radiation (λ=1.5418Å) at 40 kV and 15 mA. The samples were placed on the glass holder. The patterns were recorded in the 2θ range of 4 to 40° at a speed of 4° min^{-1}.

2.3.5. Scanning electron microscopy (SEM) spectroscopy[25]

The surface morphology of CXN, CaA, SDs and PMs were studied using a scanning electron microscope (TM4000Plus, Hitachi, Japan). Samples were mounted on a SEM-stub with double sided adhesive tape and sputter coated with platinum in the thickness of 5-6 nm under the vacuum at 10 mA for 30 s.

2.3.6. Drug content determination[21]

An amount of prepared SD equivalent to 25 mg of CXN was accurately weighed and dissolved in 25 mL methanol. Then the solution was sonicated in an ultrasonic bath for 15 min and suitably diluted with methanol. The absorbance of the samples was measured using double beam UV spectrophotometer (UV 2401 PC, Shimadzu, Kyoto, Japan) at the wavelength of 282 nm against methanol as a blank. The drug contents of the samples were calculated using the standard calibration curve at this wavelength. The percent drug contents of the samples were determined at 282 nm using the equation (1).

$$\text{Drug content (\%)} = \frac{(\text{Weight of drug entrapped within SD})}{\text{Total drug added}} \text{x100} \quad (1)$$

2.4. *In vitro* dissolution study of SDs[14]

Pure CXN, SDs and PMs equivalent to 50 mg of chloroxylenol were tested for their dissolution profiles using a USP dissolution apparatus 2 (Hanson Model SR8-Plus, Hanson Research Co., CA, USA). The study was carried out at 37±0.5°C in 900 mL DI water at 50 rpm. Aliquots of 5 mL of the samples were withdrawn at predetermined time intervals and analysed spectrophotometrically at 280 nm against water as a blank. An equal volume of fresh dissolution medium was replaced to maintain the sink condition. Each experiment was carried out in triplicate.

2.5. Data analysis

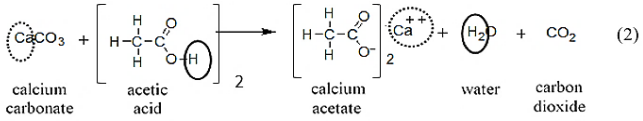
Statistical analysis of the dissolution studies was carried out using the one-way analysis of variance (ANOVA). Statistical analysis was performed with the SPSS® software package. Significance level of 0.05 was used.

3. RESULTS AND DISCUSSION

3.1. Preparation of powder samples

3.1.1. Preparation of CaA powder

Pure CaA powder was prepared from the hen ES. Calcium carbonate in the ES was dissolved in the aqueous acetic acid to form CaA. As acetic acid is a weak monoprotic acid, when it is added to water, it donates only one hydrogen ion (proton), sometimes called a deprotonation. The hydrogen in the hydroxyl part of the carboxylic group of acetic acid was lost and replaced with the calcium of salt to form calcium acetate (equation 2)[20,27].



(2)

CaA powder obtained is white, fine, and easily soluble in water. A slight odour of acetic acid is present. For white ESP 100 g, 59 g of pure CaA was obtained. The ES is ecofriendly, and thus CaA obtained from the ES is a naturally occurring substance with good physicochemical properties. Therefore, the efficacy of converting the ES to beneficial use as a pharmaceutical excipient became an idea worth investigating.

3.1.2. Preparation of SDs and PMs

CaA solution formed gel when ethanol was added[19]. The ethanol volume used in this study was the optimum for the gel formation of 30% w/v CaA aqueous solution. The addition of more ethanol into CaA solution than the required volume resulted in no more gel formation. Compared with the wet granulation method for the preparation of SDs, the gel method is technologically simple, economically accessible and inexpensive. After preparation, the SDs obtained from the gel method were free flowing, white in color, smooth and finer powder, whereas SDs with relatively less fine powder were obtained from the wet granulation method.

3.2. Physicochemical studies of CaA powder

The results obtained from specific tests for calcium, acetate and impurities complied with the requirements of the USP 43 monograph[24]. CaA content in the sample calculated on the monohydrate basis was 98.4%. The sample contained less than 25 ppm of heavy metals.

The levels of barium, nitrate and oxidizable substances in the sample were within the acceptance criteria. The pH analysis was 7.22, which was within the range of acceptance criteria (6.3-9.6). The results suggested that CaA could be used as a pharmaceutical excipient. It could be employed as a filler or diluent for solid dosage form or as a carrier for SD because of its unique properties like high aqueous solubility, free flowing properties and drug dissolution promoting effect as it released heat when dissolved in water[20].

3.3. Physical characterization of pure CaA powder

Figure 2 (left) shows the FTIR spectra of pure calcium carbonate, white ESP and brown ESP. There was a prominent absorption peak at 1,400 cm^{-1}, which was assigned to the carbonate in the basic component of the ES. The other two strong peaks at about 872 cm^{-1} and 712 cm^{-1} were attributed to the out of plane deformation modes and in-plane deformation of calcium carbonate, respectively. Both the spectra of white ESP and brown

ESP virtually showed similar peaks at the same positions of pure calcium carbonate. There was no spectral difference between them. It was postulated that the major constituent of white and brown ESP is calcium carbonate.

