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Eutectics of Oxaprozin: Synthesis, Characterization, and Evaluation for the Management of Osteoarthritis.

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Abstract

The research work described focuses development of a multicomponent solid form of the antiosteoarthritic drug Ozaprozin (OXN) to improve its biopharmaceutical properties. By using mechanochemical synthesis method to create a new solid form of OXN by combining it with coformers that possess antioxidant properties, namely ascorbic acid syringic acid, and nicotinic acid. This combination resulted in the formation of three eutectic mixtures (EMs). The formation of eutectic mixtures was confirmed using differential scanning calorimetry, and the exact stoichiometry of the mixtures (50/50% w/w) was established through phase diagrams and Tammam's triangle. The strong homomeric interaction between the individual components and steric hindrances played a role in the formation of these eutectic mixtures. The eutectic mixtures showed improved apparent solubility (five- to nine-fold) and a significant enhancement in the intrinsic dissolution rate (two- to three-fold) compared to the plain drug. This suggests that the multicomponent solid forms of OXN have the potential to enhance the drug's solubility and dissolution rate, which are crucial factors for drug absorption and bioavailability. Further studies were conducted to evaluate the biopharmaceutical performance of the eutectic mixtures. In vivo pharmacodynamic studies

demonstrated a significant improvement in the performance of the eutectic mixtures compared to the pure drug. These findings suggest that the multicomponent solid forms of OXN could potentially enhance the therapeutic efficacy of the drug in the management of osteoarthritis

1. Introduction

The field of crystal engineering has played a significant role in improving biopharmaceutical issues of poorly soluble drug molecules in recent years. Complementarity between molecular recognition sites is used in this technique to complex the drug with another molecule (coformer) in a supramolecular assembly stabilized by hetrosynthon (Bolla et al., 2022). Nevertheless, weaker interaction between binary components and lack of unique crystal packing leading to eutectic formation is regarded as an alternate significant outcome of cocrystallization. Eutectics are mixtures of two components that are completely miscible in liquid form but immiscible in solid form. The homomeric molecular interaction between components in eutectic mixtures dominates the heterogeneous interaction between components at specific molecular ratios(Bazzo et al., 2020). It is possible, however, to design cocrystal and eutectic solid forms as multicomponent solids with the same crystal engineering principles attesting to the enormous utility of multicomponent solid forms in improving the bioavailability of drugs that are insoluble in water. Eutectic mixtures have shown promising effects in tailoring biopharmaceuticals as well as their material properties(Park et al., 2020). The present study demonstrates the beneficial effect of eutectics of the anti-osteoarthritic drug Oxaprozin.

Osteoarthritis is a degenerative joint disease that affects millions of people worldwide. It occurs when the protective cartilage that cushions the ends of bones wears down over time, leading to pain, stiffness, and reduced mobility in the affected joints(Hunter and Bierma-Zeinstra, 2019). Moreover, osteoarthritis places a substantial burden on healthcare systems and the economy. It contributes to increased healthcare costs, including doctor visits, medications, and surgical interventions such as joint replacements(Leifer et al., 2022). Additionally, it may lead to work absenteeism and reduced productivity, impacting both individuals and society as a whole. Biopharmaceutics Classification System (BCS) classifies OXN as a class II drug with low aqueous solubility i.e. 0.0325 mg mL^{-1}(Alshehri et al., 2022). Furthermore, OXN was found to meet the low solubility criteria of the BCS in the pH range of 1.2-7.2(Yazdanian et al., 2004). Various strategies such as combining substituted

cyclodextrins, bile acids, and chitosan, salt formation with alkali(Mura et al., 2016), drug-drug cocrystallization(Aitipamula et al., 2016), solid dispersion of OXN with a hydrophilic carrier(TANABE et al., 1994) have been applied to address this issue. Although amorphous systems are suspective to devitrification and loss of crystallinity upon solid dispersion directed towards stability issues whereas salt formation has increased solubility and dissolving rate with limited benefits. To overcome these limitations, it is, therefore, necessary to come up with an alternative approach.

Furthermore, it is widely known that the management of osteoarthritis apart from different classes of drugs, antioxidants play a vital role in the treatment of osteoarthritis by reducing oxidative stress and inflammation, protecting cartilage from damage, and supporting cellular health. By scavenging free radicals and modulating enzyme activity, antioxidants help maintain the integrity and function of cartilage, alleviate pain, and slow down disease progression (Martello et al., 2021; Ong et al., 2020; Tudorachi et al., 2021). Accordingly, based on the benefits of multicomponent solid forms and the relationship between oxidative stress and osteoarthritis, multicomponent solid forms of OXN may show beneficial effects in osteoarthritis management.

The presence of oxazole ring and carboxylic acid group in OXN makes it a potential candidate for the development of its multi-component solid forms as reported in the literature (Aitipamula et al., 2016). As a result, a non-covalent derivative of the drug was developed with coformers that may enhance biopharmaceutical parameters, anti-inflammatory action, and reduce oxidative stress. The present study focuses on the mechanochemical synthesis of multicomponent solid forms of OXN with selected coformers (Fig. 1) viz. Ascorbic acid (AS), Syringic Acid (SY), and nicotinic acid (NI) have complementary functional moieties. In addition to being highly soluble, they are reported to show antioxidant characteristics that protect against oxidative stress and anti-inflammatory action in osteoarthritis(Hu et al., 2014; Lindsey et al., 2019; Misra et al., 2023). According to preliminary research, showing that the drug's ground product with coformers produced eutectic mixtures. These mixtures have been characterized and evaluated for apparent solubility, intrinsic dissolution rate, and in vivo pharmacodynamic studies.

2. Experimental

Materials OXN were acquired from TCI, Chemicals (India) Private Limited. Ascorbic acid (QA, with a purity of 99%), Syringic Acid (CH, with a purity of 99%), and nicotinic acid (NI,

with a purity of 99%) were obtained from Himedia Laboratories Pvt. Ltd., based in Mumbai, India, and used as received, without requiring any purification. All additional solvents and chemicals used were of analytical grade.

2.1 Preparation of Eutectic

Mixtures of OXA and selected coformers (SA, NA, and A) were individually weighed in different compositions (20:80, 40:60, 50:50, 60:40, 70:30, and 80:20, % w/w). The mixtures were subjected to solid-state grinding using an agate mortar and pestle assisted by the addition of 100 µL ethanol for 30 min. As a result, ground material was dried and stored in a desiccator for further characterization.

2.2 Differential Scanning Calorimetry

The accurately weighed (about 2mg) samples were placed into an aluminum pan, the head and body of the pan were crimped and placed into the sample holder of DSC (Q20, TA Instruments Waters (LLC, USA). The samples were heated from 30 to 400 ° C at a rate of 10 ° C. per minute for thermal examination. The experiment was carried out with nitrogen gas with a flow rate of 50ml/min and an empty aluminum pan as a control. TA Q series Advantage software (Universal Analysis 2000) was used to evaluate the data.

2.3 Hot Stage Microscopy

In order to capture microscopic images of prepared cocrystals on hot stage, a NIKON-ECLIPSE-LV100NPOL equipped with a hot stage and TMS 94 temperature controller from Linkam Scientific Instruments Ltd. was used. A hot stage was used to mount the eutectic mixture. During the heating process, the samples were heated from 50 °C to 100 °C at 10 °C/min, followed by heating to 125 °C at 5 °C/min under a microscope at 10X zoom. At 125 °C, the rate of heating was increased to 10 °C/minute, followed by 150 °C/minute, and finally 5 °C/minute at 200 °C.

2.4 Powder x-Ray Diffraction

On the X'Pert PRO diffractometer system (PANalytical, Almelo, Netherlands) with a Cu K radiation (1.54060), powder samples underwent powder x-ray diffraction (PXRD).45 kV and 40 mA were chosen as the tube voltage and current, respectively. For the diffraction

experiment on the 10-mm sample size, the divergence slit and anti-scattering slit parameters were set at 0.48°. With a step size of 0.017° and a step length of 25 s/step, each sample was inserted in an aluminium sample holder before being measured using a continuous scan between 3.5 and 50° in 2. The X'Pert High Score software was used to improve the experimental PXRD patterns.

2.5 Fourier Transform Infrared Spectroscopy

An FTIR spectrometer, the Spectrum II FT-IR (Perkin Elmer, UK), was used to assess the influence of co-processing on the structural characteristics of the drug (OXA) and Coformers (QA, NI, and CA)., The materials were combined with potassium bromide before being compressed into tiny discs. Each disk's FTIR spectra were recorded in the 4000–400 cm-1 range. After that, Spectrum software (PerkinElmer, UK) was used to process the data.

2.6 Scanning Electron Microscopy

The morphology of individual particles in the pure components and eutectic mixtures (EMs) was examined using JSM-6100; Jeol, Peabody, MA, USA scanning electron microscope. To prepare the samples for imaging, they were mounted on a metal stub using adhesive tape and then coated with a gold layer under vacuum conditions using an ion splitter (JFC-1100).

2.7 Apparent solubility studies

The study on the solubility of Oxaprozin (OXN) and its prepared eutectic mixtures was conducted using the shake flask method, as described by Higuchi and Connors in 1965 (Higuchi and Connors, 1965). In this method, an excess amount of OXN (approximately 50 mg) and its eutectic mixtures were mixed in a vial containing 5 mL of phosphate buffer with a pH of 7.4(Aitipamula et al., 2016). The mixture was agitated for 24 hours using a water bath shaker set at 200 RPM and maintained at a temperature of 37 °C. After agitation, 0.2 mL of the resulting slurry was filtered through a 0.45 µm membrane filter. The filtrate was then diluted 10 times, and the quantitative analysis of OXN was performed using high-performance liquid chromatography (HPLC). The final results of the solubility study were expressed as mean ± standard deviation (SD) values.

