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Formulation and characterization of tretinoin nanosuspension and in silico testing as an anti-inflammatory

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ABSTRACT

Introduction: Tretinoin is an effective retinoid derivative for acne therapy, but has formulation challenges due to its lipophilic nature and high crystallinity. An appropriate formulation strategy is needed to improve its stability and bioavailability. Objective: This study aims to evaluate the compatibility of tretinoin with excipients in nanosuspension formulations and their physicochemical characterization. Methods: Preliminary tests were carried out using Fourier Transform Infrared Spectroscopy (FTIR), crystallinity was analyzed by X-ray Diffraction (XRD), and thermal analysis by Differential Scanning Calorimetry (DSC). Nanosuspension characterization includes particle size, polydispersity index, zeta potential and entrapment efficiency. In silico test of the potential of tretinoin as a COX-2 inhibitor related to anti-inflammatory effects. Results: FTIR results showed no chemical interaction between tretinoin and excipients. XRD showed a decrease in tretinoin crystallinity after mixing with HPMC and PVP. DSC showed a shift in the melting point of tretinoin, indicating a physical interaction with excipients. Characterization of nanosuspension showed particle size <1000 nm, polydispersity index <0.5, zeta potential ±-20 mV, and entrapment efficiency >80%. In silico tests show that tretinoin has a binding energy of -9.57 kcal/mol against the Cyclooxygenase-2 (COX-2) enzyme with an inhibition constant of 96.03 nM. Conclusion: Tretinoin shows good compatibility and physicochemical characteristics in nanosuspension formulation, as well as potential as an anti-inflammatory agent through COX-2 inhibition.

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INTRODUCTION

Acne (acne vulgaris) is one of the most common skin diseases, especially in adolescence to young adulthood. This condition is characterized by chronic inflammation of the pilosebaceous unit involving hair follicles and sebaceous glands, and is characterized by the appearance of lesions in the form of blackheads, papules, pustules, and nodules (Vasam, Korutla, & Bohara, 2023). The factors that cause acne are very complex, including excessive sebum production, follicle

hyperkeratinization, colonization of Propionibacterium acnes bacteria, and ongoing local immune inflammatory responses. In addition to physical impacts, acne also has significant psychological effects, such as decreased self-confidence to emotional disorders (Kim & Kim, 2024),(Muhlis, 2020).

One effective topical therapy for treating acne is the use of tretinoin, a derivative of vitamin A (retinoid) which works by accelerating skin cell turnover, reducing the formation of blackheads, and has anti-inflammatory activity (Zasada & Budzisz, 2019). However, tretinoin has weaknesses in the formulation aspect due to its lipophilic nature (log P 6.3), relatively large molecular weight (300.4 g/mol), and high crystallinity level, which causes its solubility in water to be very low and its stability is easily disturbed by light and oxygen. This has an impact on low bioavailability and therapeutic effectiveness when used in conventional preparations (Tsamarah, Izzaturrahmi, & Sopyan, 2023),(Yoham & Casadesus, 2023).

In overcoming these problems, nanosuspension technology is a promising alternative drug delivery system (Fadilah & Herdiana, 2023),(Martien, Adhyatmika, Irianto, Farida, & Sari, 2012). Nanosuspension is a system of dispersion of nanometer-sized active substance particles in a liquid medium with the help of surfactants or stabilizing polymers. This technology can increase solubility, improve stability, accelerate skin penetration, and allow gradual release of drugs (sustained release)(Athallah et al., 2024),(Jacob, Nair, & Shah, 2020). In the process of developing nanosuspension preparations, an evaluation of the physicochemical characteristics is required, including material compatibility testing using Fourier Transform Infrared Spectroscopy (FTIR), crystallinity analysis using X-ray Diffraction (XRD), and evaluation of thermal properties using Differential Scanning Calorimetry (DSC) (Jafar, Putriyanti, Muhsinin, & Kencana, 2025).