Figure 2 (right) shows the FTIR spectra of commercial CaA and pure CaA obtained from the ES. Since commercial CaA used as a control in this study was a monohydrate form, the broad band in the region at 3,180 cm^{-1} was prominent and it was due to the H-bonded ν(O-H) stretching vibration. The weak band at 1,648 cm^{-1} was due to the stretching of the carbonyl C=O bond present. CaA displayed the intense band at 1,541 cm^{-1} which may be due to the result of antisymmetric C-O (ν_{as}(C-O)) stretching vibrations. The antisymmetric methyl bending ($\delta_{as}(CH_3)$) vibration was found at 1,400 cm^{-1}. The less prominent peak presented at 1,339 cm^{-1} was due to the symmetric methyl bending vibration $\delta_{as}(CH_3)$ of the acetate anion. These results agreed with those reported previously by Musumeci et al[28]. The presence of characteristic peaks of commercial CaA and the absence of spectral difference between commercial CaA and pure CaA confirmed the formation of CaA from the ES calcium carbonate. Similarly, the existence of the broad band assigned H-bonded ν(O-H) stretching at the region around 3,200 cm^{-1} of pure CaA sample indicated that it was a monohydrate form which might result from the highly hygroscopic property of CaA[29].

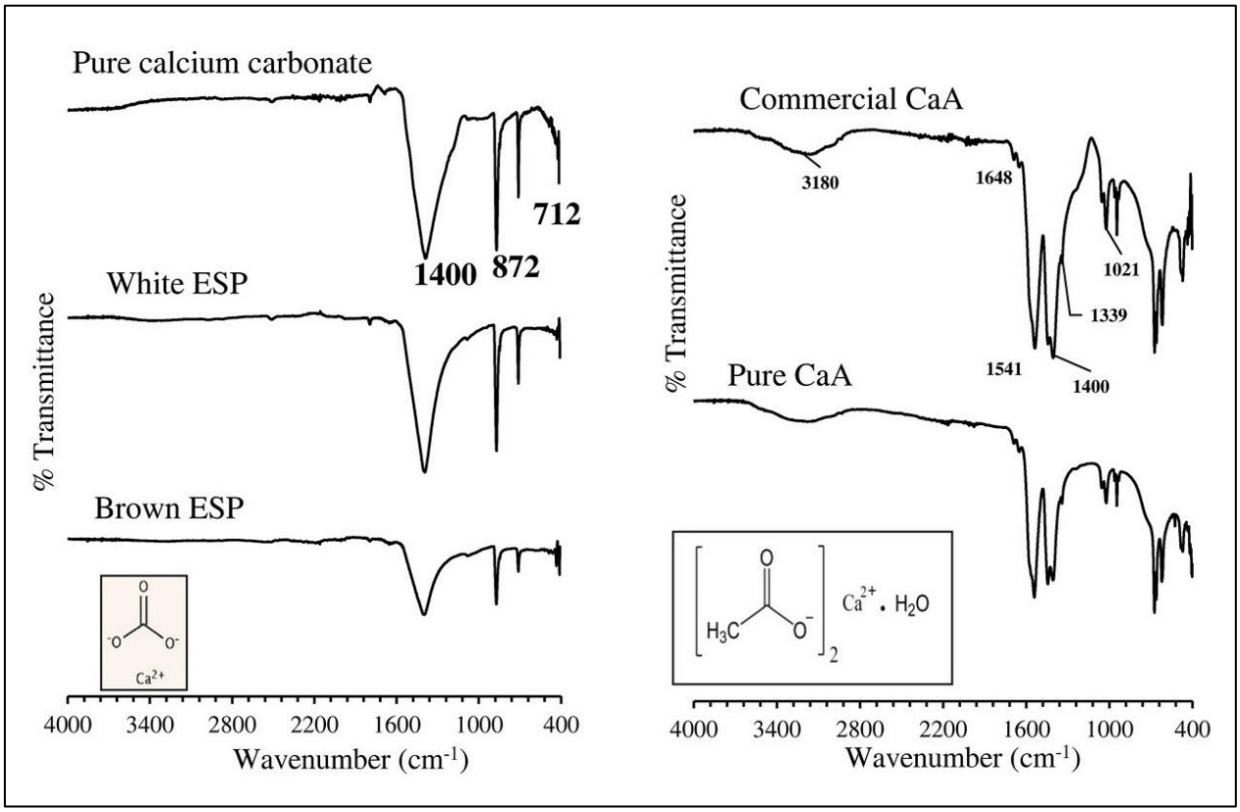


Figure 2. FTIR spectra of pure calcium carbonate, white ESP and brown ESP (left) and FTIR spectra of commercial CaA and pure CaA powder obtained from ES (right).

3.4. Solid state characterization of SDs

3.4.1. DSC

DSC is a usual way to measure the thermal properties of materials[30]. The DSC thermograms of CXN, CaA, different ratios of SDs and PMs are shown in Figure 3. The DSC thermogram of intact CXN exhibited an endothermic peak at about 118°C, while that of CaA had an endothermic peak at 178°C. The endothermic peak of CaA was not sharp and narrow when compared with the synthetic chemical. In the respective SDs, the endothermic peak of the drug showed the shifting and reduction in the intensity denoting partial molecular dispersion of drug in the carrier, which could be confirmed in PXRD study. The percentage of crystallinity (χ%) for each sample was calculated using the following equation (3)[31].

$$\chi_{.}(\%) = (\Delta H_m / W \Delta H^0_m) \times 100 \quad (3)$$

where ΔH_m (J/g) is the melting enthalpy of the sample, W is the weight fraction of the drug in the sample, and ΔH^0_m is the melting enthalpy of 100% crystalline drug. From Table 1, it was shown that much lower enthalpy in the SDs was observed in comparison to pure CXN. This might be due to a notable reduction of drug crystallinity in the SDs and dispersion of drug molecules in the system.

Table 1. Enthalpy change and percent crystallinity of chloroxylenol and solid dispersions.