2.8 Pharmacodynamic Study

In a pharmacodynamic study, female Wistar rats with adjuvant-induced arthritis were used. The rats, aged 3-6 weeks and weighing 180-320 grams, were treated with different eutectic

mixtures of oxaprozin (ET-AS, ET-SY, and ET-NI) and the study lasted for 28 days. Arthritis was induced by injecting a single dose of 0.2 mL of Freund's Complete Adjuvant subcutaneously into the subplantar region of the left hind paw. Arthritic symptoms appeared in the rats after 15 days of induction. Throughout the study, various parameters such as body weight, paw volume, and joint diameter in the left hind paw were measured on the 3rd, 7th, 12th, 15th, 21st, and 28th days. Six readings were taken and the data were presented as mean ± SEM. Statistical analysis was performed using Graph Pad Prism 5.0 software, utilizing two-way ANOVA followed by Bonferroni's post hoc test. The pharmacodynamic data were compared among the different groups, including the drug, healthy control, and arthritic control groups(Tomar et al., 2020).

3. Result And Discussion:

This section covers the comprehensive results and discussion on the designing, preparation, characterization, and evaluation of Oxaprozin and its respective eutectics. Based on previous literature studies, OXN, due to its carboxylic group and oxygen of the oxazole ring(figure 1), is a potential candidate for the generation of multicomponent solid forms (Schierle et al., 2021). The important role of antioxidants in reducing the stiffness, joint pain, inflammation, and immunity impairments associated with arthritis has been well-established (Pal et al., 2023)(Levy et al., 2009). They help to treat osteoarthritis by reducing oxidative stress, which can cause joint damage. Their anti-inflammatory effects can alleviate pain and swelling associated with the condition. Besides this, they also have chondroprotective effects, preserving cartilage integrity. They moderate signaling pathways which are involved in cartilage metabolism and inflammation, potentially slowing down disease advancements(Veen et al., 2021)(Grover and Samson, 2016). Because of this, it was intended to use selected molecules with antioxidant potential conformers to build non-covalent bonds with OXN that may achieve desired outcomes, such as enhancing biopharmaceutical performance. In this context, thermal analytical techniques were used on ascorbic acid, syringic acid, and nicotinic acid (Figure 2). The drug -coformer ground product showed all of them found to be eutectics. The prepared eutectics OX-AC (Oxaprozin: ascorbic acid), OX-SY (Oxaprozin: Syringic acid), and OXNI (Oxaprozin: Nicotinic acid) were further examined using other thermoanalytical techniques. A decreased melting endotherm in DSC served as the main defining characteristic of the eutectics. Additionally, Eutectics showed a unique V-shaped binary phase diagram and no noticeable modifications in the FT-IR and PXRD (Butreddy et al., 2021).

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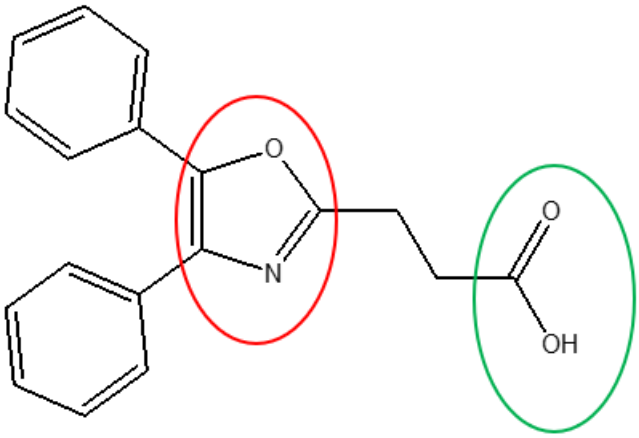


Fig 1: Chemical structure of OXN with potential functional groups.

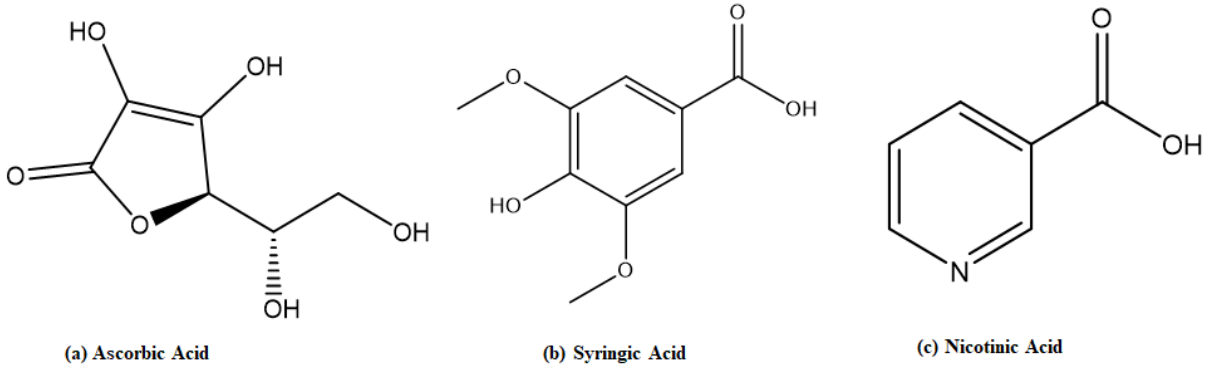
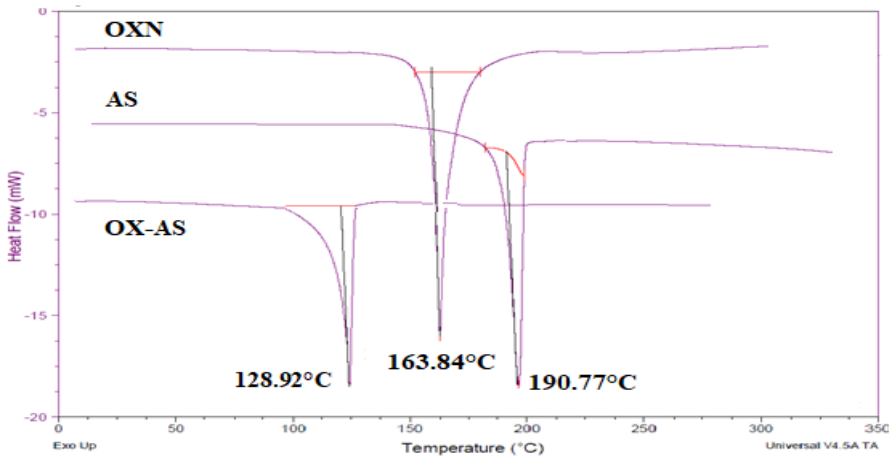


Fig. 2: Chemical structure of coformers(a-c) that produced eutectics with OXN.

3.1 Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) is a thermal analysis technique commonly used to study phase transitions and thermal properties of materials. The resulting DSC thermogram provides information about the sample's thermal behavior, including melting points, enthalpy changes, and phase transitions. All the DSC thermograms for Oxaprozin, the conformers and eutectics (OX-AS, OX-SY and OX-NI) are shown in Figure 3 (a-c).



(A)

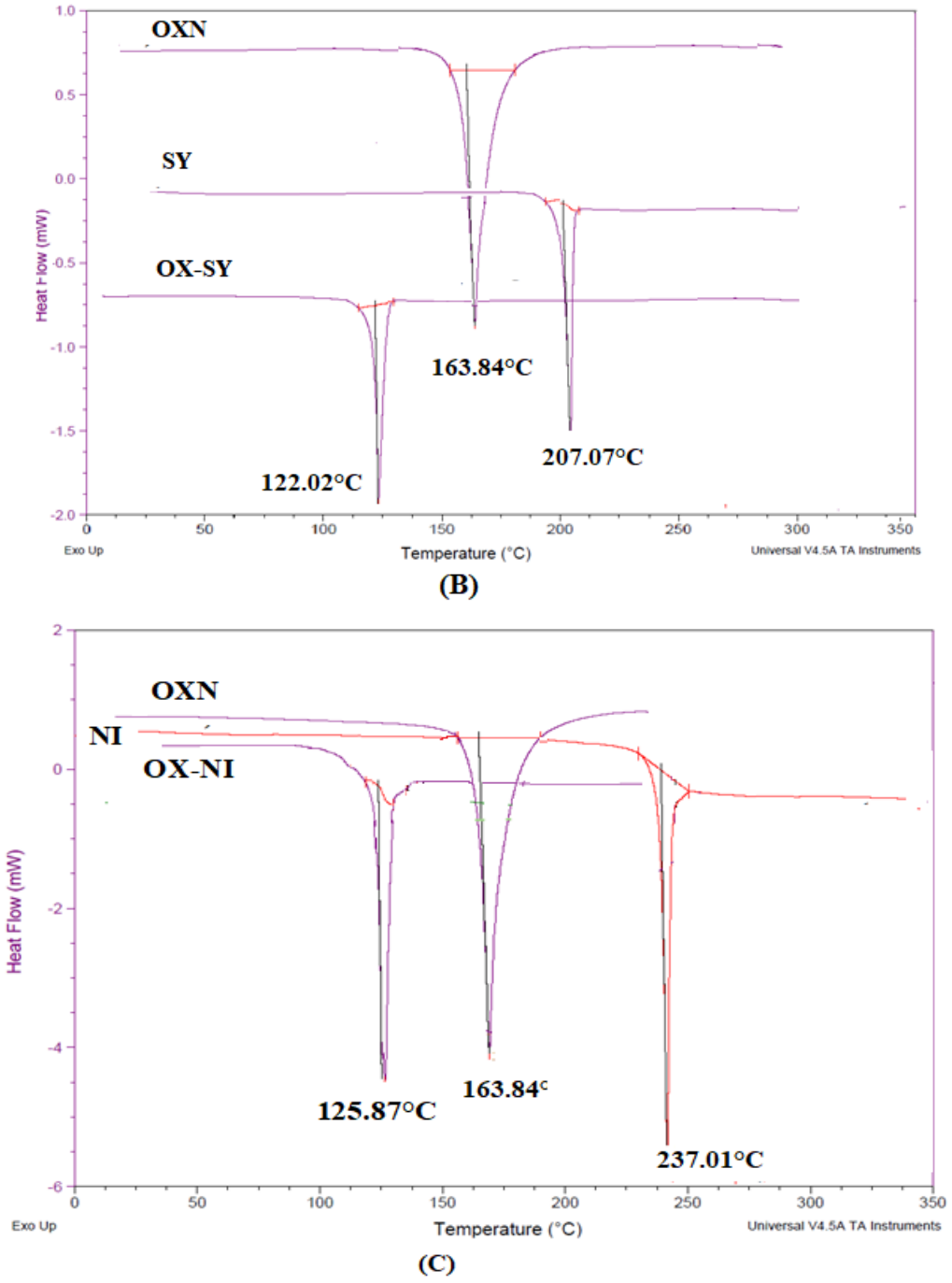


Fig 3 : DSC thermograms of (a) OX-AC(b) OX-SY(c) OX-NIC

In case of OX-AC**,** Figure 4 Demonstrates the melting endotherm for Etodolac and ascorbic acid at 163.84°C and 190.77°C, respectively. However, as the AS concentration gradually increased from 10% molar composition in the various binary compositions of OXN and AS, another endotherm formed at 120.73°C Figure 4(b). As the OXN composition was increased,

a lower melting endotherm became evident in mixtures containing Oxaprozin: ascorbic acid at specific ratios. The temperatures at which this endotherm appeared[Figure 4 (b-e)] were 120.763°C (10% oxaprozin: 90% ascorbic acid), 125.26°C (20% oxaprozin: 80% ascorbic acid), 128.92°C (30% oxaprozin: 70% ascorbic acid), and 125.23°C (40% oxaprozin: 60% ascorbic acid).