In addition, other important parameters such as particle size, polydispersity index, zeta potential, and adsorption efficiency need to be analyzed to ensure that the formed nanosuspension system is stable and effective (Aini, 2021),(Jafar, Salsabilla, & Santoso, 2022). Not only from the physical side, evaluation of the biological potential of tretinoin as an anti-inflammatory agent also needs to be considered.(Suryani, Darniwa, Musa'adah, & Akbar, 2024). One of the methods used is the in silico test, which is a simulation of the molecular interaction between tretinoin and target enzymes that play an important role in the inflammatory process (Yoham & Casadesus, 2023).

By combining the nanosuspension formulation approach and in silico analysis, this study is expected to provide a comprehensive picture of the potential of tretinoin as an effective and stable anti-inflammatory therapeutic agent in topical preparations.

RESEARCH METHOD

Tool

The tools used in the study were digital scales (Mettlertoledo®), spray bottles, ultraturax homogenizer (IKA T25 digital), analytical balance (Mettler Toledo), magnetic stirrer (IKA® C-mag HS 10), sonicator probe (Ivymen System CY - 500), hot plate (Oxone®), particle size analyzer (PSA) instrument (Malvern instruments Ltd), pH meter (Mettlertoledo®), Vivaspin (Eppendrof, Germany), UV spectrophotometer (Shimadzu UV), Fourier Transform InfraRed (FTIR) instrument (Aligent Technologies Cary 630 FTIR), Transmission Electron Microscopy (TEM, JEOL JEM 1400, Japan), Differential Scanning Calorimetry (DSC) instrument, X-Ray Diffraction (XRD) instrument, general glassware (Pyrex®) used and SPSS data analysis software (SPSS Inc., Chicago, IL).

Material

The ingredients used are the active substances Tretinoin (Beutue Lab), Aquades, Methanol PA (PT. Merapi Utama Pharma), surfactant Polyoxyl 40 hydrogenated castor oil (Cremophore®), Plantacare 1200®, Polyvinylpyrrolidone (PVP)® and Hypromellose (HPMC)®.

Detailed Procedure

Preparation and Inspection of Materials. Tretinoin as an active ingredient is checked for suitability using a Certificate of Analysis (CoA) document, while additional ingredients are tested for quality using the Handbook of Pharmaceutical Excipients (HOPE) reference (Primary, 2024).

Characterization of Raw Materials

Initial analysis was carried out using the Fourier Transform Infrared Spectroscopy (FTIR) method to evaluate the possibility of chemical interactions between tretinoin and additional ingredients, such as HPMC and PVP. The spectrum results showed a typical pattern of each compound and ensured that no new peaks were formed, indicating the compatibility of the ingredients in the formulation system. Furthermore, crystallinity analysis was carried out using X-ray Diffraction (XRD) to determine the physical properties of the ingredients and their mixtures. The XRD results showed a decrease in the intensity of the crystallinity peak in the mixture of tretinoin with HPMC and PVP, indicating a change in structure from a crystalline form to a semi-amorphous form, which has the potential to increase the solubility and release of active substances in the nanosuspension system. To strengthen these results, thermal analysis was also carried out using Differential Scanning Calorimetry (DSC). This technique is used to determine the melting point and thermal properties of individual ingredients and their mixtures (de Barros Lima et al., 2015; Jafar et al., 2025).

Preparation of Tretinoin Nanosuspension

Nanosuspension is made using the sonoprecipitation method, namely by dissolving tretinoin in DMSO (organic phase) then dropping it into the water phase containing surfactants and polymers while stirring, then sonicating using a probe sonicator (Shah, Khunt, Bhatt, Misra, & Padh, 2015).

Characterization of Nanosuspension

Nanosuspension characterization includes testing particle size, polydispersity index (PDI) using a psa or Particle Size Analyzer and zeta potential using a zeta sizer. The sample was diluted with distilled water up to 10 mL, placed in a cuvette, and analyzed to determine the size distribution and stability of the preparation (Jafar et al., 2025).

Entrapment Efficiency (EE%)

EE% was analyzed using the centrifugation method with Vivaspin, followed by measurement of the supernatant using a UV spectrophotometer at 352 nm to calculate the free drug content and determine the entrapment efficiency (Jafar et al., 2025).