Pure drug (ΔH J/g) chloroxylenol 148.119	*Samples (ΔH J/g)	% Change in ΔH	% Crystallinity
	B1:1 = 76.794	48.2	103.7
	B1:2 = 56.597	61.8	120.5
	B1:3 = 40.785	72.5	108.4
	B1:4 = 27.192	81.6	91.8
	C1:1 = 74.711	49.6	104.0
	C1:2 = 44.353	70.1	90.7
	C1:3 = 32.757	77.9	88.1
	C1:4 = 23.358	84.2	78.8
	PM1:1 = 76.473	48.4	103.9
	PM1:2 = 49.152	66.8	100.0
	PM1:3 = 35.648	75.9	97.4
	PM1:4 = 27.768	81.3	93.7

*Solid dispersions prepared by (B) gel method, (C) wet granulation method

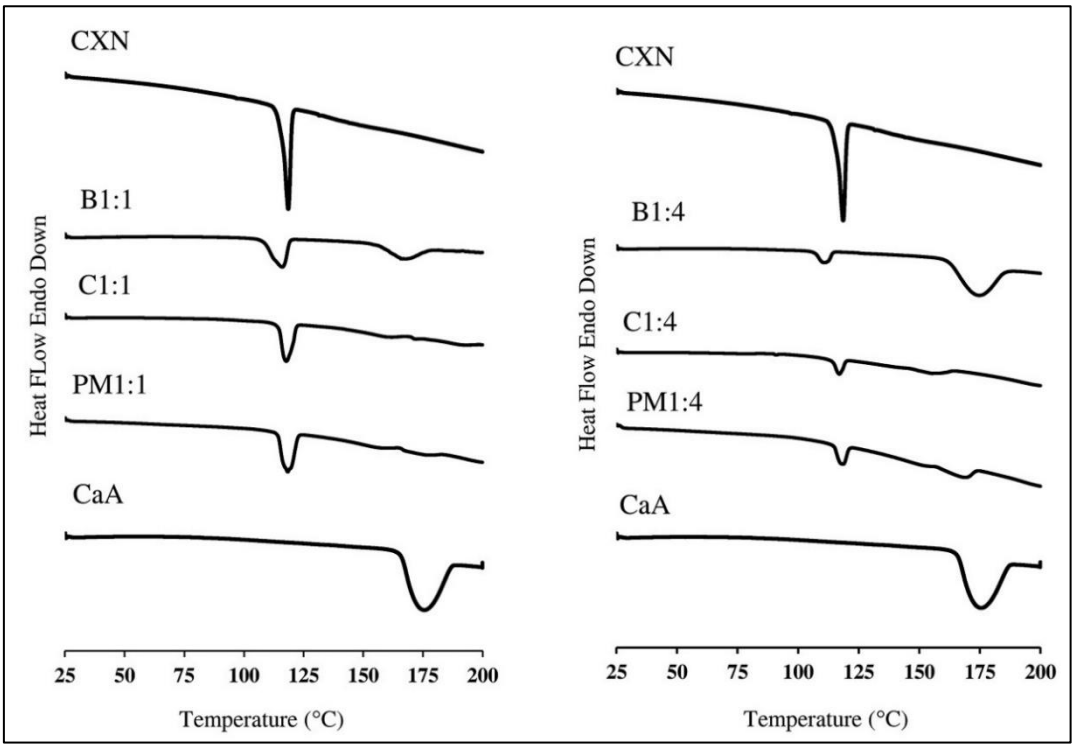


Figure 3. DSC thermograms of pure CXN, pure CaA, SDs by (B) gel method, (C) wet granulation method and PMs; 1:1 ratio (left) and 1:4 (right).

3.4.2. TGA

TGA is a useful technique to observe the weight change of a sample as a function of temperature and to evaluate the thermal stability of materials[31]. The weight loss profiles of CXN in SDs are shown in Figure 4 (left). From TGA thermograms, CXN possessed one step of weight loss, while CaA possessed two steps of weight loss (Figure 4 right).

The initial two-stage weight loss of CaA at ~100-160°C and ~160-200°C with a total percentage weight loss of 9.4% found in this study was attributed to the loss of bound water molecules[32]. The second step from 400-460°C was assigned to the melting of CaA[33]. In the initial two-stage weight loss, the first loss was the loss of CaA monohydrate $Ca(CH_3COO)_2.H_2O$, and its subsequent formation of semi-hydrate calcium acetate $Ca(CH_3COO)_2.½H_2O$ that released water and became dehydrated at 160-200°C (equations 4-5).

$$Ca(CH_3COO)_2.H_2O \rightarrow Ca(CH_3COO)_2.½H_2O + ½H_2O \quad (4)$$

$$Ca(CH_3COO)_2.½H_2O \rightarrow Ca(CH_3COO)_2 + ½H_2O \quad (5)$$

The temperature 400-460°C indicated that the dehydrated CaA decomposed into calcium carbonate and acetone (equation 6) with a combined percentage weight loss of 25%.

$$Ca(CH_3COO)_2 \rightarrow CaCO_3 + CH_3COCH_3 \quad (6)$$

The thermal decomposition of CaA observed in this study was in good agreement with other published data[32,34].