The melting endotherm associated with pure AS experienced a shift from 190.77°C to lower temperatures of 182.18°C, 169.38.°C, 155.24°C, and 139.07°C as the molar ratios of etodolac to syringic acid varied to 10:90, 20:80, 30:70, and 40:60, respectively. Beyond this stage, when the concentration of oxaprozin reached 50%, a single endothermic peak emerged at 128.92°C, indicating the formation of a eutectic mixture.

Continuing to increase the percentage of etodolac in the binary mixture beyond 50%, subsequent melting endothermic peaks were observed at temperatures of 135.23°C, 137.41°C, 149.22°C, 155.42°C, and 163.84°C. These temperatures corresponded to the molar ratios of (60:40), (70:30), (80:20), and (90:10), respectively. However, these melting endotherms were accompanied by the emergence of new endotherms at temperatures of 125.23°C, 124.35°C, 123.68°C, and 121.94°C. These temperatures corresponded to the compositions of 60:40, 70:30, 80:20, and 90:10 of OXN (oxaprozin) and AS (ascorbic acid), respectively. These new endotherms were attributed to the presence of excess unreacted OXN in the mixture.

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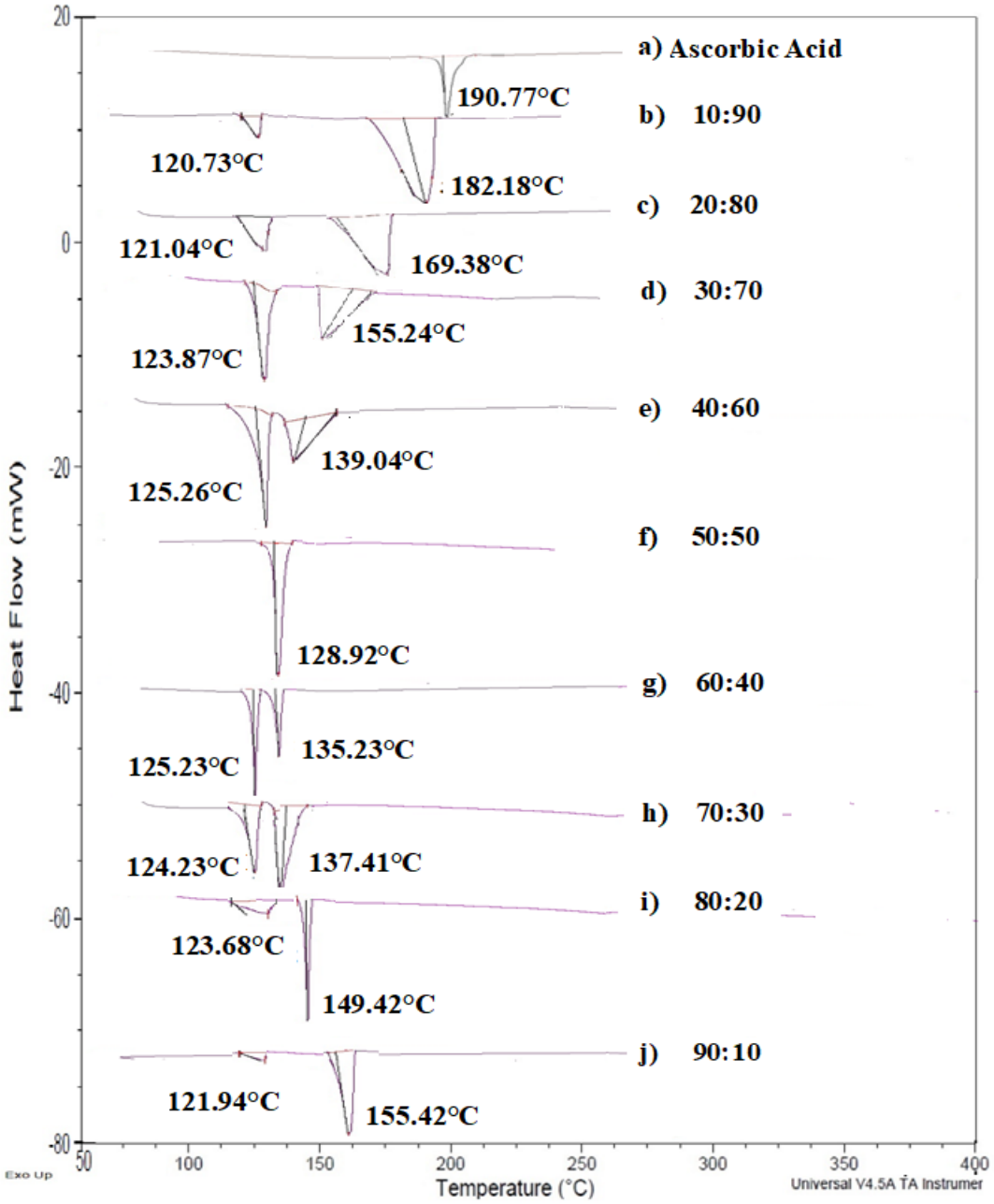

Fig 4 : DSC thermograms of Ascorbic acid and all compositions of OX-AS

The different endothermic peaks can be found in **Figure 4** (a-i), and a clear comparison is made with the endothermic peaks of OXN and AS . The consistent position of the low melting endotherm across all compositions is referred to as the solidus. The 50:50 composition of Oxaprozin: ascorbic acid , which exhibits a single eutectic endothermic peak, represents the true stoichiometry at which the eutectic OX-AS is formed.

Therefore, the presence of the low melting endotherm in the DSC analysis, the absence of new peaks, and the reappearance of the individual peaks of the constituents in the FT-IR and PXRD spectra of the eutectics serve as additional confirmation of the formation of the eutectic phase.

Likewise, in the case of the eutectic **OX-SY** depicted in Figure , the low melting endotherm was observed at temperatures of 117.56°C, 122.01°C, 123.68°C, and 121.21°C as the ratio of OXN and SY varied between (10:90, 20:80, 30:70, , and 40:60). A single melting endothermic peak corresponding to the 50:50 ratio appeared at 122.02°C, which serves as the consistent solidus point of OX-SY and is lower than both Oxaprozin (melting point = 163.84°C) and Syringic acid (melting point = 207.03°C). This composition also exhibits the highest enthalpy of the eutectic. Subsequently, a peak corresponding to SY appeared at temperatures of 123.87°C, 126.58°C, 121.43°C, and 124.98°C.