In silico test

In silico testing began with validation and molecular docking simulations were performed using AutoDock 4.2.3 and MGLTools 1.5.6. Redocking was performed by re-docking the native ligand to the target protein to ensure the accuracy of the method, which was considered valid if the RMSD value was ≤ 2 Å (Shivanika, Kumar, Ragunathan, Tiwari, & Sumitha, 2020). The docking simulation of the test ligand was continued with the gridbox setting based on the validation results, using the LGA algorithm. The results were analyzed based on the binding free energy (ΔG) and inhibition constant (Ki) values, then visualized using Discovery Studio Visualizer and VMD to identify the interaction of active residues of the protein with the ligand (Elfita, Apriadi, & Dianmurdedi, 2022).

RESULTS AND DISCUSSIONS

The results of the study in the first stage all the materials used have passed the quality control stage according to pharmaceutical standards. Tretinoin, as the main active ingredient, shows physical and chemical characteristics that are in accordance with the specifications in the CoA, such as the

form of yellow powder and low solubility in water. Excipients consisting of surfactants (Cremophor and Plantacare) and polymers (HPMC and PVP) were also checked for quality based on the Handbook of Pharmaceutical Excipients (HOPE) reference and were declared to meet the quality standards for use in nanosuspension formulations (Primary, 2024).

The results of the FTIR analysis showed no significant changes in the spectrum of the mixture between tretinoin, PVP, and HPMC. No new absorption peaks or band shifts were found, indicating that there was no chemical interaction between the components. This proves that tretinoin is chemically compatible with both polymers in the nanosuspension formulation system (de Barros Lima et al., 2015; Jafar et al., 2025).

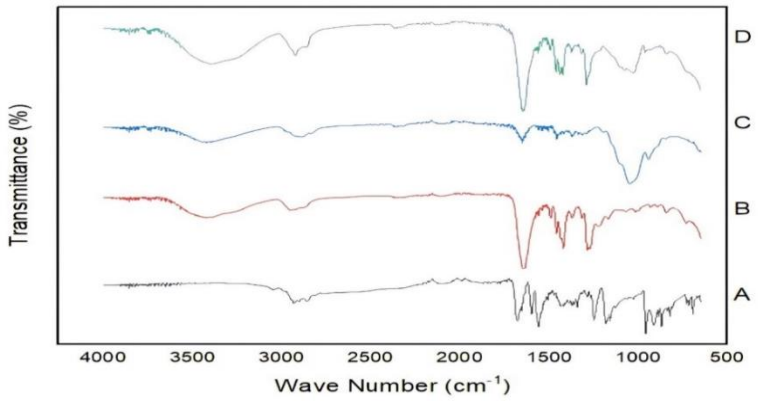


Figure 1. FTIR (A) tretinoin, (B) PVP, (C) HPMC, (D) tretinoin, PVP and HPMC

The XRD data results show that tretinoin in pure form is crystalline with sharp diffraction peaks, while PVP and HPMC are amorphous. The mixture of tretinoin with both polymers shows a decrease in the intensity of the crystal peak, indicating a change to a semi-amorphous form. This supports the increase in solubility and stability of the nanosuspension system (Jafar, Sucipto, & Supriadi, 2024).

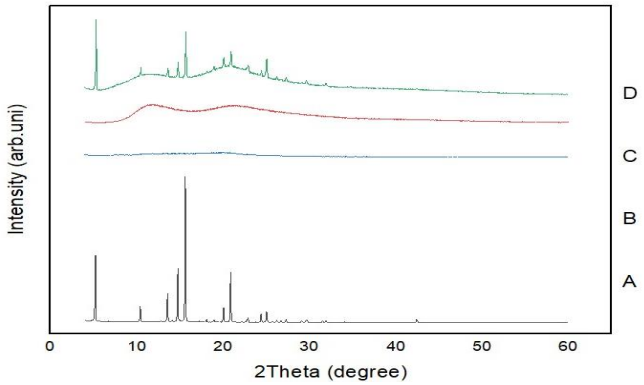


Figure 2. XRD tretinoin (A), PVP (B), HPMC (C), tretinoin, PVP And HPMC (D)

The results of DSC analysis showed a shift or decrease in the melting peak of tretinoin after being combined with PVP and HPMC. This shift indicates a physical interaction between the active ingredient and the polymer which causes a decrease in crystallinity. This is useful in increasing the dissolution and potential bioavailability of tretinoin in nanosuspension formulations (de Barros Lima et al., 2015; Jafar et al., 2025).