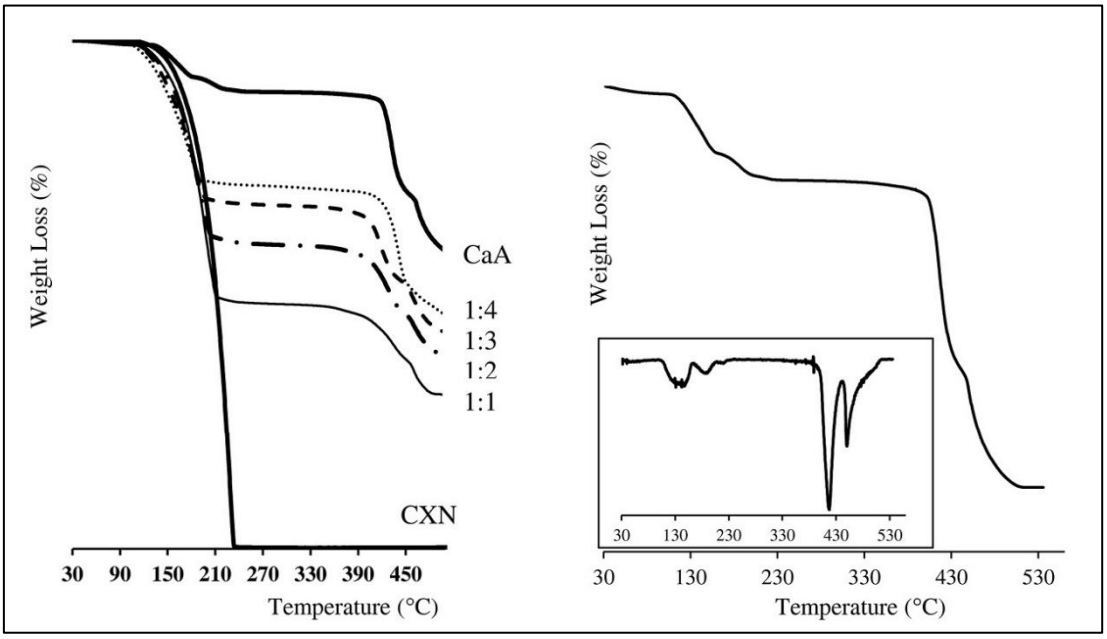


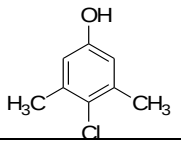
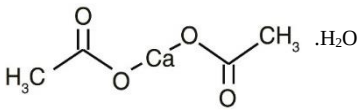
Figure 4. TGA thermograms of pure CXN, pure CaA, SDs prepared by gel method (left) and TGA and DTG (small figure) thermograms showing the thermal decomposition of CaA monohydrate (right).

SDs exhibited two degradation events, which could be related by the release of CXN from the samples and subsequent degradation of CaA itself. The degradation temperature (T_d) of CXN in the samples (<200°C) was slightly lower than that of pure drug (onset at 147°C and peak at 230°C).

3.4.3. FTIR

FTIR studies were conducted to investigate any interactions between drug and CaA in the form of SDs. Figure 5 represents the FTIR spectra of each component, their SDs and PMs. Table 2 shows the major functional groups of drug and CaA. Pure CXN displayed a broad band due to the phenolic O-H stretching at 3,311 cm^{-1}, the aromatic C-C stretching at 1,586 cm^{-1} and 1,464 cm^{-1}, the in-plane O-H bending at 1,313 cm^{-1}, the in-plane and the out-of-plane C-H bending at 1,163 cm^{-1} and 848 cm^{-1}, respectively, and a peak due to C-Cl stretching at 636 cm^{-1}. In the present study, the region of interest was in the range of 1,600-600 cm^{-1} in all spectra because it was a suitable region to examine the influence of the vibration modes at the specific peaks of CXN[35]. The O-H group provided three vibrations (stretching, in-plane bending and out-of-plane bending)[29]. In this study, the O-H stretching vibration was found at 3,311 cm^{-1} in pure CXN powders. The in-plane and out-of-plane vibrations of O-H group were assigned at 1,313 cm^{-1} and 514 cm^{-1}, respectively. The peak observed at 3,180 cm^{-1} in the spectrum of CaA was due to the O-H stretching vibration. Although the overlapping of the characteristic peaks of CXN and CaA occurred, the characteristic peaks of

Table 2. Major peaks of chloroxylenol and calcium acetate monohydrate.

	Chloroxylenol	**Calcium acetate**
		
Functional group	**Wavenumber (cm^{-1})**	**Wavenumber (cm^{-1})**
O-H stretching	3311	3180
C=O stretching	-	1648
C-C stretching	1586, 1464	-
Asymmetric C-O stretching	-	1541
Asymmetric methyl bending	-	1400
Symmetric methyl bending	-	1339
In-plane O-H bending	1313	-
In-plane C-H bending	1163	-
CH_3-in plane bending	-	1021
Out-of-plane C-H bending	848	-
C-Cl stretching	636	