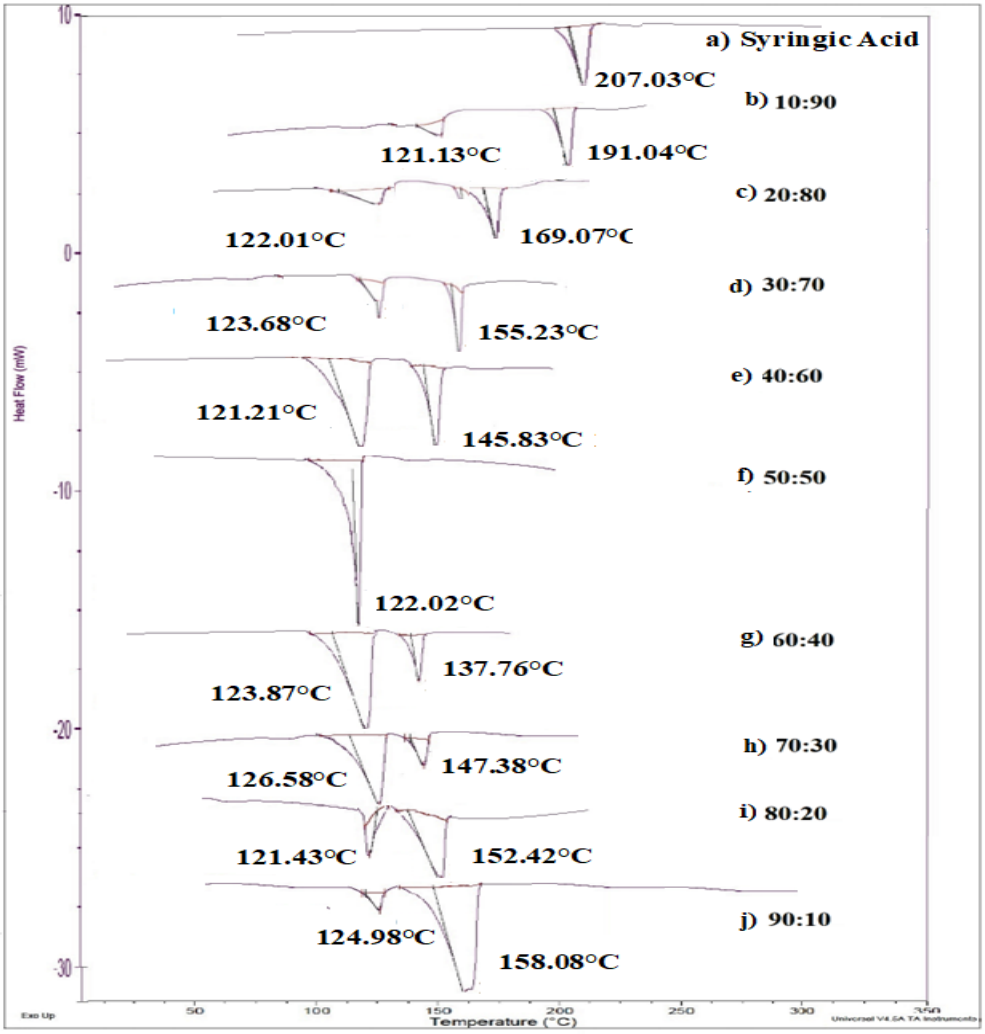

Fig 5: DSC thermograms of Syringic acid and all compositions of OX-SY

Similarly, in the eutectic OX-NI shown in Figure 6, lower melting endothermic peaks were observed at temperatures of 115.13°C, 117.48°C, 119.25°C, and 120.08°C for compositions of etodolac and nicotinic acid in ratios of 10:90, 20:80, 30:70, and 40:60, respectively. In the 50:50 composition of Oxaprozin: Nicotinic acid, a single eutectic endothermic peak appeared at 125.87°C, representing the solidus point of OX-NI. This temperature was found to be lower than the melting points of both constituents. Furthermore, this particular composition exhibited the highest enthalpy of fusion for the eutectic phase.

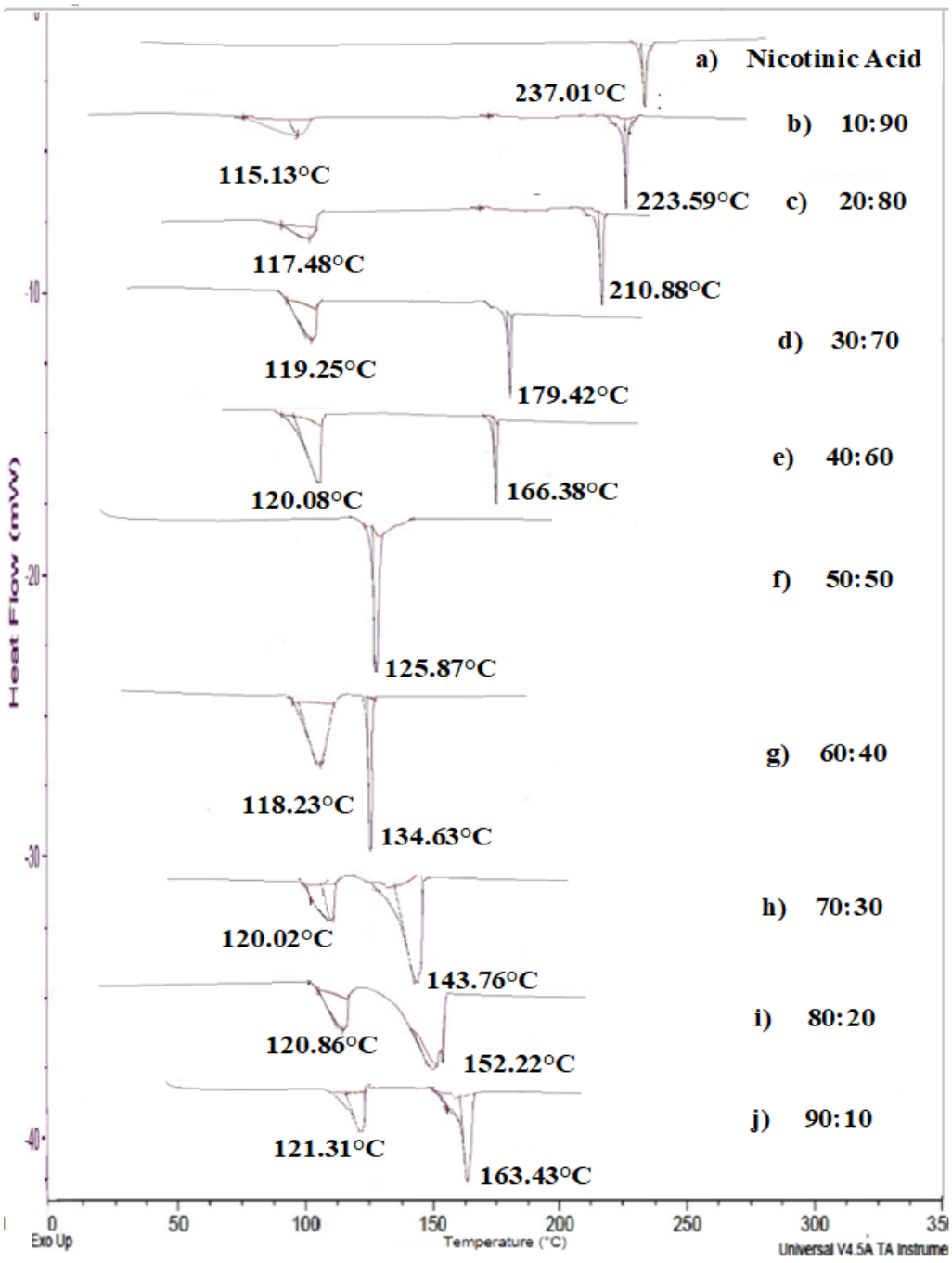

Figure 6: DSC thermograms of Ascorbic acid and all compositions of OX-NI

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3.2 Binary Phase Diagrams:

Binary phase diagrams are essential tools for understanding the formation of eutectic mixtures in pharmaceutical systems. These diagrams provide a graphical representation of the relationship between temperature, composition, and the resulting phases present in a mixture. Binary phase diagrams are constructed primarily to identify the intersection point where the two lines representing variable liquidus temperatures meet along the line representing the solidus temperature or eutectic melting points on the axis. The actual composition corresponding to this eutectic point is characterized by a single melting eutectic endotherm that aligns with the solidus point. This point of convergence is referred to as the true stoichiometry of the eutectic.

To analyze the eutectics, the temperature was plotted against the mole fraction of oxaprozin, resulting in distinct V-shaped binary phase diagrams for all three eutectic mixtures. The solidus represents a constant and uniform melting point that appears consistently across nearly all compositions at approximately the same temperatures. In contrast, the remaining compositions exhibit an extra melting endotherm, which corresponds to unreacted and excess reactants. This additional endotherm has a variable temperature and is referred to as the liquidus point for all compositions(Li et al., 2020; Sathisaran et al., 2018)

The binary phase diagrams were constructed using the compositions depicted in Figure 7, Figure 8 , and Figure 9. All of the studied eutectics displayed ideal behavior, demonstrating a decrease in melting points for the 50:50 molar ratios. Specifically, OX-AS exhibited a melting point depression at 128.92°C, OX-SY at 122.02°C, and OX-NI at 125.87°C for the 60:40 molar ratios, respectively.