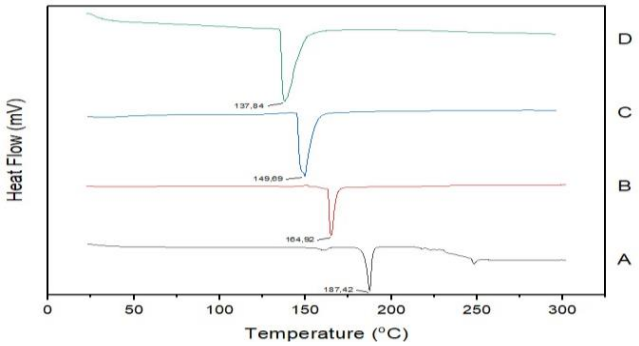


Figure 3. DSC tretinoin (A), PVP (B), HPMC (C), tretinoin, PVP And HPMC (D)

The preparation of nanosuspension by sonoprecipitation method successfully produced a tretinoin dispersion system in a stable colloidal form. The gradual addition of the organic phase to the aqueous phase containing surfactants and polymers resulted in a homogeneous dispersion, marked by the formation of a golden yellow solution (Ahmadi Tehrani, Omranpoor, Vatanara, Seyedabadi, & Ramezani, 2019). The sonication process helps reduce the particle size and increase the stability of the nanosuspension system. The tretinoin nanosuspension formed was then characterized including particle size, polydispersity index, zeta potential (Jafar et al., 2022).

Table 1. Formulation and characterization of tretinoin h-1 nanosuspension

H-1 Formulation						Z-Ave		ZP
						nm	PdI	mV
Code	Tretinoin	Cremophor	Plantacare	HPMC	PVP			
F1	0.05	-	-	-	-	936.9±18.94	0.36±0.13	-32±0.64
F2	0.05	2.5	-	-	-	528.26±24.98	0.51±0.37	-21±3.03
F3	0.05	-	2.5	-	-	264.2±52.04	0.49±0.04	-44±4.34
F4	0.05	-	-	1.75	-	329.83±5.09	0.27±0.04	-13±0.32
F5	0.05	-	-	-	1.06	509.13±11.18	0.33±0.02	-14±0.23
F6	0.05	2.5	-	1.75	-	877.6±1123.8	334±577.1	-23±0.76
F7	0.05	2.5	-	-	1.06	3311.3±984.0	1±0.00	-25±1.54
F8	0.05	-	2.5	1.75	-	42.09±5.17	0.45±0.06	-44±10.83
F9	0.05	-	2.5	-	1.06	161.13±92.08	0.32±0.02	-26±2.70
F10	0.05	2.5	2.5	1.75	1.06	58.92±3.57	0.34±0.11	-35±5.25

Table 2. Formulation and characterization of tretinoin h-30 nanosuspension

H-30 Formulation						Z-Ave		ZP
						nm	PdI	mV
Code	Tretinoin	Cremophor	Plantacare	HPMC	PVP			
F1	0.05	-	-	-	-	1035±77.50	0.57±0.08	-21.16±0.55
F2	0.05	2.5	-	-	-	245.5±145.31	0.39±0.18	-22.86±1.98
F3	0.05	-	2.5	-	-	525.5±252.35	0.59±0.27	-38.06±3.00
F4	0.05	-	-	1.75	-	162.6±0.87	0.24±0.03	-18.96±1.50
F5	0.05	-	-	-	1.06	353.9±3.45	0.21±0.00	-20.83±0.93
F6	0.05	2.5	-	1.75	-	115.14±53.76	0.24±0.03	-32.36±9.59
F7	0.05	2.5	-	-	1.06	1548±626.6	1±576	-23.66±1.59
F8	0.05	-	2.5	1.75	-	108.6±37.44	0.33±0.04	-25.5±0.72
F9	0.05	-	2.5	-	1.06	301±106.8	0.41±0.11	-35.4±2.10
F10	0.05	2.5	2.5	1.75	1.06	102.0±17.15	0.81±576	-13.96±1.75