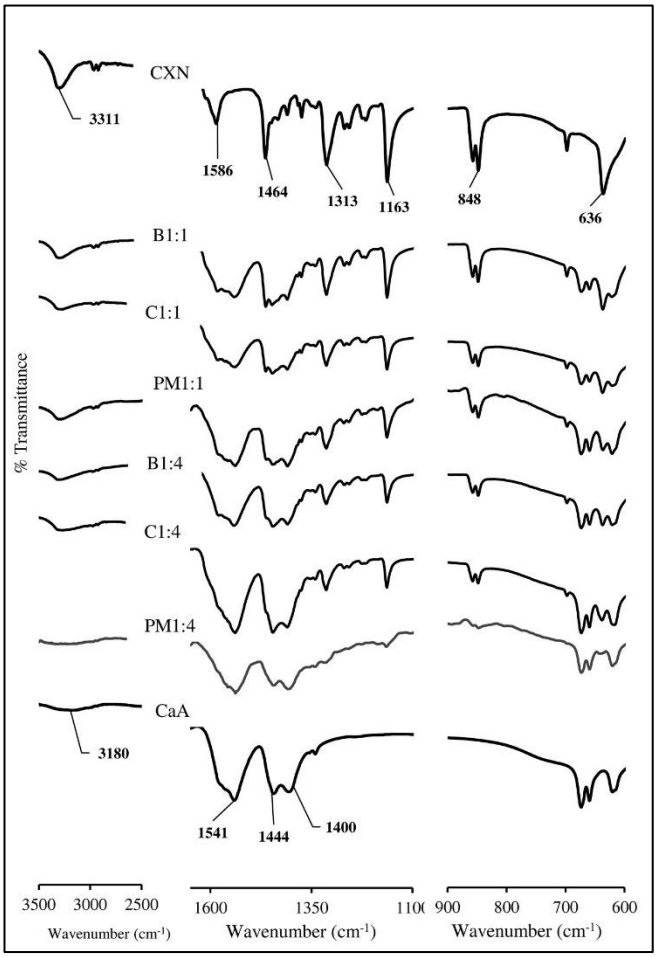


Figure 5. FTIR spectra at specified regions of pure CXN, pure CaA, SDs by (B) gel method, (C) wet granulation method and PMs.

CXN at 1,464 cm^{-1}, 1,313 cm^{-1} and 848 cm^{-1} originating from the C-C stretching, in-plane O-H bending and out-of-plane C-H bending, respectively were observed in all SDs.

It was found that there was no difference between the spectra of the sample prepared from the gel method and the sample prepared from the wet granulation method. Additionally, no spectral difference between samples from these two methods and the PMs was observed. It implied that CXN and CaA did not physically and chemically interact. However, the peak of 1,464 cm^{-1} was not clearly found in physical mixtures. It might be due to the intermolecular hydrogen bonding between the terminal hydroxyl group of CXN and water molecule of CaA[36].

3.4.4. PXRD

The PXRD patterns of CXN, CaA, SDs and PMs are shown in Figure 6. Intact CXN showed strong characteristic diffraction peaks at 2θ of 13.8°, 20.6°, 24.6°. 28.3° and 34.4°, matching the results found in Trivedi's work[21], regarding the characterization of biofield treated CXN. In the diffraction spectra of SDs, the characteristic peaks of CXN were present on lower intensity at 2θ of 13.8° and 24.6°. This finding confirmed the presence of CXN within the SDs.

The decrease in crystallinity was designated by reductions in peak intensity. Shifts or formation of new peaks/loss of peaks or complete diffuse patterns might be due to the possible loss of drug crystallinity or complexation[37]. Since the gel formation of CaA in ethanol made some of the drug molecules separate from one another, it hindered the formation of typical drug crystals. On the other hand, the gel structure acted as an inhibitor of crystal growth. This result suggested the incorporation of drug molecules into CaA particles. PXRD of SD1:4 prepared by the gel method showed a decrease in the degree of crystallinity, as evident by the disappearance of peak of CXN at 34.4°. It could be predicted that the majority of CXN had been converted to the amorphous form.

SDs prepared by the wet granulation method exhibited less crystallinity because of the lower number and height of the peak. CaA particles were already formed before preparation of SDs, however, the crystallinity of CaA was decreased after preparation due to mixing and grinding in the mortar. The drug in solution surrounded the CaA particle. After the fast solvent evaporation, the drug left on the surface of the particle was in amorphous form[38]. The results agreed with the observation from SEM analysis. PMs also showed low intensity peaks arising from CXN and CaA, indicating that crystallinity of drug and/or the carrier may have altered, probably leading to the improvement of drug dissolution.

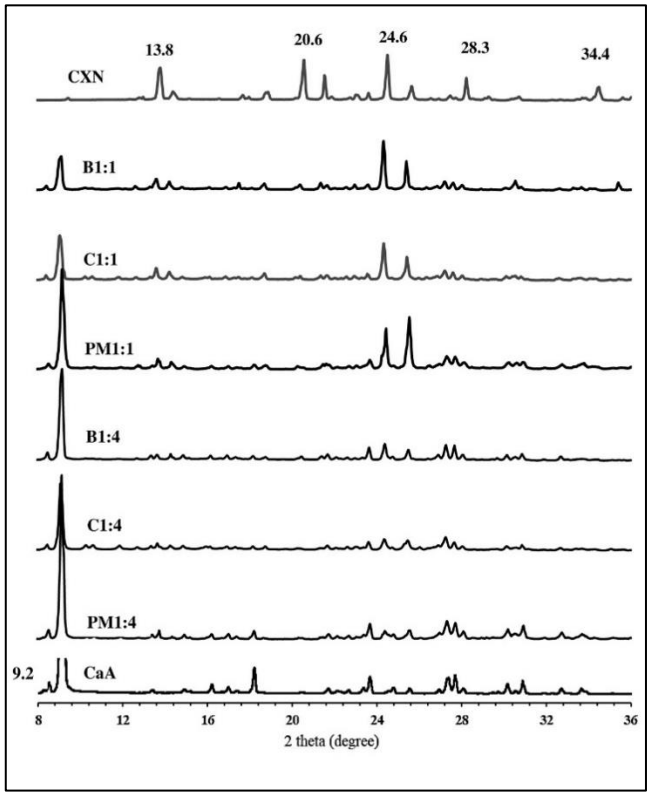


Figure 6. PXRD patterns of pure CXN, pure CaA, SDs by (B) gel method, (C) wet granulation method and PMs.