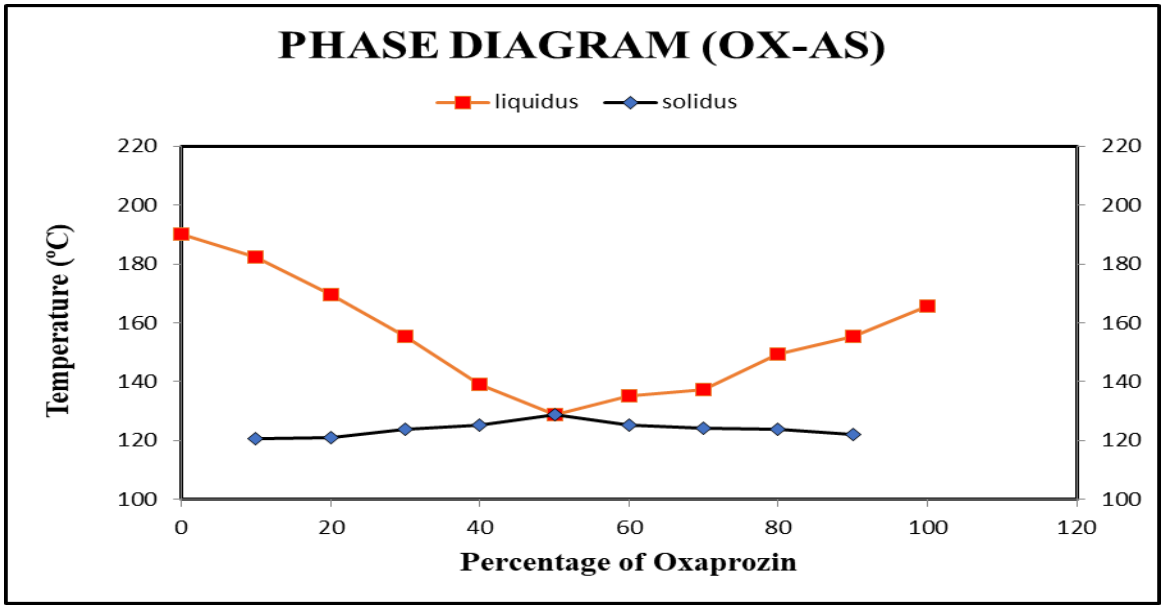

Fig 7 : Binary phase diagram of OX-AS

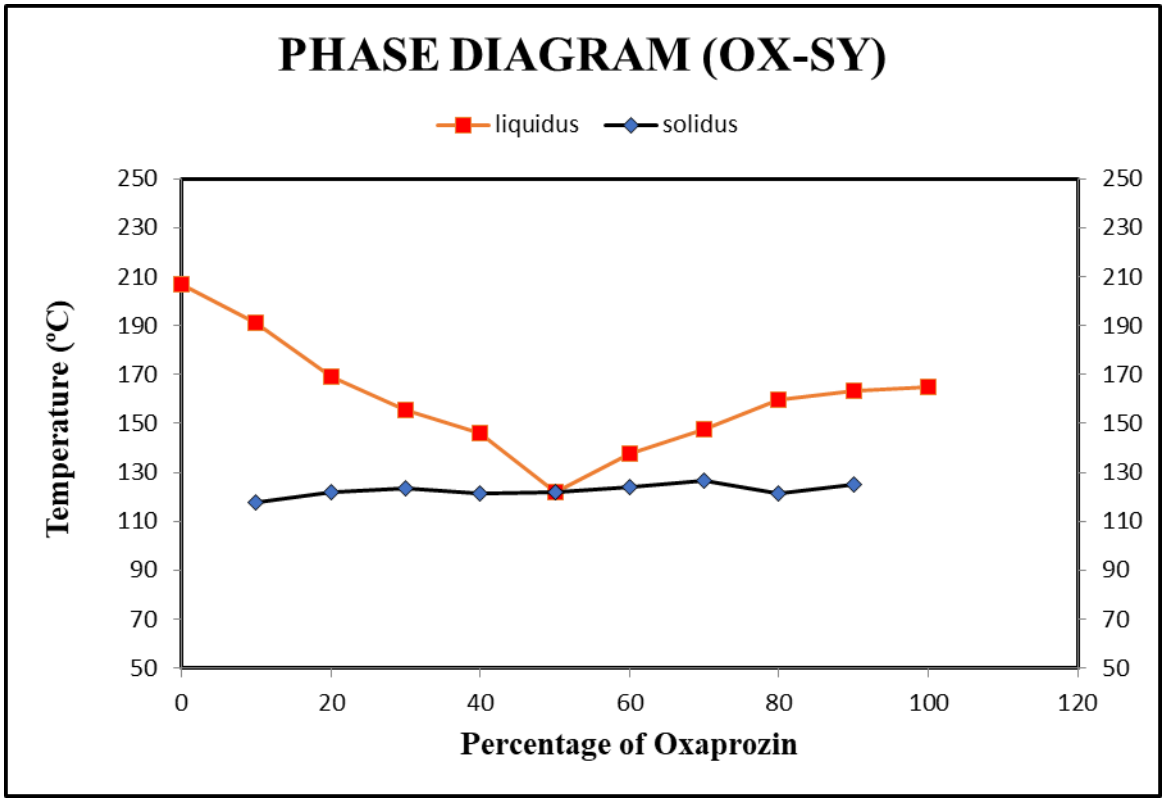

Fig. 8 : Binary phase diagram of OX-SY

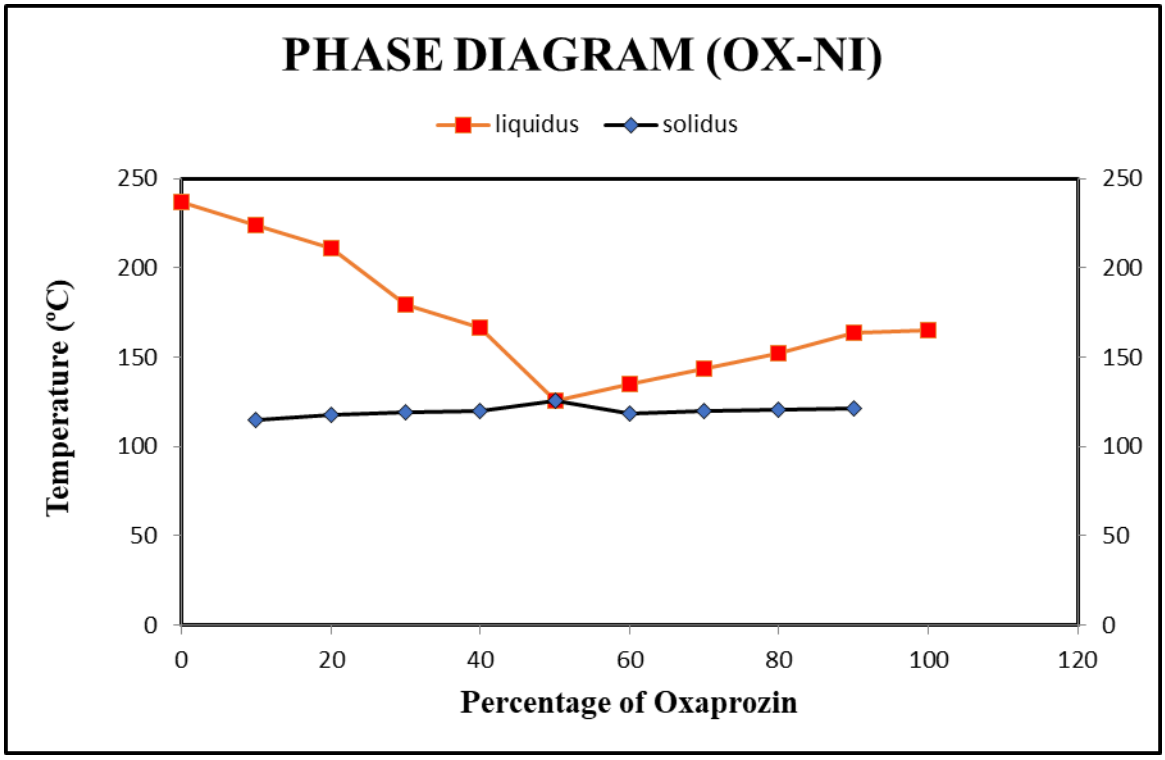

Fig. 9: Binary phase diagram of OX-NI

3.3 Tammam's triangle

Tammam's triangle is a concept used in the identification of the maximum enthalpy in eutectic mixtures. In Tammam's triangle, the axes represent the molar ratios of the two components in the eutectic mixture. The triangle is constructed by connecting the three points on the graph: pure component A(Drug), pure component B (Coformer), and the eutectic composition. In the binary phase diagram, this point of maximum enthalpy is identified as the solidus point, indicating the absence of any excess or unused reactant. The maximum enthalpy point in the eutectic mixture corresponds to the highest point on Tammam's triangle. By analyzing the enthalpy values at different compositions within the triangle, the point with the maximum enthalpy can be determined. This graph exhibits an inverted V shape, with the peak representing the maximum enthalpy of fusion (ΔH fusion, eutectic) for the pure eutectic composition. The identification of the maximum enthalpy in a eutectic mixture using Tammam's triangle can provide insights into the energy released or absorbed during phase transitions and the overall stability of the system(Górniak et al., 2013).

In the case of the eutectic **OX-AS,** Tammam's triangle (Figure 10) aligns with the findings from the binary phase diagram. It reveals that the composition with a 50:50 ratio of oxaprozin

to ascorbic acid at the apex exhibits the maximum enthalpy of fusion of eutectics [ΔH fusion(eutectic)] at 300.84 J/g.

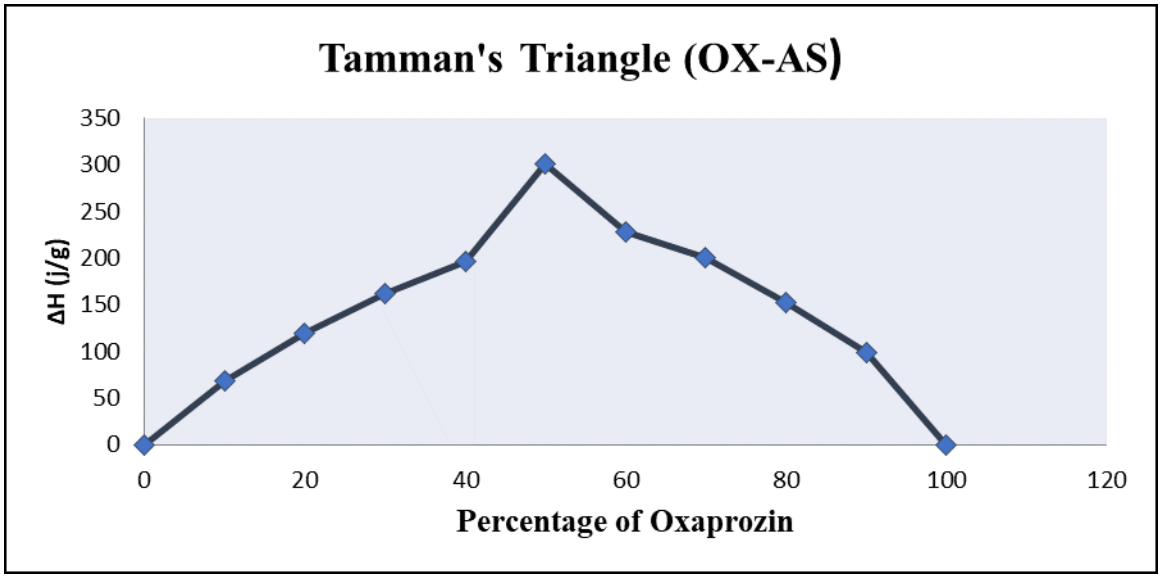

Figure 10: Tammam's triangle of OX-AS

Similarly, in the eutectic OX-SY, Tammam's triangle (Figure 11) portrays the stoichiometry of 50:50 for oxaprozin and syringic acid, which corresponds to the composition with the highest enthalpy of fusion of the eutectic phase [ΔHfusion(eutectic)] at 362.85 J/g among all the compositions.