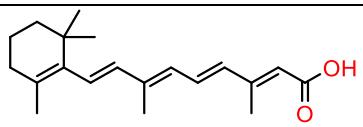
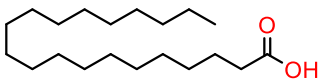
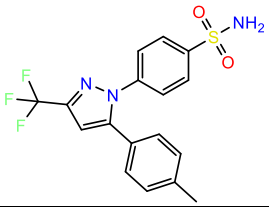
Characterization of particle size on day 1 (D-1) showed that the smallest particle size was in formula F8 at 42.09±5.17 nm, followed by F10 (58.92±3.57 nm) and F9 (161.13±92.08 nm). Meanwhile, on day 30 (D-30), the smallest particle size was in formula F10 at 102.0±17.15 nm, followed by F6 (115.14±53.76 nm) and F8 (108.6±37.44 nm). In general, all formulas showed particle

sizes in the range of <1000 nm, indicating fairly good particle stability during storage, although there was a slight increase in size in some specific formulas (Jafar et al., 2025).

On D-1, the best (smallest) PdI value was shown by formulas F4 (0.27±0.04) and F5 (0.33±0.02), indicating a homogeneous particle size distribution. On D-30, formula F5 showed the smallest PdI value of 0.21±0.00, followed by F4 and F6 with values of 0.24±0.03. Most of the formulas still showed PdI values <0.5 indicating a stable and uniform dispersion system, although in some formulas there was an increase in PdI values indicating slight inhomogeneity during storage (Jafar et al., 2022). The zeta potential value at H-1 showed that formulas F3 (-44±4.34 mV) and F8 (-44±10.83 mV) had the best electrostatic stability, while at H-30, formulas F3 (-38.06±3.00 mV) and F9 (-35.4±2.10 mV) still showed high stability. Generally, a large negative zeta potential value (>-25 mV) indicates the presence of a strong enough repulsive force between particles to maintain system stability, so that most formulas remain in the range of good electrostatic stability after 30 days of storage (Jafar et al., 2024).

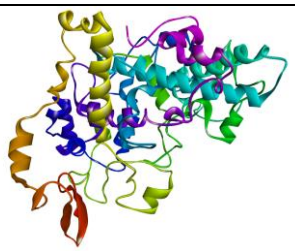
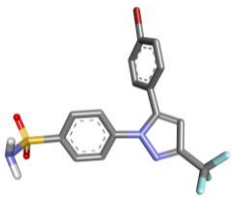
In silico assay was conducted to evaluate the potential of tretinoin as an anti-inflammatory agent through its interaction with the target enzyme Cyclooxygenase-2 (COX-2) taken from the RSCB PDB protein database with PDB ID 6COX. Molecular docking of 6COX with tretinoin was conducted to evaluate the potential of tretinoin as a COX-2 inhibitor so that it can be seen whether tretinoin can inhibit the activity of this enzyme. The structure of tretinoin was obtained from the PubChem database, then converted and optimized to ensure a stable conformation (Torres, Sodero, Jofily, & Silva-Jr, 2019). Celecoxib is a well-known COX-2 inhibitor and has been clinically proven to be an effective nonsteroidal anti-inflammatory drug (NSAID). Celecoxib can be used as a reference molecule to compare the effectiveness of tretinoin as a COX-2 inhibitor. 6COX-arachidonic acid docking was performed to evaluate how arachidonic acid binds to COX-2 and compare it with tretinoin and celecoxib. Arachidonic acid serves as a control molecule to understand the nature of the enzyme-substrate interaction. Arachidonic acid is a natural substrate of the enzyme Cyclooxygenase-2 which is converted into prostaglandins, important mediators in the inflammatory process (Carol A. Rouzer and Lawrence J. Marnett).