3.4.5. SEM

Information on the crystal shape, particle size of the pure materials and their morphology in SDs could be determined using SEM technique. The micrographs for the pure CXN, CaA, SDs and PMs were recorded to study their morphology (Figure 7). CXN existed in the form of well-defined crystals of cluster shape with sharp edges. CaA appeared as timber or flute-like columns. PXRD and SEM results confirmed the solid phase of CaA and they were compared to those of literatures and found to be well consistent[39-40].

SDs prepared by the gel method exhibited rod-like microstructure (Figure 7B). An increased surface area was available for CXN to attach itself to the surface of the SD or inserting into the crystalline carrier. The presence of CXN could not easily be seen within the carrier in the SDs as the morphology of the drug changed completely in the SDs due to the fine dispersion of the drug in the carrier.

SDs prepared by the wet granulation method looked relatively uneven and rough surface which provided additional surface for the deposition of drug particles (Figure 7C).

Basically, PM is the combination of two components and should be seen as individual crystals. In the PMs (Figure 7PM), the CXN crystals that were mixed with the excipient or adhered to its surface retain the crystalline structure of the drug as small lumps of irregular size.

The difference in surface features between SDs and PMs was attributable to the arrangement of the molecules differently during manufacturing procedures[14]. From the SEM photographs, it was possible that CXN in SDs existed either in amorphous form or partially crystalline form. Furthermore, it was expected that the amorphous state or small particle size of CXN along with an increased surface area and the close contact between the drug and the hydrophilic carrier might be responsible for the improved wettability and enhancement of the dissolution of the drug, as confirmed by the results of dissolution presented in Figure 8.

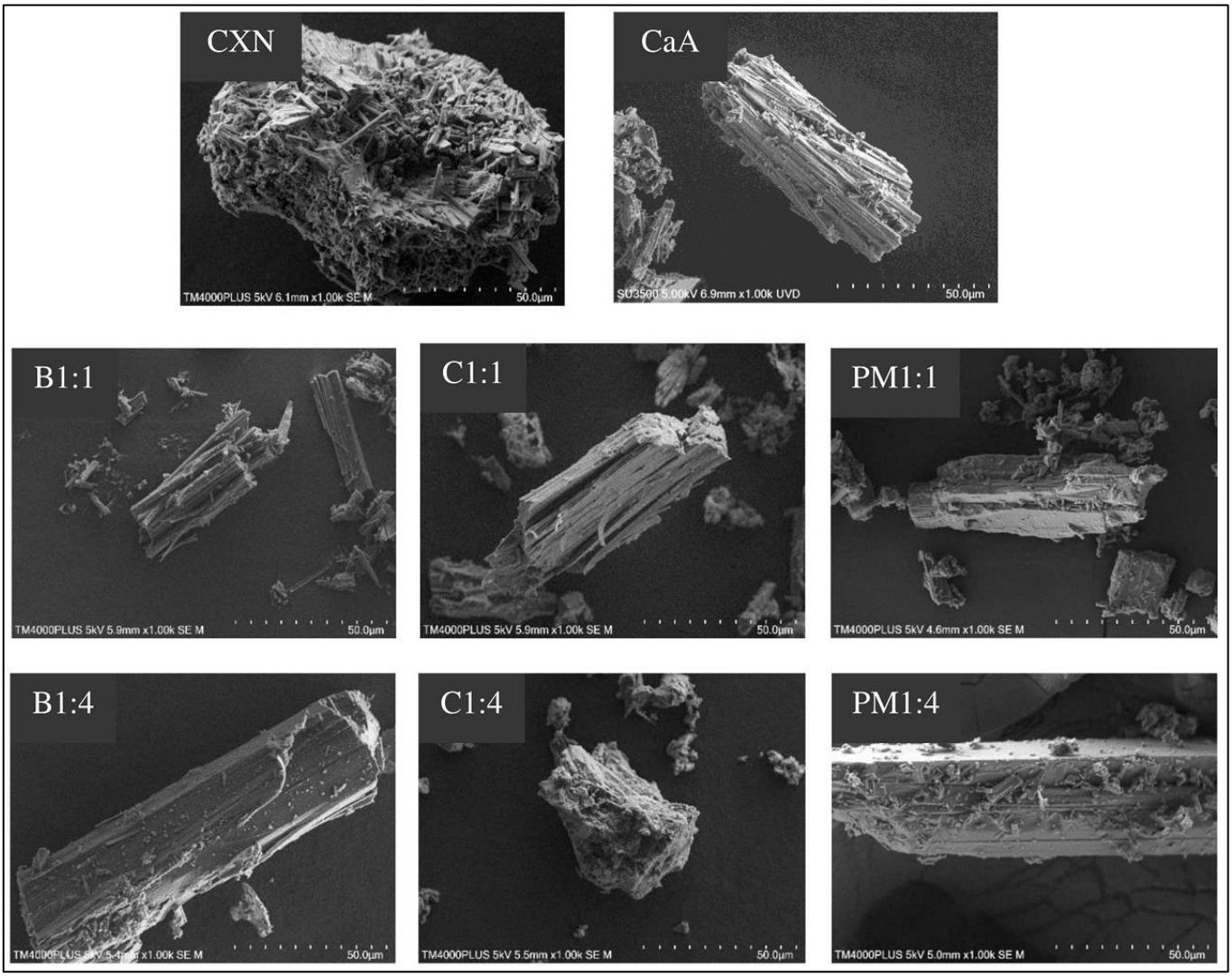


Figure 7. SEM photographs of pure CXN, pure CaA, SDs by (B) gel method, (C) wet granulation method and PMs taken at a magnification of 1000x.