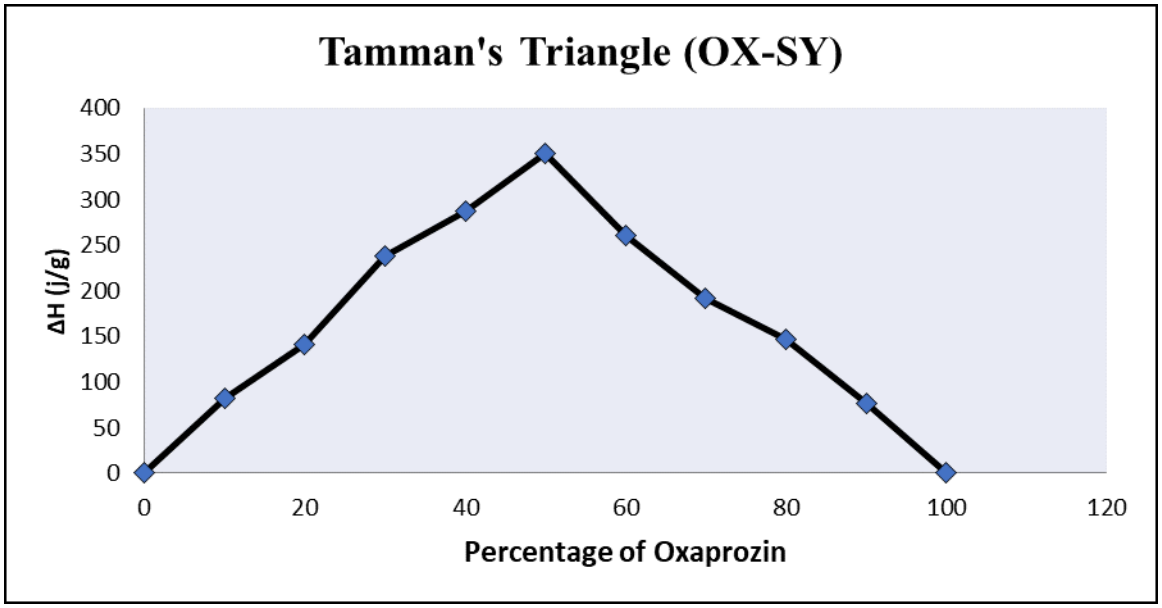

Figure 11: Tammam's triangle of OX-SY

Furthermore, in the eutectic OX-NI, Tammam's triangle (Figure 12) demonstrates that the composition with a 60:40 ratio of oxaprozin to nicotinic acid possesses the maximum enthalpy of fusion of the eutectic [ΔHfusion (eutectic)] at 248.4 J/g among all the compositions.

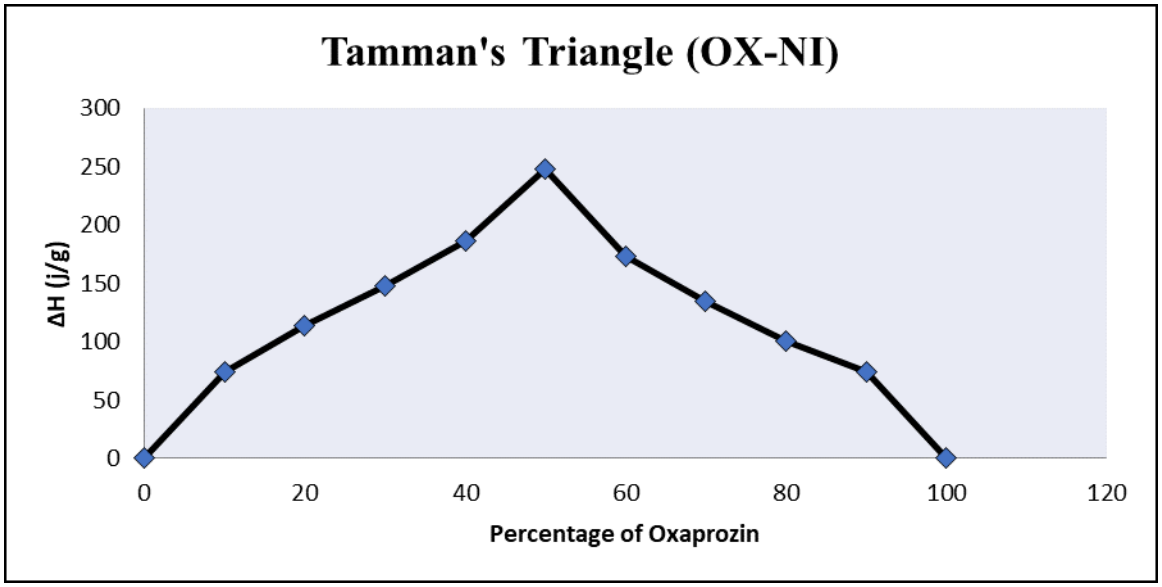

Fig. 12: Tammam's triangle of OX-NI

3.4 Hot stage Microscopy

This technique is based on a commonly accepted principle of transforming solids into liquids through an endothermic fusion process. In the eutectic system, this transformation occurs when the solids absorb latent heat of melting at their specific melting point temperature. However, this process is intricate due to the presence of two distinct crystalline molecules in close proximity within the microenvironment of seemingly single crystalline entities. These molecules maintain their own intact lattice arrangement and are mutually stabilized by weak non-covalent forces. The presence of an extensive and complete melting endotherm indicates a pure single entity, while varying melting temperatures suggest a partial conversion to a eutectic state with some residual excess preliminary substance(Chaiya et al., 2021; Wicaksono et al., 2020). The Figure (13), figure(14) and figure(15) demonstrates the melting of OX-AS, OX-SY and OX-NI with complete and uniform melting around 128.92°C, 123.86°Cand 125.87 °C respectively.

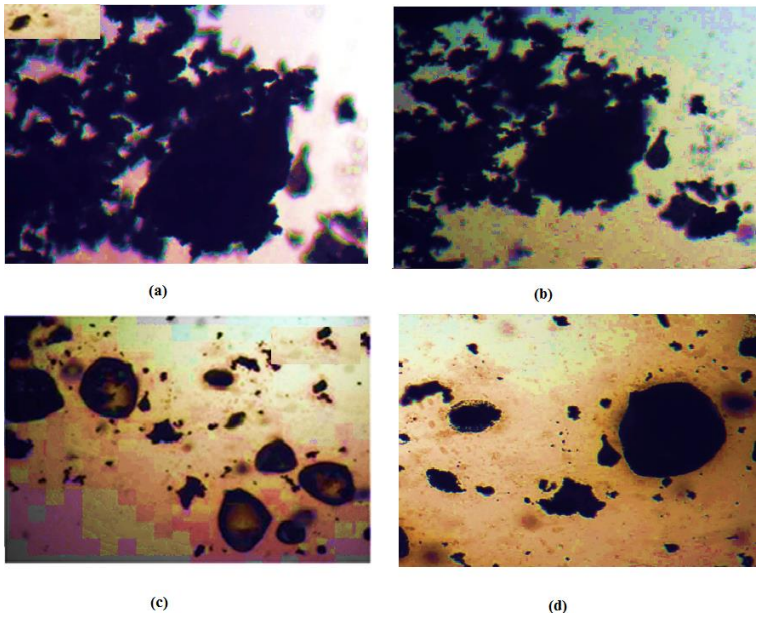

Fig 13: OX-AS before melting, (b) OX-AS starting to melt, (c) OX-AS melting at 128.92°C and (d) OX-AS fully melted

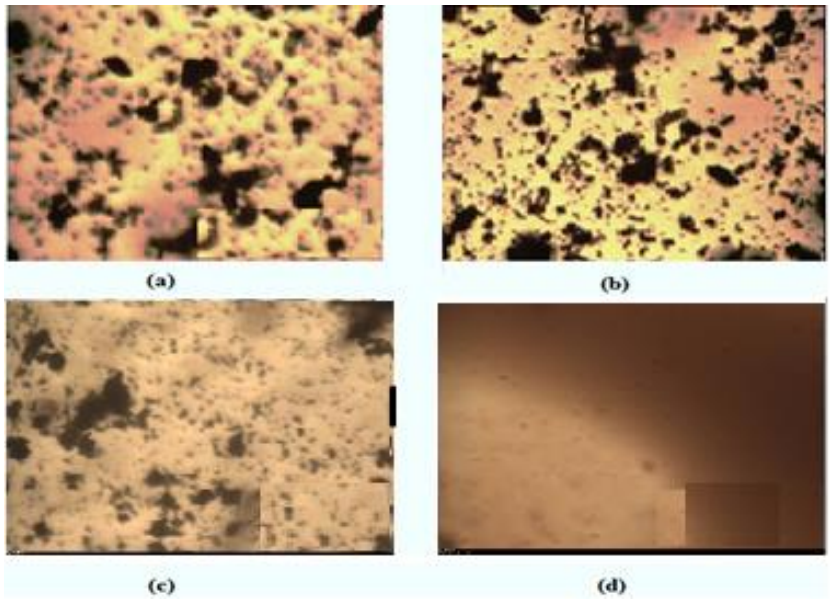

Fig. 14: OX-SY before melting, (b) OX-SY starting to melt, (c) OX-SY melting at 122.02°C and (d) OX-SY fully melted

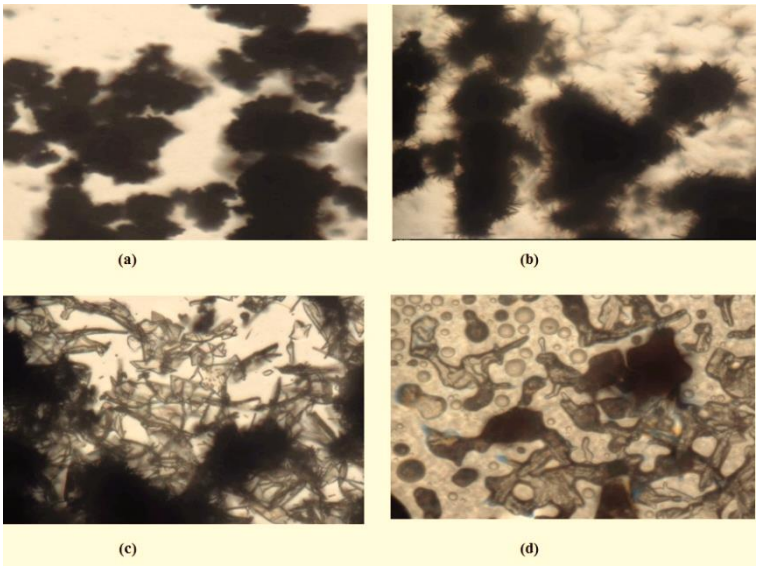

Fig. 15: OX-NI before melting, (b) OX-NI starting to melt, (c) OX-NI melting at 125.87°C and (d) OX-NI fully melted

3.5 PXRD and FTIR Analysis

The use of vibrational spectroscopic methods like FT-IR, in combination with Powder X-ray diffraction techniques, is crucial for analysing and characterizing the microstructure of solid substances. Spectroscopic methods usually have a specific threshold of detection below which they are not able to detect deviations in the strength of interactions or changes in the structures for chemical analysis.