Table 3. Test ligand preparation

Ligand	2D Structure	Pubchem CID
Tretinoin		5280343
Arachidonic acid		10467
Celecoxib		2662

The results of the prediction of physicochemical properties show that tretinoin has a log P of 6.30, a molecular mass of 300.44 g/mol, and two hydrogen acceptors and one hydrogen donor, which are still in accordance with Lipinski's Rule of Five. The HOMO-LUMO energy gap value of 4.657 eV indicates good electronic stability and a fairly low level of molecular reactivity (F. Zheng et al., 2023).

Table 4. Target proteins

GDP Code	Macromolecules	Natural ligands
6COX		

The protein structure of COX-2 (PDB ID: 6COX) was prepared by removing water molecules and native ligands, then adding hydrogen atoms using Discovery Studio to improve the accuracy of the docking simulation. Validation of the docking method was carried out through redocking using a native ligand (Celecoxib) which produced a Root Mean Square Deviation (RMSD) value of 1.84 Å, which is within the accepted validation limit (RMSD ≤ 2 Å). The RMSD value is very important in molecular docking to measure the difference in atomic positions between two molecular structures (between the tretinoin docking pose and the reference structure). Docking can be said to be accurate if the two positions have very small differences. If the RMSD value between the tretinoin position and the reference ligand is in the range of ≤ 2 Å, then the two poses are considered similar enough so that the docking is considered accurate (Eric W. Bell and Yang Zhang). The natural ligand produces a binding energy of -10.99 kcal/mol with an inhibition constant of 8.24 nM, and forms several important interactions with the active residues of COX-2 (Wiyono & Diyah, 2023).

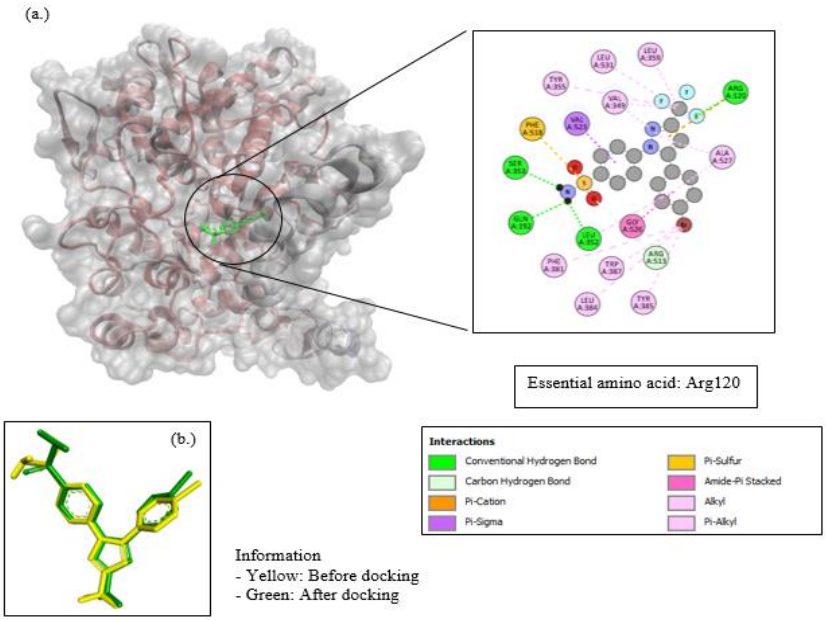


Figure 4. Molecular docking visualization validation of natural ligand of cyclooxygenase-2 protein (a) overlay visualization (b).

Molecular docking validation on COX-2 enzyme has been performed to evaluate the accuracy and stability of ligand interaction with the active site of the enzyme. The results obtained showed an RMSD value of 0.900 Å, indicating that the docking method used has a good level of reproducibility. In addition, the resulting binding energy was -10.99 kcal/mol, indicating a strong

affinity between the ligand and the COX-2 enzyme. The interactions observed included the formation of ten hydrogen bonds with several important residues, namely Arg120, Gln192, and Leu352. The Arg120 residue has been confirmed as a key residue in the interaction of the ligand with COX-2. The hydrogen bonds formed on this residue play an important role in stabilizing the enzyme-ligand complex, thereby increasing the binding affinity. In addition, the presence of residues Gln192 and Leu352 in the interaction indicates an additional contribution in strengthening the complex formed. Based on these validation results, it can be concluded that the docking approach used is able to predict ligand interactions with good accuracy, and shows that the ligand has the potential as a strong COX-2 inhibitor.