3.4.6. Drug content determination

The amount of CXN in SDs and PMs was analysed by using spectrophotometer at 282 nm and the results are shown in Table 3. The drug contents of SDs ranged from 95.06% to 101.61% and 96.93% to 100.33% for gel method and wet granulation method, respectively. The percent drug content of PM was found in the range of 98.80% to 99.82%. The percent drug content of all formulations was within the acceptable limits (90%-110%), indicating the suitability of method for the preparation of SDs.

Table 3. Percentage drug content of chloroxylenol in solid dispersions.

No.	*Formulations	% Drug content (mean ± SD, n=3)
1	B1:1	99.91 ± 1.70
2	B1:2	95.06 ± 2.58
3	B1:3	101.61 ± 0.51
4	B1:4	99.99 ± 3.46
5	C1:1	96.93 ± 2.95
6	C1:2	99.14 ± 1.47
7	C1:3	100.33 ± 1.11
8	C1:4	99.82 ± 1.11
9	PM1:1	99.40 ± 0.39
10	PM1:2	99.57 ± 0.68
11	PM1:3	98.80 ± 2.03
12	PM1:4	99.82 ± 0.68

*Solid dispersions prepared by (B) gel method, (C) wet granulation method

3.5. *In vitro* dissolution study of solid dispersions

The dissolution profiles of the SDs are shown in Figure 8. Pure drug showed a poor dissolution profile (i.e., only 29.11% of drug was released at 45 min), whereas PMs revealed a slight improvement in dissolution profiles due to the presence of carrier in the respective mixtures. Immediate release dosage forms are those with at least 85% of the labelled amount which dissolves within 15, 20-30, or 45 min[41]. *In vitro* release studies revealed immediate release profiles of drug at all ratios of SDs prepared by both methods at 45 min as presented in Figure 8(i) and Figure 8(ii). All SDs showed a significant improvement in drug release at 30 min compared to pure drug (p<0.05). SD prepared by the gel method showed fast release of CXN (93.64-113.22%), and SD prepared by the wet granulation method released (92.41-94.88%) from the wet granulation method in water medium at 60 min followed by a gradual release over 2 h. CaA, a hydrophilic carrier, might enhance compound hydration, and thus led to faster dissolution. If the carrier is soluble in the dissolution medium, the release of SD is the dissolution-controlled mechanism[42]. SDs based on hydrophilic carriers dissolved immediately in water and a steady concentration of dissolved drug was attained within first few hours of dissolution of these SDs[43]. Based on the release profiles, SDs showed a very slightly decrease in the release pattern of drug after the maximum release. It was due to the CaA, when it dissolved, generated heat to the surrounding[20] and subsequently more drug was dissolved. Temperature was an important factor for drug solubility. Generally, an increase in temperature would result in increased solubility and dissolution of most drugs. An external increase in temperature would inhibit the dissolution of some drugs with exothermic behaviour as an excess of heat was already generated and lower solubility was achieved if temperature was increased. And thus variation in temperature might directly enhance the rate of dissolution[44]. After the maximum release, the drug concentration in solution was slightly decreased. As the heat from the exothermic reaction of CaA in water dissipated quickly in the surrounding dissolution medium, the temperature of the dissolution medium was decreased. This led to the decrease in the solubility of the drug.

In SDs, dissolution rate was enhanced as a consequence of increasing carrier concentration up to ratio of 1:4. Hence, SD1:4 was selected to compare the effectiveness of the gel method and the wet granulation method. Figure 8(iii) represents the dissolution profiles of PMs. Dissolution rate of PMs was higher than the pure CXN, which might be because of high hydrophilicity of the carrier[45].

Figure 8(iv) compares the dissolution profile of SDs and PM prepared in 1:4 ratio. At 30 min, SD prepared by the gel method revealed 105.88% drug release, while SD prepared by the wet granulation method showed 91.64% drug release. Statistical analysis showed that there was a significant difference (p=0.047) in the percent drug release between SDs prepared by the gel method and the wet granulation method at 30 min. Thus, SD1:4 prepared by the gel method is better in drug release than SD1:4 prepared by the wet granulation method. However, no statistically significant difference (p>0.05) in dissolution at 30 min was observed between samples from these two methods of less carrier ratios.