When evaluating eutectics such as OX-AC, OX-SY, and OX-NI using FT-IR, it was found that the vibrational frequencies showed no significant changes (Figure 16). Even if there were slight shifts in the frequencies, they were negligible and not indicative of any substantial structural changes. Similarly, the X-ray diffraction techniques were inconclusive as the presence of a minor component within the matrix of a major component, without any bonding, did not cause significant alterations in the characteristic 2θ values compared to the parent molecules (Figure 17). Therefore, the powder X-ray diffraction data provided limited support since no new peaks appeared in any of the eutectic mixtures, and none of the existing peaks were missing. Attempting to produce single crystals of eutectic mixtures through solution crystallization proved to be highly challenging. This difficulty likely arose from the differing solubilities of the two components in the solvent, causing them to crystallize at different points. Additionally, the eutectics had weak interactions and incomplete bonding, further inhibiting their crystallization into single crystals.

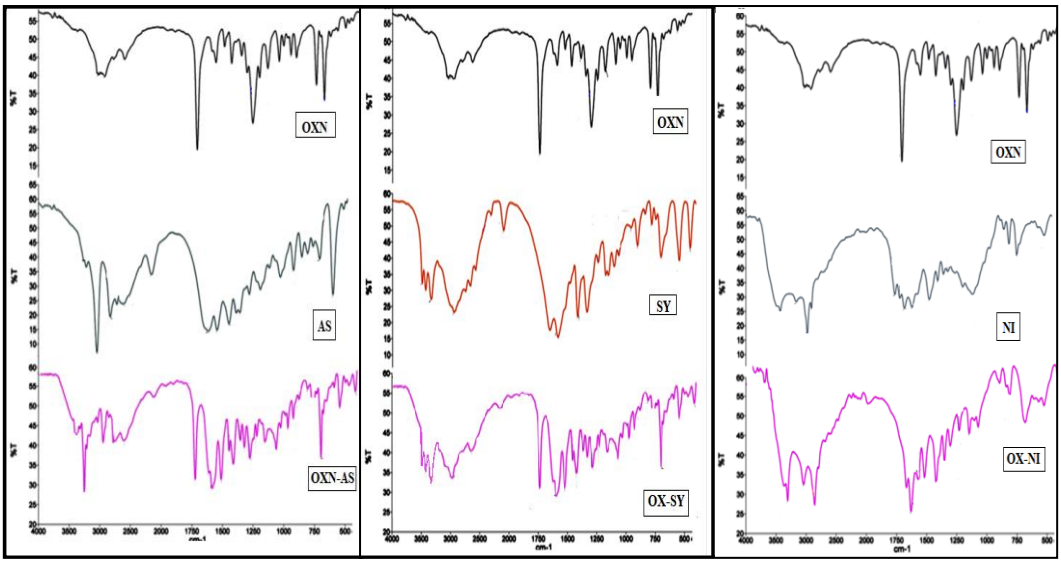

Fig. 16 : FTIR spectra of Oxaprozin (OXN), Coformers (AS, SY and NI) and corresponding eutectics (OX-AS, OX-SY and OX-NI)

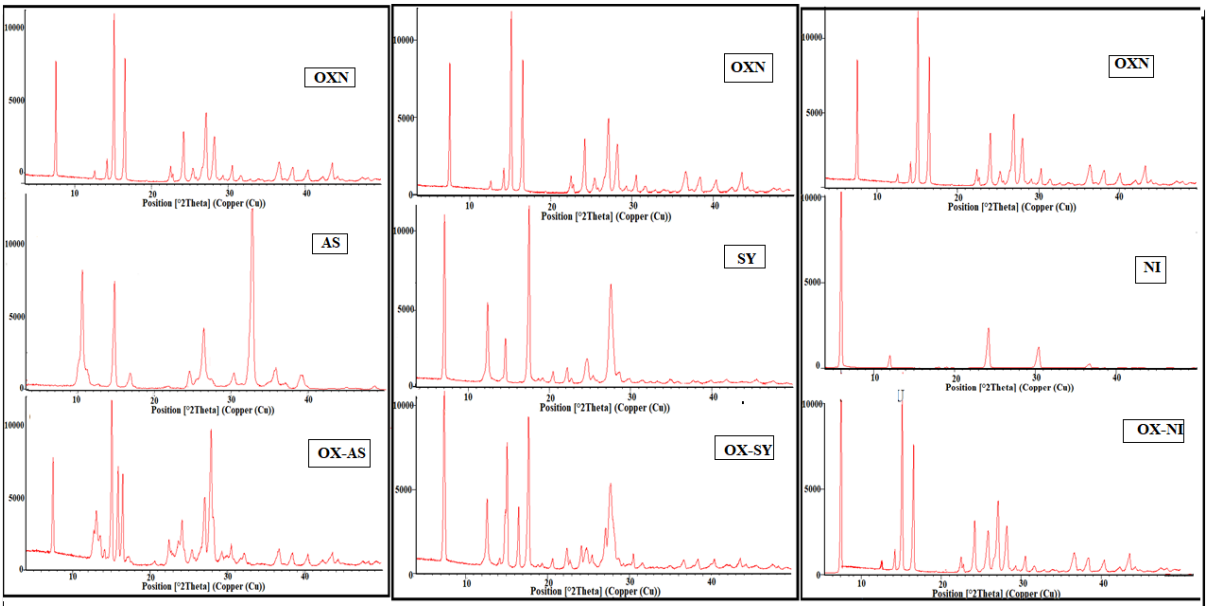

Fig. 17 : PXRD pattern of Oxaprozin (OXN), Coformers (AS, SY and NI) and corresponding eutectics (OX-AS,OX-SY and OX-NI)

3.6 Scanning Electron Microscopy

SEM, (Scanning electron microscopy) is another beneficial technique in the study of prepared pharmaceutical eutectic (OX-AS, OX-SY and OX-NI), providing valuable information about the morphology and surface characteristics of the eutectic mixture. By capturing SEM pictures of the eutectic mixture, any changes in the crystalline structure of the components, such as alterations in particle shape or size, formation of new crystal structures, or the

presence of amorphous regions can be observed. These observations can be crucial in understanding the physical transformations that occur during eutectic formation.

Figure 18 demonstrates variable particle shapes . In SEM images, OXN particles appeared as irregularly shaped, non-uniform entities. The irregular shape of oxaprozin particles is influenced by factors such as crystallization, agglomeration and particle growth, a during the manufacturing process. Likewise, Ascorbic acid (AS) forms plate-like crystals that vary in size from 20-100 µm, syringic acid (SY) is observed as tiny needle-shaped crystals showing sharp edges, measuring approximately 5-25 µm in size., while nicotinic acid (NI) forms elongated columnar globular crystals ranging from 90-200 µm. When examining the eutectics under a scanning electron microscope, noticeable changes were observed compared to the parent molecules. The crystals of the eutectic system containing syringic acid (OX-SY) appear as irregularly shaped clusters in the size range of 4-20 µm. The eutectic systems with ascorbic acid (OX-AS) and nicotinic acid (OX-NI) display agglomerates of unevenly shaped crystals without a well-defined shape, ranging in size from 10 to 100 m.

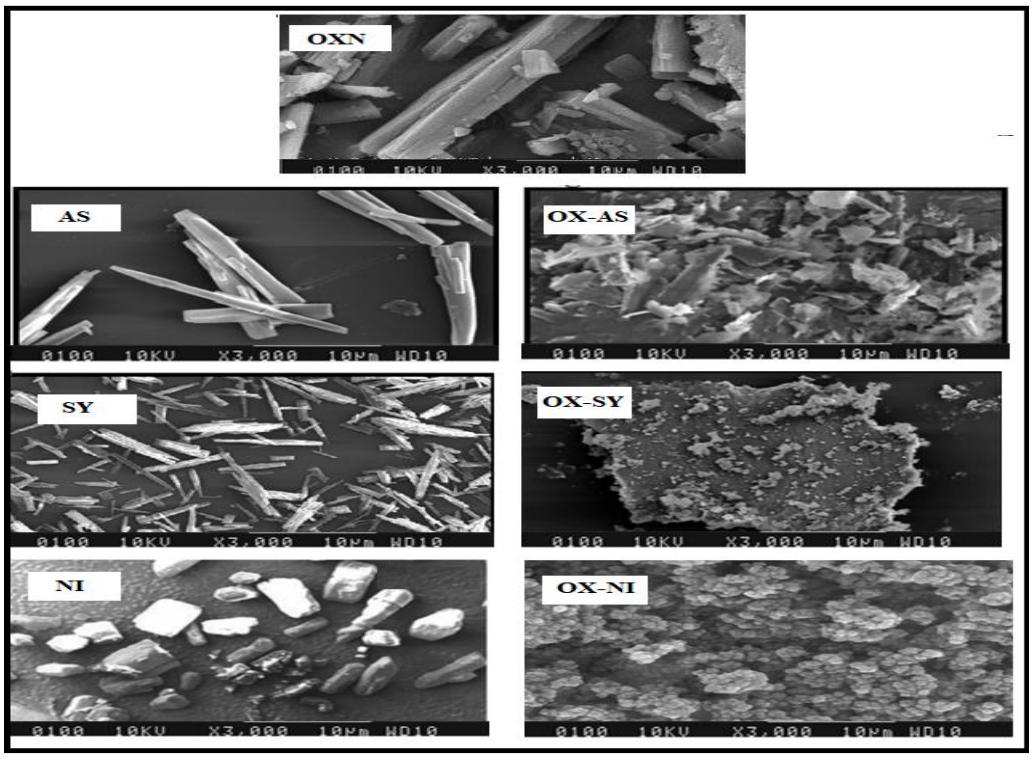

Fig. 18 Scanning electron microscopy images of Oxaprozin (OXN), Coformers (AS, SY and NI) and corresponding eutectics (OX-AS, OX-SY and OX-NI)