Table 5. Molecular docking results of ligand with target

Ligand	Binding energy (kcal/mol)	Inhibition constant	Hydrogen Bond (HB)	Hydrophobic bond (HI)
Natural ligands	-10.99	8.78 nM	8 HB: Arg120, Gln192, Leu352	11 HI: Val532, Ala527, Gly526, Leu384, Val349, Leu359, Leu531, Tyr385, Trp387
Tretinoin	-9.57	96.03 nM	3 HB: Gln192, Ser353, His90	9 HI: Phe518, Leu352, Val523, Met522, Tyr385, Trp387
Arachidonic acid	-7.93	1.53 uM	2 HB: Arg120, Tyr355	14 HI: Val349, Leu352, Ala516, Val523, Ala527, Ile517, His90, Tyr385, Trp387, Phe518
Celecoxib	-10.44	22.42 nM	4 HB: Arg120, Gln192, Leu352	15 HI: Val349, Ser353, Val523, Leu384, Met522, Leu359, Tyr355, Tyr385, Trp387, Ala527

Essential amino acid: Arg120

Molecular docking simulation can analyze the position of a ligand to the target and the chemical bonds that occur so that the affinity of the compound to a target can be predicted. Molecular docking simulation for test ligands is carried out to obtain the interaction and affinity of the test ligand to the active site of the COX-2 enzyme. The free energy of binding is a measure of the ability of the ligand to bind to the target. The smaller the free energy of binding, the higher the affinity between the target and the ligand, and vice versa, if the free energy of binding is greater, the affinity between the target and the ligand is lower (Rasyid, Mardiyanti, Arief, & Saputri, 2023).

The free binding energy (ΔG) value indicates the stability of the ligand to bind to the receptor. The more negative the Gbind value, the better the stability level, so that the bond between the ligand and the receptor is stronger and the Ki value indicates the concentration of inhibitor required to inhibit target performance (Syarafina, Safithri, Bintang, & Kurniasih, 2022). Test ligands that have inhibition constant values of less than 100 μM are considered as potential inhibitors, while inhibition constant values greater than 100 μM are not strong inhibitors (X. Zheng & Polli, 2010). Low binding free energy values indicate a stable ligand-target complex (Hairulazam et al., 2021). Molecular docking was performed on cyclooxygenase-2 (COX-2) enzyme with PDB ID 6COX using AutoDock 1.5.6 software. The results obtained showed that the natural ligand had the highest binding energy of -10.99 kcal/mol with an inhibition constant of 8.78 nM, indicating the strongest interaction compared to other tested ligands. Celecoxib, which is known as a selective inhibitor of COX-2, showed a binding energy of -10.44 kcal/mol with an inhibition constant of 22.42 nM. Tretinoin was recorded to have a binding energy of -9.57 kcal/mol with an inhibition constant of 96.03 nM, while arachidonic acid showed the lowest binding energy of -7.93 kcal/mol with an inhibition constant of 1.53 μM.

The stronger binding energy of celecoxib indicates that celecoxib has a higher affinity for COX-2 than tretinoin. The insignificant difference between tretinoin and celecoxib suggests that tretinoin may be an effective alternative, especially if pharmacokinetic aspects (such as solubility, skin penetration, and stability) are improved through nanosuspension formulation. Although celecoxib has better binding energy and inhibition constant than tretinoin, tretinoin still shows significant potential as an anti-inflammatory agent. This can be seen from the fairly strong binding

energy and inhibition constant which is still in the nanomolar range, tretinoin can function as an effective COX-2 inhibitor, especially in the context of topical treatment (inflammatory acne).