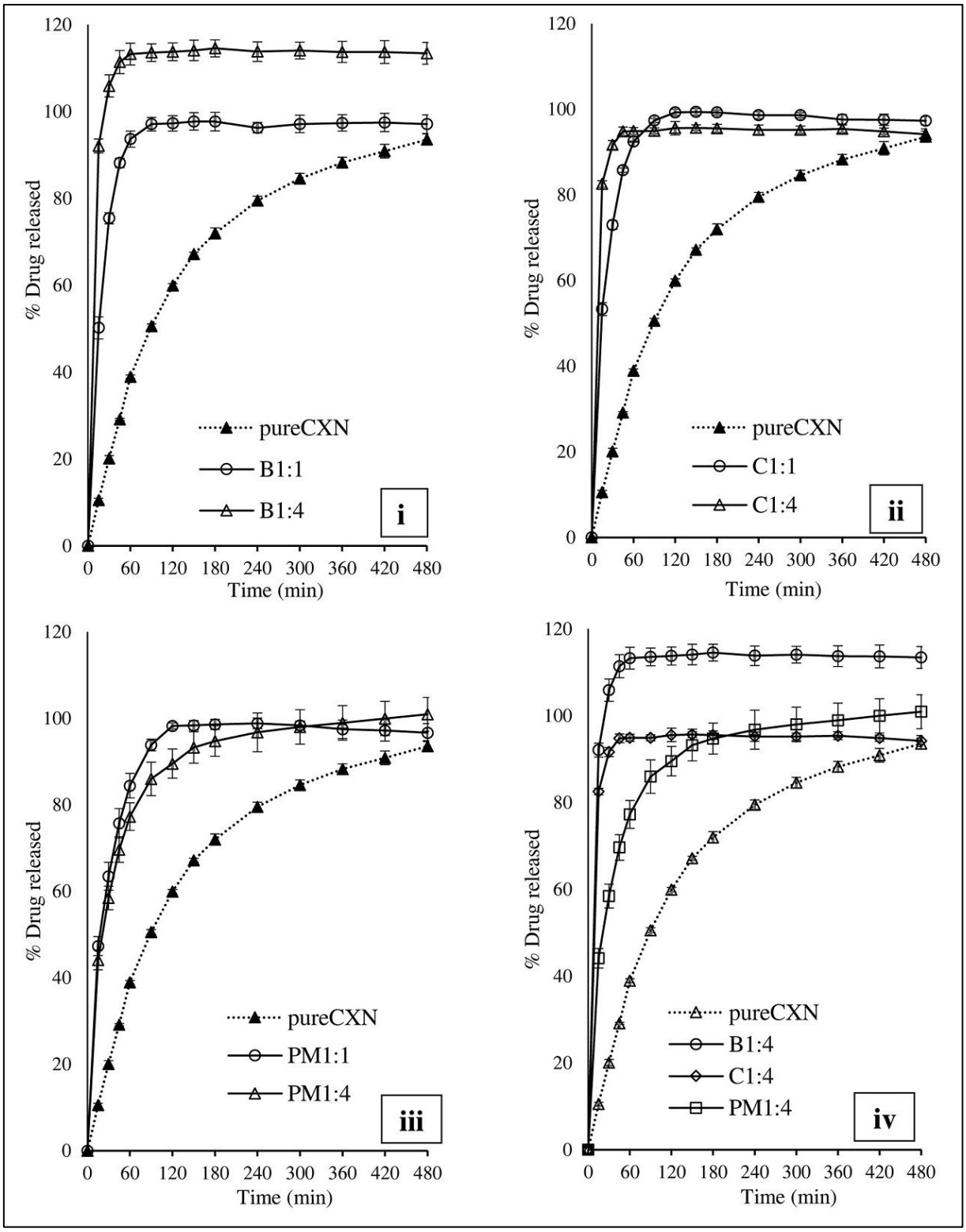


Figure 8. Dissolution profiles of CXN in (i) SDs by gel method, (ii) SDs by wet granulation method, (iii) PMs and (iv) dissolution profiles of CXN in 1:4 ratio of SDs (B) by gel method (C) by wet granulation method and PM.

From these results, it was observed that SDs prepared by the gel method appeared to be most effective in drug release as well as in dissolution rate compared to pure CXN and PMs. Moreover, the gel forming technique used in this study was advantageous to prepare SDs of poorly soluble drugs using the hydrophilic carrier, CaA from the ES waste because of minimizing pharmaceutical excipient cost, using the environmentally safe carrier, less toxic organic solvent as ethanol and relatively short preparation time.

The improved dissolution could also be attributed to a reduction in particle size of the drug (supported by SEM study), partial drug transformation into an amorphous state (supported by PXRD study), its deposition

on the surface of the carrier, and improved wettability. A solid dispersion consisted of a hydrophilic carrier in which the drug was dispersed molecularly or as very small particles. The mechanisms by which the dissolution rate of the drug is improved are: (i) reduction of particle size of the drug, resulting a larger surface area available for dissolution (ii) increased wetting properties of the drug, and (iii) the higher energy state of the amorphous form compared to the crystalline form leads to a higher solubility of drug [45].

4. CONCLUSION

In this study, CaA powder was successfully prepared from the ES by the chemical reaction with aqueous acetic acid. The resulting salt of calcium was CaA monohydrate. As CaA was hydrophilic in nature, it could be a filler or diluent for solid dosage form or a promising carrier of SD for poorly water soluble drugs. SDs were prepared by the gel method and the wet granulation method using CXN as a model drug. Consequently, the gel forming technique was technologically simple, time-saving to prepare SDs with good qualities and cost-effective as ethanol could be recovered and reused. The results of this study clearly indicated that the dissolution rate of CXN could be enhanced by preparing SDs using different methods and certain proportions of carriers. Improved drug dissolution was likely resulted from molecular dispersion of drug in the hydrophilic carrier, at least partial transformation of crystalline to amorphous CXN, and thus improved drug wettability by the dissolution medium. This was more obvious in SDs prepared by the gel method as compared to the wet granulation method, seemingly due to the effects of CaA forming gel in ethanol, and thus hindrance of crystal growth of drug particles in gel structure. Salt formation, inhibition of crystal growth and increase in wettability by the carrier could be possible mechanisms for the improvement in dissolution. The carrier improved the wettability by forming a layer around the drug, thus lowering the hydrophobicity of CXN. The loss of drug crystallinity in the SD systems were confirmed by DSC, PXRD and SEM. Therefore, the current study demonstrated the high potential of the gel forming technique for obtaining SDs for poorly water-soluble drugs using CaA as a hydrophilic carrier.

5. ACKNOWLEDGEMENT

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Conflict of interest
None to declare.

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Ethics approval
None to declare.

Author contribution
ZM conducted the experiments, data collection and prepared the manuscript. PPL conceived and designed the experiments, contributed reagents, materials and analysis tools, supervised the project and edited the manuscript. All authors have read the manuscript and agreed to approve the manuscript submission.

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