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4. Apparent solubility and dissolution studies

The solubility and dissolution properties of eutectic mixtures of OXN were investigated in a phosphate buffer with a pH of 7.4 at a temperature of 37°C. The highest absorption of oxaprozin occurs in the intestines during oral administration, making this condition a suitable representation of the human intestinal environment. All eutectic mixtures (EMs) demonstrated significant improvement in solubility and dissolution rate, following the order: OX-AS > OX-SY > OX-NI > OX. Solubility studies revealed that the OX-AS eutectic mixture was approximately 8.7 times more soluble than the pure OXN, followed by the OX-SY eutectic mixture, which was 6.1 times more soluble. The OX-NI eutectic mixture was 4.1 times more soluble throughout most of the experiment. The maximum solubility (Smax) and dissolution profiles are Demonstrated in Figure 19 and Figure 20. The solubility results were determined at 4 hours and 24 hours. The eutectic mixture of OX-AS and OX-SY achieved maximum solubility levels after 180 minutes, whereas the OX-NI eutectic mixture reached maximum solubility after 200 minutes. This can be attributed to the favorable thermodynamic properties of eutectics, such as higher free energy, weaker intermolecular interactions, and increased molecular mobility between the constituents of the mixture. The variation in solubility enhancement among different eutectic mixtures can be attributed to the differential intrinsic solubility of the coformer. Additionally, the improved dissolution rate in EMs is attributed to the drug's better dispersibility in the hydrophilic coformer and increased wettability resulting from the coformer's solubilization, which accelerates the drug's dissolution in the medium. The intrinsic solubility of the coformer significantly influences the solubility of the complex in a multicomponent system. In this study, coformers with high aqueous solubilities, such as AS, exhibited better solubility profiles in the eutectic mixtures compared to other coformers (SY and NI).

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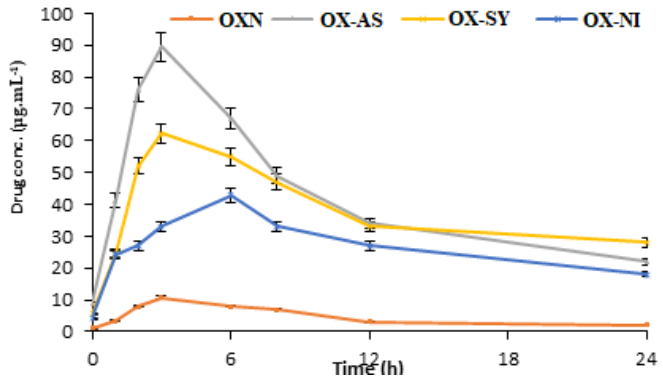

Fig 19: Apparent solubility of Oxaprozin and its respective eutetics.

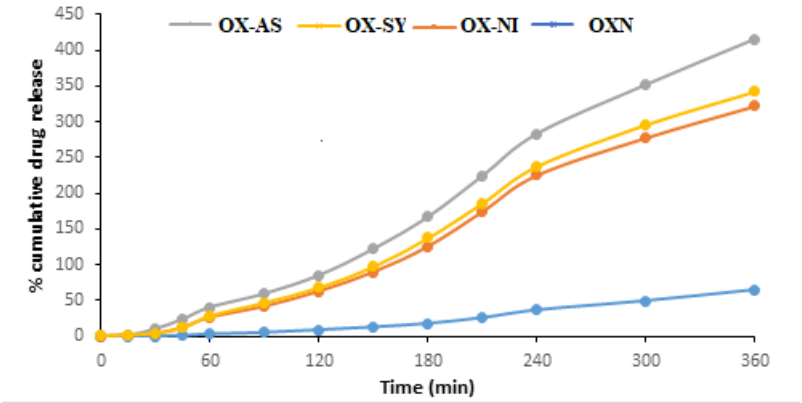

Fig 20: Dissolution profile of Oxaprozin and its respective eutetics

5. Pharmacodynamic Study:

The anti-osteoarthritic activity of all the eutectics of OXN was evaluated in Wistar rats with arthritis induced using complete Freund's adjuvant. The treatment with the standard drug (OXN) and eutectics (OX-AS,OX-SY and OX-NI) began on the 13th day after disease induction, as described in the experimental section. Various parameters were measured, including body weight, paw volume,), and left joint diameter. Initially, the treatment groups experienced weight loss, which gradually recovered after the start of treatment. Compared to , all the eutectics (OX-AC > OX-SY > OX-NI) showed greater reductions in paw volume, , and the maximum reduction in left joint diameter (Fig. 21). These findings indicate that eutectics exhibit improved anti-inflammatory effects and potentially offer faster pain relief, possibly due to enhanced solubility and pharmacokinetic properties of the eutectics.

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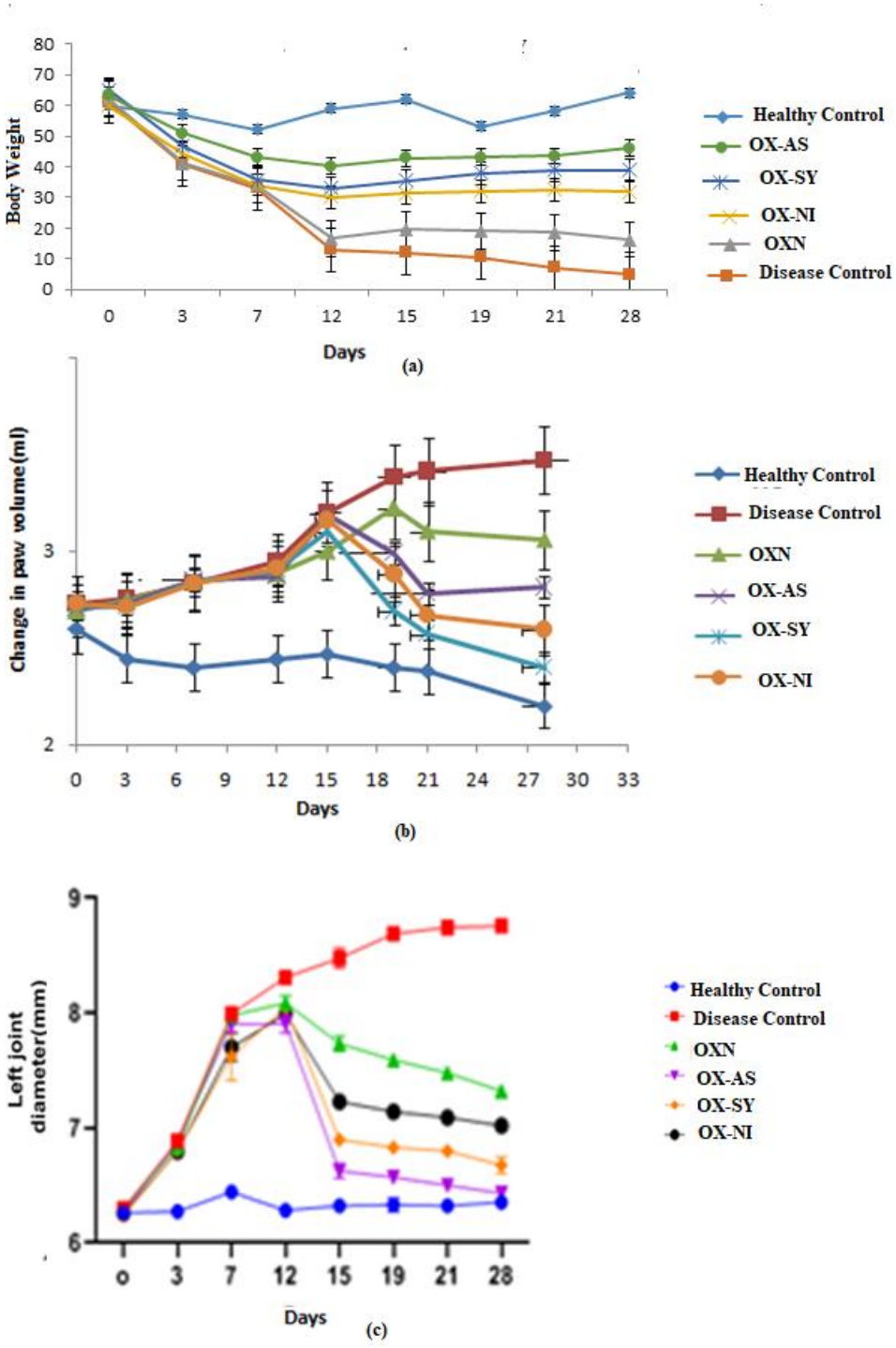

Fig 21. Effect of co-crystals on (a) body weight (b) change in paw volume (c) reduction in joint diameter of rats in FCA-inducedarthritis

6. Conclusion:

The utilization of pharmaceutical eutectic mixture has shown promise in addressing the solubility issues related to limited bioavailability of Oxaprozin. By employing the mechanochemical solvent drop-grinding method with ethanol as the solvent, three eutectic mixture of oxaprozin were successfully synthesized with ascorbic acid, syringic acid, and nicotinic acid. The choice of coformers was based on a CSD search, and the resulting cocrystals were characterized using advanced analytical techniques such as DSC, FTIR, PXRD, HSM and SEM.The prepared eutectic mixture exhibited enhanced solubility and dissolution parameters, as evidenced by increased solubility and intrinsic dissolution rates. Subsequently, pharmacodynamics studies were conducted which were in accordance with the above spectroscopic results. The eutectic mixtures (EMs) formed by combining Oxaprozin with multiple components offer two-fold benefits when compared to the plain drug. Firstly, they exhibit enhanced biopharmaceutical properties, and secondly, they possess significant antioxidant properties.This research highlights the potential of utilizing crystal engineering techniques in drug development through crystallization, offering new opportunities for the advancement of pharmaceuticals.

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