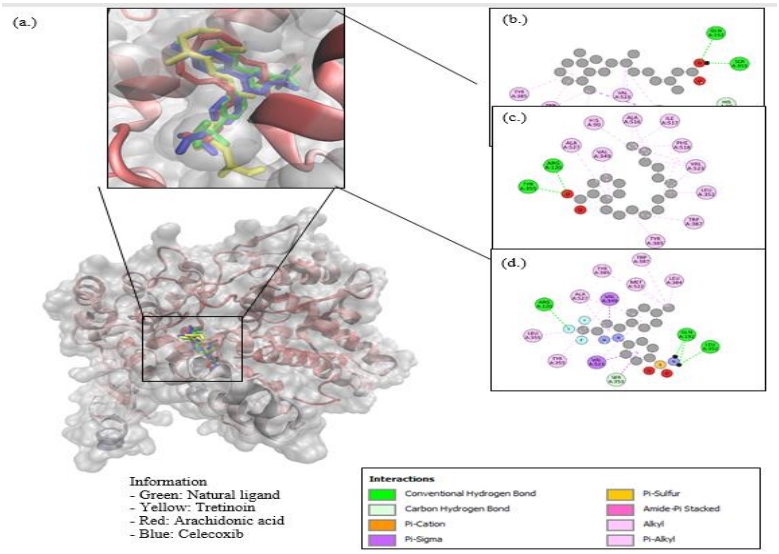


Figure 5. Molecular docking visualization of ligand-protein (a.), tretinoin (b.), arachidonic acid (c), celecoxib (d)

Simulation of tretinoin molecular docking with COX-2 showed that tretinoin has a binding energy of -9.57 kcal/mol, with an inhibition constant of 96.03 nM. The molecular interaction of tretinoin with COX-2 involves three hydrogen bonds with residues Gln192, Ser353, and His90, and nine hydrophobic interactions with residues Phe518, Leu352, Val523, Met522, Tyr385, and Trp387. Visualization results using Discovery Studio Visualizer and VMD show that tretinoin is able to bind tightly to the active site of the COX-2 enzyme, especially at key residues that play a role in regulating inflammation (Ahmad, Khan, Jamal, Alzahrani, & Albiheyri, 2023).

These findings strengthen the potential of tretinoin as a COX-2 inhibitor in the anti-inflammatory mechanism. A more negative binding energy indicates a stable tretinoin-protein complex, while a low inhibition constant indicates a fairly good binding affinity. Although tretinoin has a lower affinity than celecoxib, these results indicate its potential as an anti-inflammatory agent that can be optimized through a nanosuspension formulation approach to improve its stability and bioavailability (Kim & Kim, 2024).

CONCLUSION

The results of the material characterization showed that tretinoin is compatible with HPMC and PVP excipients, as evidenced by FTIR analysis which showed no chemical interactions in the form of new absorption peaks. XRD analysis showed a decrease in the intensity of the tretinoin crystallinity peak after being combined with the polymer, indicating a transition to a semi-amorphous form. DSC results showed a shift in the melting point of tretinoin, indicating a physical interaction between the active ingredient and the excipient. Nanosuspension characterization showed a particle size of <1000 nm, a polydispersity index of <0.5 reflecting a homogeneous particle distribution, a zeta potential of ±-20 mV indicating electrostatic stability, and an entrapment efficiency of >80% indicating the system's ability to optimally maintain tretinoin in the preparation. In addition, in silico tests showed that tretinoin has good binding affinity to COX-2, with a binding free energy of -9.57 kcal/mol and an inhibition constant of 96.03 nM, indicating its potential as an anti-inflammatory agent. The interaction of tretinoin with COX-2 involves three

hydrogen bonds and several hydrophobic interactions at the active residues of the enzyme. Visualization using Discovery Studio Visualizer showed that tretinoin forms a stable ligand-protein complex, supporting its mechanism of action in inhibiting inflammation. These results indicate that the tretinoin nanosuspension system is not only superior in terms of physicochemical properties, but also supports its pharmacological mechanism of action in inhibiting the inflammatory process in the skin.

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